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Progress Report

October 1985 – September 1986

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Prepared by
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

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LBL/JSU/AGMEF CONSORTIUM

PROGRESS REPORT

OCTOBER 1985 - SEPTEMBER 1986

Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

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INTRODUCTION

Fiscal Year 1986 represented the first year of U.S. Department of Energy funding for the activities of the Consortium that comprises the University of California Lawrence Berkeley Laboratory (LBL), Jackson State University, (JSU), and the Ana G. Mendez Educational Foundation (AGMEF). The Memorandum of Understanding establishing the LBL/JSU/AGMEF Consortium provides for the conduct of joint scientific research between the participating institutions as well as the strengthening of the academic and research capabilities of JSU and AGMEF.

The LBL/JSU/AGMEF Consortium is the first, and continues to be the only, such collaboration between a National Laboratory, a Black university, and an Hispanic university system. Fully consistent with its mission, LBL is supporting the development of the basic research and development capabilities of AGMEF and JSU. At the same time, JSU, an Historically Black College/University (HBCU) is providing the major academic development program for AGMEF, an Hispanic university system.

BACKGROUND

Numerous national research studies have shown that minority youth -- Black, American Indian, Mexican-American, and Puerto Rican students graduating from secondary schools on the mainland or in U.S. territorial possessions -- are minimally prepared academically to pursue college-level mathematics and science. Those who do enroll in university courses typically perform at lower academic levels than their non-minority counterparts. In addition, minority students who attend predominantly minority universities and colleges must cope with the limited capacity of these institutions to offer academically diverse mathematics, natural science, and computer science curricula.

There are several reasons for the dilemma cited above. Minority institutions generally do not have a broad array of scientific or applied research equipment. Many of the faculty have not had substantial research opportunities, others have not yet earned doctoral degrees. Consequently, this limits graduate level programs in the sciences at minority institutions. Indeed, many minority colleges lack strong basic undergraduate mathematics and science programs. Thus, these institutions are poorly equipped to provide the enriched academics or academic support systems needed to help a typical minority student succeed in the sciences. Ultimately, these conditions are the barriers that inhibit full participation of minority institutions in the mission and programs of federal agencies such as the Department of Energy, National Institutes of Health, NASA, and others.

As early as 1978, Lawrence Berkeley Laboratory (LBL) began a program to increase the involvement of scientists and researchers in minority institutions in the scientific programs that contribute to the Department of Energy's mission.

After a number of formal and informal meetings over a two year period, LBL and the Department of Energy (DOE) sponsored a "Conference on Energy Research at Historically Black Universities." Sixty participants represented nineteen historically black institutions, five national laboratories, the Department of Energy, and two majority institutions. The conference proceedings document well the objectives, research group projects, and summary recommendations. Of

particular interest here is the recommendation that a cooperative program be developed between the national laboratories and historically Black colleges/unversities. (Cf. Proceedings: Conference on Energy Research at Historically Black Universities. Lawrence Berkeley Laboratory report LBL-12615, 1980.)

By the next year, the first historic milestone was reached toward implementing that recommendation. With the stated goal of enhancing the "computer sciences and scientific research programs at both institutions, and...strengthening LBL's minority recruitment program," a Memorandum of Understanding establishing a joint, cooperative program between Jackson State University and Lawrence Berkeley Laboratory was signed on September 10, 1981. Substantial progress was achieved on each of the program's seven principal components.

- Collaborative Joint Research Projects
- JSU-LBL Staff Exchange Appointments
- Remote Access by JSU Researchers to LBL Computers
- Mini-courses, Lectures, and Seminars
- Scientific Support Services
- Equipment Loans

A striking outgrowth of the JSU-LBL collaboration was the September 1983 agreement between Lawrence Berkeley Laboratory, Jackson State University, and the Ana G. Mendez Educational Foundation (AGMEF) and its affiliate institutions (Puerto Rico Junior College, Metropolitan University and University of Turabo). A formal Memorandum of Understanding joined AGMEF, JSU, and LBL in a consortium designed to advance the science and technology programs of JSU and AGMEF. This is the first, and continues to be the only, such collaboration between an Hispanic university system, a Black university, and a National Laboratory. The goal of this alliance is basic and direct: to develop and effect a long-term, comprehensive program that will enable the campuses of the Mendez Foundation and Jackson State University to provide a broad, quality offering in the natural and computer sciences, to increase the number of minority students entering these fields, and to contribute to scientific knowledge and the federal government's science mission through research.

Both Jackson State University and the three campuses of the Mendez Foundation education system have entered into this collaborative effort in pursuit of common goals: (1) provide campus faculty with improved research opportunities, (2) provide campus faculty with improved research sessions, (3) improve the academic support system delivered to enrolled minority students, (4) develop strong undergraduate and graduate programs in mathematics, computer science, natural science, general science, technology, engineering, and other math-based disciplines, (5) develop pre-college programs that better prepare minority students for a college program, (6) increase the number of graduates from math-based degree programs, and (7) develop an institutional capability to engage in competitive research and academics.

Substantial progress has been achieved on a number of the components and objectives of the consortium.

- o M.S. and Ph.D. programs in natural and computer sciences
- o Faculty internship program and opportunities
- o Joint AGMEF/JSU/LBL faculty and research appointments
- o Student Co-operative programs
- o Development of undergraduate programs
- o Development of a Graduate Program for AGMEF students
- o Development of strong computer science academic offerings
- o Identification of funding sources to strenghten AGMEF curricula
- o Pre-College programs

PRINCIPALS

Lawrence Berkeley Laboratory

The Lawrence Berkeley Laboratory is a major national laboratory operated by the University of California under contract with the U.S Department of Energy. The laboratory's basic mission is to 1) perform multidisciplinary research in general science and energy sciences; 2) develop and operate major national experimental facilities for use by qualified investigators; and 3) provide educational and training opportunities for the next generation of scientist and engineers.

The connection between LBL and the Berkeley Campus of the University of California forms the basis of its unique capabilities to conduct basic research in an environment that combines academic quality with national research and development experiences and facilities. The LBL/JSU/AGMEF relationship is fully consistent with and directly supports the laboratory's mission.

Jackson State University

The Jackson State University School of Science and Technology is dedicated to addressing the problems of underrepresentation of minorities in scientific and engineering fields, and the participation of minorities in the nation's energy programs. Over the past several years, JSU has accelerated the development of its science and technology programs. It clearly offers the required institutional framework, a rich human resource, and state-of-the-art equipment necessary to stimulate strong, competitive academic and research programs that respond to the needs of minorities both nationally and internationally.

Ana G. Mendez Educational Foundation

The Ana G. Mendez Educational Foundation is the second largest private university system in Puerto Rico. It administers and directs three institutions with a combined enrollment over 17,500: the Universidad del Turabo, the Universidad Metropolitana, and Puerto Rico Junior College, a two year technical college. Most recently the AGMEF established the first privately operated educational television station in the Caribbean, WMTJ-TV, Channel 40.

The AGMEF's mission is to expand post-secondary education opportunities for the thousands of Puerto Rican youth that lack access to higher education, and to attract students with high potential who, because of their cultural and financial background, would otherwise be denied a college education.

CONSORTIUM ACTIVITIES - FISCAL YEAR, 1986

In Fiscal Year 1986, the Consortium's activities focused on five basic areas:

- Faculty Development
- Curriculum Development
- Student Development
- Collaborative Research Development
- Pre-College Programs

FACULTY DEVELOPMENT

Both Jackson State and the Ana G. Mendez Foundation have strong faculty development components as part of their programs. Foremost among the ways that LBL supports faculty development at JSU and AGMEF are the "Summer Faculty" Programs. In addition, JSU has made academic appointments to LBL staff. These activities have created the basis for the peer relationships between researchers at JSU, AGMEF, and LBL. AGMEF Faculty training at JSU is an important component of faculty development. Further faculty enrichment is supported by lecture an seminar series at JSU and at AGMEF campuses.

LBL/AGMEF Summer Faculty Program

Since the summer of 1984, faculty from the three AGMEF schools (Puerto Rico Junior College, Metropolitan University, and Turabo University) have spent approximately two months each summer at LBL to gain exposure to current research and research methodology in their fields. The interests of the faculty are matched with on-going research activities at LBL and the faculty work with LBL scientists in their respective fields. The faculty participated at LBL as Visiting Researchers.

The summer of 1986 saw four AGMEF faculty working for eight weeks at LBL on various projects. Two of the faculty worked in the LBL Applied Sciences Division, one in the Biology & Medicine Division, and one in Data Processing. Table 1 lists the AGMEF faculty and their areas of research. It is notable that, in one case, a strong possibility of a joint, co-operative research project has grown out of this summer experience (see "Collaborative Research Development" below).

TABLE 1. LBL/JSU/AGMEF 1986 SUMMER FACULTY PROGRAMS AGMEF FACULTY

Visiting Faculty	LBL Collaborator	Division	Research Area
Carlos Candelario	W. Bagot	Administration	Data Processing
Fernando Diaz	T. Novakov	Applied Science	Atmospheric aerosol sampling; performing measurements on the 71 samples taken at UMET. As part of this work, a proposal is being submitted to NSF for a joint, collaborative research project to expand the scope of the work by developing methods to measure gaseous mercury concentrations in the atmosphere and to establish a sampling station at the El Yunque Rain Forest.
Margarita Irizary	A. Chatterjee	Biology & Medicine	Radiation damage to DNA.
Wallis Medina Verges	T. Novakov	Applied Science	Atmospheric aerosol research: 1) analysis of aerosol samples taken in Puerto Rico, and 2) effects of cigarette smoke on the S (IV/S Total Ratio).

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LBL/JSU Staff & Faculty Appointments

As with the LBL/AGMEF program, Jackson State faculty work at LBL to gain exposure to current research methodology in their fields. The summer program with JSU has, perhaps, placed a greater emphasis on developing co-operative research programs and a number of such investigations are underway (see "Co-operative Research Development" below).

Eight JSU faculty came to LBL in the summer of 1986 for times varying from two to twelve weeks. With one exception, the faculty were hired as LBL Staff Scientists during their stay. Table 2 lists the JSU faculty and their areas of research.

One LBL scientist, Dr. Margaret Hutchinson of the Computing Division, received an academic year appointment (September 1985 - May 1986) at JSU to assist the Computer Science Department in the training of undergraduate/graduate majors.

AGMEF Faculty Training at Jackson State

Through this component, a major effort was made to increase the AGMEF faculty capabilities for research and teaching through formal studies. Emphasis was focused on existing AGMEF faculty with strong science backgrounds to complete advanced degrees in their major fields. Table 3 lists the fourteen AGMEF faculty members were enrolled in Masters of Science degree programs in the School of Science and Technology at JSU during 1985-86.

The training provided to these individuals has resulted in a more capable AGMEF faculty in computer science and biology. Hence, the AGMEF is well on its way to developing a self-sufficient capacity to offer and deliver a full range of quality science programs for its students.

TABLE 3. AGMEF FACULTY ENROLLED IN MS DEGREE PROGRAMS AT JACKSON STATE UNIV.

Name	Major	Graduation
Aponte, Jorge	Computer Science	July, 1986
Berchal, Juan	Computer Science	July, 1986
Candal, Isabel	Computer Science	July, 1986
Candelario, Carlos	Computer Science	May, 1986
Esteves, Juan	Computer Science	May, 1987
Figueroa, Carlos	Computer Science	Dec, 1987
Garcia, Edgardo	Computer Science	May, 1986
Liard, Felix	Computer Science	July, 1987
Menendez, Leida	Biology	July, 1986
Reveron, Carmen	Biology	May, 1987
Rivera, Candido	Computer Science	Dec, 1987
Rondon, Jose	Computer Science	May, 1987
Santiago, Luis	Computer Science	Dec, 1987
Sevillano, Wanda	Computer Science	May, 1986

TABLE 2. LBL/JSU/AGMEF 1986 SUMMER FACULTY PROGRAMS JACKSON STATE UNIVERSITY

Visiting Faculty	LBL Collaborator	Division	Research Area
Prem Bhalla	A. Shoshani	Computer Science	Behavior of multidimensional file structure (Grid File) under parallel disk allocation. Also literature surveys of 1) scientific and statistical data bases, 2) query optimization techniques, 3) sampling and antisampling procedures, 4) statistical summary tables and related query languages, and 5) file organization and performance.
William Brewer	I. Turiel	Applied Science	Water heater energy use: modifying algorithms used in existing computer model, performed parametric analyses of the impact of various design options on energy efficiency.
Mohammed Heydari	W. Johnston	Computer Science	Remote Procedure Calls
R. Shayne Johnston	A. Sessler	Physics	Optical guiding in a free Electron Laser. A proposal to DOE is being prepared.
Bee Lee Lim	W. Bagot	Administration	Data Processing: examine feasible options for an effort reporting system that would use departmental PCs to upload effort data to an IBM mainframe for processing and to download generated reports.

Table 2
Page 2

Visiting Faculty	LBL Collaborator	Division	Research Area
Cass Parker	T. Novakov	Applied Science	Atmospheric aerosol research.
Hiroyasu Tachikawa	F. McLarnon	Applied Science	Constructed special electrochemical cells to be used at JSU to characterize electrode reactions by Raman Spectroscopy. This work is being conducted under a subcontract that LBL has placed with JSU.
Wen-Hsun Yang	A. Chatterjee	Biology Medicine	Effect of oxygen on enhancement of strand break in the gamma-ray radiated DNA of SV-40 virus. A paper, "Two dimensional electrophoresis for analysis of SV-40 viral DNA with polyacrylamide gel block in agarose gel," is close to publication.

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Seminar Presentations at Jackson State

This component of the program has provided the natural/computer science faculty and students an opportunity to meet and interact with prestigious, noteworthy scientists and intercept the knowledge which these scientists shared. JSU faculty and students were target audiences of several seminars in the natural/computer sciences. Table 4 lists the seminar speakers and topics.

These seminars have consistently provided the faculty and students with personal/educational enrichment which has enhanced their interest, knowledge, and research capabilities in the sciences. Ultimately, this has impacted the quality of research, teaching efforts, and learning opportunities in the School of Science and Technology at JSU.

TABLE 4. SEMINAR PRESENTATIONS, AT SCHOOL OF SCIENCE AND TECHNOLOGY, JACKSON STATE UNIVERSITY

Speaker	Seminar Presentation
Dr. J. Ivan Legg Washington State University	"Transition Metal Ions on Probes of Biological Functions"
Dr. Lynn Jelinski AT&T Bell Laboratories	"The Molecular Details of Motion in Bulk Polymer - Results from Solid State D_2 in NMR"
Dr. Elon Close Lawrence Berkeley Laboratory	"Discrete Models: Their Effective Construction and Solution"
Mr. Adrian Rodriguez Lawrence Berkeley Laboratory	"Radiation Biophysics"
Dr. Jim Clock University of Kentucky	"Mechanism of Aging"
Dr. Brend Holmes Naval Research Laboratory Washington, D.C.	"Polymer Chemistry"
Dr. Walter McIntyre National Center for Atmospheric Research	"USAN: A University Satellite Network"

AGMEF Distinguished Lecturers Series:

The Distinguished Lecturers Series are in-service training activities aimed at spreading current scientific knowledge among the Scientific Community in Puerto Rico and involving the participation of graduate students as well as Science Faculty of AGMEF's institutions and other universities in the Island.

The first cycle of lectures for the academic year of 1985-86 began on February 19, 1986 with the lecture given by Dr. George Hillyer on the topic of the Usage of New Advances in the Studies of Immunodiagnostic and Immunity in Bilharzia at the Universidad Metropolitana.

Three other lectures were given from March through May: "Use of Radiosensitizers in Cancerous Tissues" by Dr. Gabriel Infante from Catholic University of Puerto Rico; "Chemical and Biochemical Process in DNA: Damage Studies with Ionizing Radiation" by Dr. Aloke Chatterjee of LBL; and "Astrophysics" by Dr. William Bruckman of UPR. These lectures were all given at UMET.

University of Turabo had two lectures given by UPR professors from the Graduate Program in Biology and they are: "Biogeography of the Spherodactylus Gender in the Antilles Region" by Dr. Richard Thomas and "Hormonal Control of Vitelogenesis in the Tropical Lizards" by Dr. Magda H. Morales.

Dr. Gladys Escalona de Motta, UPR professor at the Neurobiology Laboratory in the Medical Science Campus, conferred on "New Advances in the Study of Activated Cells" at PRJC's lecturer series.

CURRICULUM DEVELOPMENT IN NATURAL SCIENCE AT AGMEF FOR THE ACADEMIC YEAR 1985-86

The following curriculum development activities were accomplished throughout the academic year 1985-86 in the Natural Sciences area:

Metropolitan University (UMET)

1. Honors Program in Natural Science was established.

2. New laboratory experiences for Physics 203-204 were designed.

3. Physics 460 was offered for the first time together with an operating electronic laboratory.

4. Handbooks for Math courses 111-112, 100, 105, 107 add 108, 98 and 99 were revised.

Metropolitan University (UMET) con't.

5. The course Molecular Biology was designed.

6. The Academic Board approved a new course, Modern Physics.

7. A MISIP proposal was approved to revise the curriculum in courses such as: Anatomy and Physiology, Human Biology, Molecular Biology, and Microbiology.

8. Collaboration efforts were made with Channel 40 to prepare a new course in Biology.

Puerto Rico Junior College (PRJC)

1. New academic programs were created:

   a) Associate Degrees in Science and Technology

      (1) Institutional Assistant Pharmacist - August 1986
      (2) Industrial Pharmacist Technician - implementation pending on market study
      (3) Ultrasonic Diagnostic Technician - August 1986

2. Development of the proposals for New Academic Programs: (in progress)

   a) Associate Degrees in Science and Technology

      (1) Digital Technology (Maintenance and Robotic Services)
      (2) Forest Resources Technician
      (3) Medical Emergency Technician (paramedics) - 110 hours certification for Municipal Ambulance Drivers (combined efforts with PROSEE) - 840 hours certification (combined efforts with Dean of Extension) - Associate Degree - Paramedic Technician

   b) General Education

      (1) Associate Degree in Communications

         - Production Technician in:

            o Radio/TV
            o Commercial Design
            o Instructional Technology

         - Transfer degree in Communications

            o Journalism
            o Publicity
            o Public Relations

(2) Associate Degrees in Psychology/Social Work

- Crisis Intervention specialist (courses will be incorporated to all health related sciences)

c) Business Management

(1) Associate Degree in Microcomputer Applications

3. Development of market study to assess the pertinence of the following courses (study was conducted in the summer '86 DOE/LBL funds):

a) Industrial Pharmacy

b) Industrial Quality Control

(1) Food
(2) Chemistry
(3) Pharmaceutical

4. The Academic Board approved the creation of new courses.

a) The following courses respond to CDU's initiatives.

(1) Math 110 - Introduction to College Math
(2) Spanish 110 - Introduction to College Spanish
(3) English 110 - Introduction to College English

5. Starting in January '87, remedial courses in Math, Spanish, and English will be eliminated and substituted for the above courses mentioned.

6. Program inactivation

a) Automotive Technology (last group of students graduated this semester)

7. Program activation

a) Natural Resources Management (program will be strengthened with the expansion of the specializations in Forest Resources Management and Fishing).

<u>Universidad del Turabo (UT)</u>

1. Implemented B.S. in Chemistry

2. A proposal for a B.S. in Applied Math was presented to the Curriculum Committee, Academic Board, and Administrative Council.

3. Two new courses in Math were presented:

   a) Problem solution

   b) Number theory

4. Two new laboratories were constructed; one for Instrumental Analysis and another for Chemistry Physics.

5. The second group of students were admitted to the Honor Program.

6. Two professional enhancement workshops were designed for science teachers at the Public Department of Education.

7. MISIP was initiated for curriculum revision in chemistry, laboratory remodeling, and preparation of new courses.

8. Started working with Project CAUSA.

STUDENT DEVELOPMENT

In support of the Student Development efforts of both JSU and AGMEF, two programs are conducted at LBL. AGMEF conducts three additional programs aimed at student enrichment: Science honors, undergraduate research, and a student lecture program.

Semester Co-operative Program

Under the LBL/JSU Semester Co-operative Program, students from Jackson State may come to LBL for a full, academic semester to work with LBL staff scientists. The program is offered to a limited number of students who meet stated eligibility requirements and who are majoring in a natural or physical science, mathematics, computer science, or engineering. Students are selected on the basis of their academic standing, faculty recommendations, and interviews conducted by LBL scientific staff. LBL provides participating students with round-trip transportation, housing and board in family homes, health insurance, and a modest living expense allowance.

The Semester Co-operative Program is designed to be as complete an academic/research experience as possible. Participating students are assigned to work for LBL staff scientists who assign their day-to-day work and the course of their work during their stay. Depending on the specific placement, students work on aspects of experiments, do general laboratory research work, and work with state-of-the-art scientific or computer equipment. An agreement with the University of California, Berkeley enables the students to enroll in courses on the UC campus. This has been especially useful in the case of courses that are not available at the student's home campus.

Five JSU students participated in the Spring, 1986 Semester Co-operative program. Two worked in LBL's Computer Science Division; two worked in the Biology & Medicine Division; and one worked in the Applied Sciences Division.

At the request of their scientific sponsors, three of the the students remained to participate in the Summer program (described below). Table 5 lists the students and their areas of research.

TABLE 5. 1986 SPRING SEMESTER CO-OPERATIVE PROGRAM, PARTICIPANTS

Student	Scientific Sponsor	Division
Corliss Austin*	A. Rodriguez	Biology & Medicine
Raquel Cobbins*	E. J. Ainsworth	Biology & Medicine
Robert Holt*	E. Theil	Engineering (Computer Science)
Vanessa Miller	C. Lederer	Computer Science
Stacy Robinson	R. Ritschard	Applied Sciences

* Remained to participate in Summer Program

Summer Internship Program

Students from Jackson State and the two Universities of the Ana G. Mendez Educational Foundation (Metropolitan and Turabo) participate in the LBL Summer Internship Program. As with the Semester Co-operative program, summer internships are offered to a limited number of students who meet stated eligibility requirements and who are majoring in a natural or physical science, mathematics, computer science, or engineering. Students are selected on the basis of their academic standing, faculty recommendations, and interviews conducted by LBL scientific staff. LBL provides participating students with round-trip transportation, and the students become Laboratory employees with a "Student Assistant" status.

Participating students work for LBL staff scientists who assign the students their day-to-day work and the course of their work during the summer. Depending on the specific placement, students work on aspects of experiments, do general laboratory research work, and work with state-of-the-art scientific or computer equipment.

The bulk of the students' time is spent on their assigned work with LBL staff scientists. However, additional assignments and events round out the program so that the laboratory experience can be as complete as possible.

- Tours of LBL and LLNL expose students to the wide range of scientific research being carried out today and to state-of-the-art scientific and computer equipment.

- Early in the program, students write short statements of their research goals for the summer. In this way, they are able to quickly focus on the work that they will be performing during the summer. By meeting with the staff of the Office of Minority Outreach Programs (OMOP) and fellow students, the students are provided a further opportunity to articulate their work and goals.

- Toward the end of the program, the students complete a paper on their summer work (these papers are presented in Appendix A). In addition to written papers, the students present their work to a seminar of their peers and scientific sponsors.

Seventeen students participated in the 1986 Summer Internship Program. Nine of the Students were from AGMEF universities; eight were from Jackson State. Of the seventeen, five worked in Computer Science (either in the LBL Computer Science Division or the Real Time Systems Group of the Engineering Division); nine worked in the Biology & Medicine Division; two worked in the Applied Sciences Division; and one worked in the Physics Division. Table 6 lists the students from the AGMEF schools and the areas they worked in. Similarly, Table 7 lists the students from JSU.

Science Honors Programs:

The Universidad del Turabo established an Honors Program in the Fall of 1984 with 17 high achieving freshmen. The Program offers a special curriculum and a series of enrichment activities, as well as a substantial Honors Scholarship. The program currently has 27 students in General Natural Science, Biology and Chemistry. The UT Honors program had 25 freshmen in 1986. The UMET initiated a similar Honors Program in General Natural Science and Biology in the Fall of 1986 with 15 students.

Undergraduate Research Program:

The UT and UMET both have established undergraduate research programs through formal undergraduate research courses. Most research is undertaken as a follow-up by students participating in the LBL Summer Program. A total of 22 students have completed or are currently involved in undergraduate research projects.

Student Lecture Program:

A student lecture program was established as an on-going activity to expose students to the latest developments in the various fields. Scientists from Puerto Rico and the mainland have offered over 15 lectures since the program began.

TABLE 6. LBL/JSU/AGMEF 1986 SUMMER STUDENT INTERNSHIP PROGRAM PARTICIPANTS FROM AGMEF UNIVERSITIES

Students	Sponsors	LBL Div	Research Area
Jacqueline Colon-Sanchez	C. Lederer	Computer Science	SPSSX and TELL-A-GRAPH Languages
Jose Emmanuelli-Rivera	R. Maderas	Physics	High Energy Elementary Particle Physics
Angel Malavet-Rodriguez	J. Schooley	Biology & Medicine	The Study of the Role of Macrophages in the Inhibition of Erythropoieses
Jannette O'Neill-Gonzalez	S. Ebbe	Biology & Medicine	Effect of Thromobocytopenia on Artherosclerosis in Rabbits with Dietary Hypercholesteolemia
Andres Padilla-Nevarez	E. Close	Computing	SPSSX and TELL-A-GRAPH Languages
Wanda Reyes-Coraliz	T. Novakov	Applied Science	Atmospheric Aerosol Chemical Sampling
Sylvia Torrens-Santiago	N. Brown	Applied Science	Nitrous Oxide Formation in Combustion
Rosalyn Torres	V. Paxson	Engineering (Computing)	Language Translation and Software Maintenance
Cynthia Velez-Garcia	R. Leven	Biology & Medicine	Effect of Thrombopetic Substances on Megakaryiocyte Metabolism

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TABLE 7. LBL/JSU/AGMEF 1986 SUMMER STUDENT INTERNSHIP PROGRAM PARTICIPANTS FROM JACKSON STATE

Students	Sponsors	LBL Div	Research Area
Corliss Austin	A. Rodriguez	Biology & Medicine	The Effects of Irradiation on Normal Kidney Tissue
Raquel B. Cobbins	J. Ainsworth	Biology & Medicine	Aspects of Radiation Research: The Radiation Biophysics Group
Pauline Francis	V. Jacobson	Engineering (Computing)	Performance Analysis of Ethernet
Robert Holt	E. Theil	Engineering	Software Maintenance and Program Conversion
Pat Camille Jacobs	E. Blakely	Biology & Medicine	Cellular and Molecular Radiobiology of Charged Particicle Radiation Damage
Andrea Moman	J. Afzal	Biology & Medicine	Tumor Radiobiology
Michael Proctor	R. Schwarz	Biology & Medicine	Procollagen Secretion and Synthesis
Doris J. Tribune	M. Esposito	Biology & Medicine	Genetic Recombination during Mitosis and Meiosis of Saccharomyces Cerevisiae

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COLLABORATIVE RESEARCH PROJECTS

Jackson State

Throughout 1985-86, the LBL-JSU venture has crystallized into a program with increasing definition and purpose. The original intent was to build the program around faculty collaborative research efforts. There have been numerous meetings between LBL scientists and JSU faculty in attempts to define these scientific relationships. Table 8 lists some of the research that has been jointly pursued by LBL and JSU scientists.

TABLE 8. COLLABORATIVE RESEARCH EFFORTS, LBL-JSU

"Theory of Free Electron Lasers"	Dr. Shayne Johnston	JSU
	Dr. Andy Sessler	LBL
"Effect of Radiation on Cells"	Dr. Balwant Sekhon	JSU
	Dr. Aloke Chatterjee	LBL
	Dr. Wen-Hsun Yang	JSU
"NMR Carbon Fixation Studies"	Dr. Richard Sullivan	JSU
	Dr. Rolf Melhorn	LBL
"Building Energy Analysis"	Dr. Sonny Bolls	JSU
	Dr. Ron Ritschard	LBL
"Characterization of Ambient Carbonaceous Aerosols in Southeastern United States"	Dr. Cass Parker	JSU
	Dr. Ray Dod	LBL
	Dr. Tihomar Novakov	LBL
"Chick Tendon Proteoglycans"	Dr. Denise Chan	JSU
	Dr. Mina Bissell	LBL

These arrangements have been formed on the basis of "equal partners" in research rather than the usual paternalistic relationships which have developed out of similar circumstances in previous years among faculty from other organizations. A major factor which contributes to the "peer relationship" between the JSU researchers and LBL scientists is the annual Research Conference held on the JSU campus during the Spring Semester.

During the Research Conference held April 28-29, 1986, several select LBL scientists and JSU professors made presentations and discussed research concepts for future joint proposals. A total of twenty-one presentations were made; fourteen by JSU researchers and seven by LBL scientists.

Ana G. Mendez Educational Foundation

Atmospheric Sciences Research:

For the first time, UMET faculty are involved in a major research project. Working in collaboration with LBL scientists in Atmospheric Sciences, UMET faculty are determining levels and movement of pollutants in Puerto Rico as part of a worldwide study. This should result in several publications and a major Conference to be held in Puerto Rico by 1988.

Seed Grants:

In 1985 the AGMEF instituted a seed grant program to provide faculty with initial funding for research initiatives. Small grants are awarded to faculty to encourage research and to seek external funding. Several faculty have initiated modest research projects involving students who participated in the Summer Internship programs.

PRE-COLLEGE PROGRAMS

Two Puerto Rico pre-college programs at LBL were available to teachers and students from Puerto Rico and Mississippi. Additionally, AGMEF has undertaken a wide-ranging, comprehensive pre-college program in collaboration with the Puerto Rico Department of Education.

Science Award Week

The Puerto Rico Public Department of Education (PDE) and Ana G. Mendez Educational Foundation (AGMEF) coordinated the participation of science students and teachers in a series of programs which took place at Lawrence Berkeley Laboratory (LBL) last summer. The Ana G. Mendez Educational Foundation, through the Consortium established with JSU/LBL, has added another contribution to the enhancement of science and math programs in PDE's intermediate and high school levels.

Felipe Carrasquillo and Adalberto Melendez, both PDE's students at Jose S. Alegria School and Rafael Rocca High School respectively, participated in the program called Science Award Week. Both students were science award winners in the past State's Science Fair. Through this program, these students, along with 18 students from other High Schools in the mainland, have acquired new experiences and have been exposed to highly complex lab research work. Petra Camacho and Doris Irizarry were the two teachers, who along with Carrasquillo and Melendez, participated in the Science Award Week program. These professors were chosen from a group of top professionals who assisted various students in research work for the Science Fair. The program provides high school students who have demonstrated ability and interest in science and mathematics with an opportunity for direct interaction and exposure to research scientists, facilities, and projects at LBL.

Summer Teacher Program

The LBL Summer Program is a six-week program with approximately 20 high school teachers. The participating teachers are assigned to research groups that match their interest, educational background, and current teaching responsibilities. The program focuses on the teachers' ability for scientific analysis and critical thinking. Placing secondary school teachers in the research laboratory as active participants creates a unique challenge and opportunity for them to see another dimension to science and scientists. Approximately eighty percent of the time is spent in the research laboratory; twenty percent of the time is spent in lectures, seminars, or tours.

Esilda Reyes and Luis Sepulveda from the Puerto Rico Public Department of Education and James Freddie Joyce from the Holmes County School District in Mississippi participated. The program ran from June 23 through August 1.

Comprehensive Activities to Update Academics (CAUSA)

A wide ranging pre-college program has been undertaken with the 700,000 students of the Commonwealth of Puerto Rico's Department of Education system to increase the number of students with high potential for a career in science to enter and remain in the science pipeline. The program, Comprehensive Activities to Upgrade Science Academics Campaign (CAUSA), has included:

A Comprehensive Pre-College Development Plan with the Department of Education of Puerto Rico for a ten year collaborative program and the signing of an MOU with the Department of Education;

Training 130 elementary and secondary school science teachers in science (biology, chemistry, and physics) and in teaching techniques, and;

Establishment of the Academic Academy to train and motivate 180 gifted students from the Island's public schools. These students were selected from 630 students who took the College Board Examination Tests. This program offers these students special enrichment activities in the sciences during Saturdays at the Institutions' labs.

It was recognized early on that a major effort was required in developing pre-college initiatives if the number of good, well trained and motivated pre-college students entering the science pipeline was to increase and if the AGMEF institutions were to have a significant pool of students for college science majors. This required a comprehensive development program to upgrade the quality of science education in the Island's 700,000 student public school system.

After a year of intensive, collaborative planning with Puerto Rico's Department of Education, the AGMEF signed a Memorandum of Understanding with the Department of Education to carry out a ten year development program in

Teacher Training, Curriculum Development and Student Programs; Comprehensive Activities to Upgrade Science Academics (Campaign C.A.U.S.A.). The major achievements to date are:

Teacher Training:

Eighty elementary school science teachers trained through a formal three credit course at the UT in the specific content they teach and in teaching techniques, responding directly to the new curriculum.

Forty high school Biology and Chemistry teachers trained through a formal three credit course in Curriculum Differentiation at UT. Teachers received training in their field and designed a new Biology and Chemistry curriculum for the system that will be tested in the Island's public schools this year.

Under LBL and AGMEF sponsorship, two outstanding public high school science teachers who participated in a six week program at LBL to expose them to up-to-date science education techniques and to research in their fields.

Also under LBL and AGMEF sponsorship, two other outstanding teachers were sent to participate in Science Education Week at LBL, to expose them to a research environment and advances in their field and science education.

Student Development/Academic Academy:

The most significant achievement in the Pre-College Component has been the implementation of the Academic Academy. Through a $335,000 grant by the Carnegie Corporation, the AGMEF has identified 180 gifted 9th to 12th graders among 2,300 applicants to participate in a Saturday program of enriched curriculum and research in science and mathematics, lectures, field trips, English and counseling. The purpose of the program is to train and motivate them to enter a career in the math based fields. Also through this program, enrichment activities and special curriculum in science and math have been designed for use in the Island's public school system, and for the first time, a system for identifying gifted junior and senior high school students in Puerto Rico has been designed and is being tested for system wide use.

SUMMARY

The momentum of the program has provided much success for the participating institutions. Accomplishments have surpassed our most optimistic expectations. The program has had a major impact on developing minority science faculty for teaching and research. It has enhanced and broadened academic programs and facilities. A number of student development options in training and graduate/undergraduate research has been created.

These attainments are primarily the product of an honest, open, and intimate collaboration between the participating institutions; systematic inter-institutional planning; and a strong commitment to the long-term development program. It is evident that the program has grown significantly in recent years, and will continue to respond to changing needs.

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4/7/87

APPENDIX

LBL/JSU/AGMEF CONSORTIUM

PROGRESS REPORT

OCTOBER 1985 – SEPTEMBER 1986

STUDENT PAPERS

SUMMER INTERNSHIP PROGRAM

1986

APPENDIX: STUDENT PAPERS
SUMMER INTERNSHIP PROGRAM, 1986

SPSSX and TELL-A-GRAPH Languages A-1
(Jacqueline Colon-Sanchez, Metropolitan University)

High Energy Elementary Particle Physics A-19
(Jose Emmanuelli-Rivera, University of Turabo)

The Study of the Role of Macrophages in the Inhibition of Erythropoieses A-29
(Angel Malavet Rodriquez, University of Turabo)

Effect of Thromobocytopenia on Artherosclerosis in Rabbits with Dietary Hypercholesterolemia A-35
(Jannette O'Neill-Gonzalez, University of Turabo)

SPSSX and TELL-A-GRAPH Languages A-49
(Andres Padilla-Nevarez, Metropolitan University)

Atmospheric Aerosol Chemical Sampling A-71
(Wanda Reyes-Coraliz, University of Turabo)

Nitrous Oxide Formation in Combustion A-81
(Sylvia Torrens-Santiago, University of Turabo)

Language Translation and Software Maintenance A-91
(Roslyn Torres, Metropolitan University)

Effect of Thrombopoetic Substances on Megakaryiocyte Metabolism A-147
(Cynthia Velez-Garcia, University of Turabo)

The Effects of Irradiation on Normal Kidney Tissues A-151
(Corliss Austin, Jackson State University)

Aspects of Radiation Research A-161
(Raquel B. Cobbins, Jackson State University)

Performance Analysis of Ethernet A-169
(Pauline Francis, Jackson State University)

Software Maintenance and Program Conversion A-181
(Robert Holt, Jackson State University)

Cellular and Molecular Radiobiology of Charged Particle Radiation Damage A-189
(Pat Camille Jacobs, Jackson State University)

Tumor Radiobiology A-193
(Andrea Moman, Jackson State University)

Procollagen Secretion and Synthesis A-209
(Michael Proctor, Jackson State University)

Genetic Recombination During Mitosis and Meiosis of Saccharomyces Cerevisiae A-215
(Doris J. Tribune, Jackson State University)

1728D

TELL-A-GRAF AND SPSXX LANGUAGES

Jacqueline Colon-Sanchez
Metropoliton University

Research Sponsor: Dr. Claudette Lederer
Information and Computer Science Division

On June 16, I started on a project that required my preparation in different kinds of computer programming. The first week at the Lawrence Berkeley Laboratory was dedicated to learning about all the software that I needed for my job this summer. The first one is the Visual Editor. It is a software program that allows you to make different texts and gives you the opportunity to store this text in a file. When you have a file, you can read, write and erase part of the text without making a new one again. If we compare the EDT EDITOR with the VI EDITOR we can conclude that the VI EDITOR has a large group of commands that allow you to work easily on your text.

TELL-A-GRAF is an interactive graphic package written in Fortran that is widely used at Lawrence Berkeley Laboratory for graphic display and for producing hardcopy for publications in reports. My job is to produce a compendium of scientific and statistical chart examples that will help users understand this product. The examples were taken from a data set that had previously been used to produce Lawrence Berkeley Laboratory research reports. This software is so powerful it has a large series of commands that are divided in groups. The instructions in TELL-A-GRAF are written in English; one of the reasons it is so friendly to use. When you work in TELL-A-GRAF the main instructions are full sentences but there are some special commands such

as "skinny" (instructions that make the bars thin) that are known to TELL-A-GRAF. An example of a full sentence instruction is "GENERATE A PLOT" (this instruction produces a plot chart).

A week later my director sent me to the Computer Center under the supervision of Claudette Lederer and Elon Close. They gave me two projects to do during the summer session (7 weeks). The first one is the revision of a book that intends to teach how use TELL-A-GRAF to make scientific graphics. For that, I made a compendium of chart examples and corrected the bad instructions in this book. The revision that I made, was done on an ADM3A terminal with total option to graphics (this terminal is connected to a main computer VAX 780).

With TELL-A-GRAF you can realize many types of graphics in the same type of chart. That means that you can produce one plot chart but with different kind of lines in the configuration aspect. It allows you to realize two different bar charts, the horizontal bar chart and the vertical bar chart. We can also produce pie charts. The user can input the labels in English and TELL-A-GRAF can print the same label in Russian, Greek, or Swedish if you so decide.

To produce a graph with TELL-A-GRAF you need some special sofware and hardware packages. First you will need a terminal that has total options for graphics and a printer that can produce a hardcopy of your graphic. Then you need to have access words or a path that will allow you to use TELL-A-GRAF: (ISSSCO) and also (TAG) to call the sofware TELL-A-GRAF. The first one is Generate. This word instructs TELL-A-GRAF to produce some type of instructions (a specific instruction is given to inform the type that you want to produce).

Another instruction is GO. This one means that all the commands are done and that it can show you on the screen what your graphic will be like. The most important instruction is the period at the end of all the instructions because in that way TELL-A-GRAF recognizes that the instruction is finish.

Another part of TELL-A-GRAF is Page, a command that can produce different front pages of a text. You can use one variety in type of letters of fronts. The size of the letters that you want to use depends on the number that you input when giving the command.

The second project is another revision of a reference book in TELL-A-GRAF. For this project, I had to make data fields; and make it possible for the program to read the data field and then run the program. This project was not finished because of lack of time but half of the program is corrected. In this part, I continued working with TELL-A-GRAF but with more powerful instructions. To make the data fields, I used the VI EDITOR and SPSSX.

The SPSSX Batch System is a comprehensive tool for managing, analyzing and displaying data. It has many capabilities; one of them is to be able to input from almost any type of data file and device-independent graphics.

At the end of the summer session I felt very happy because my goal has been realized; I go back to my island with new knowledge in the computer field and with the opportunity to teach to all my fellow students in the University all the new concepts that I have had. This will be possible because I work in the students tutor program. Another important point is that here I met people like Claudette Lederer, Elon Close, Donald Rondeau and Dr. Chatterjee. All of

them are really well prepared professionals who are very interested in the student's progress.

This learning experience gave me the satisfaction of helping scientists in their research and simulations. This program helps to prepare the future of tomorrow.

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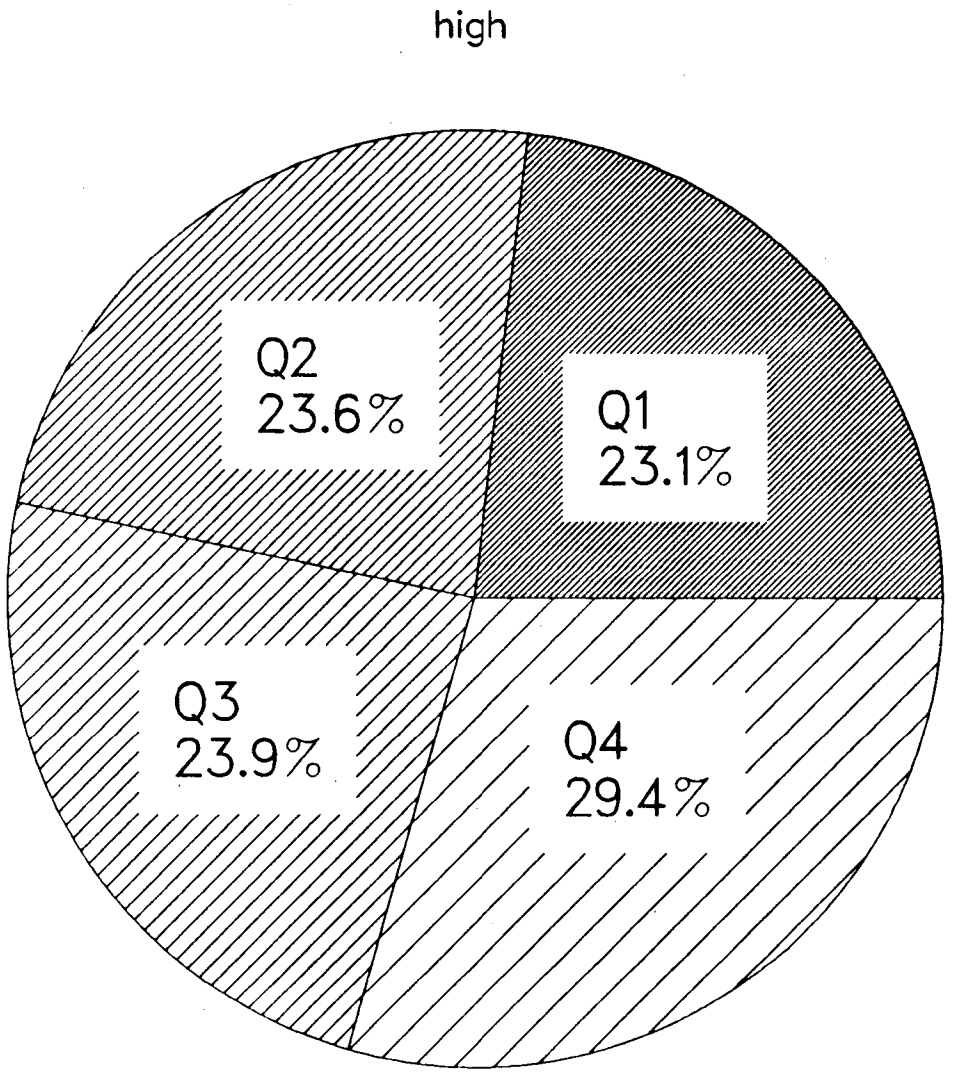

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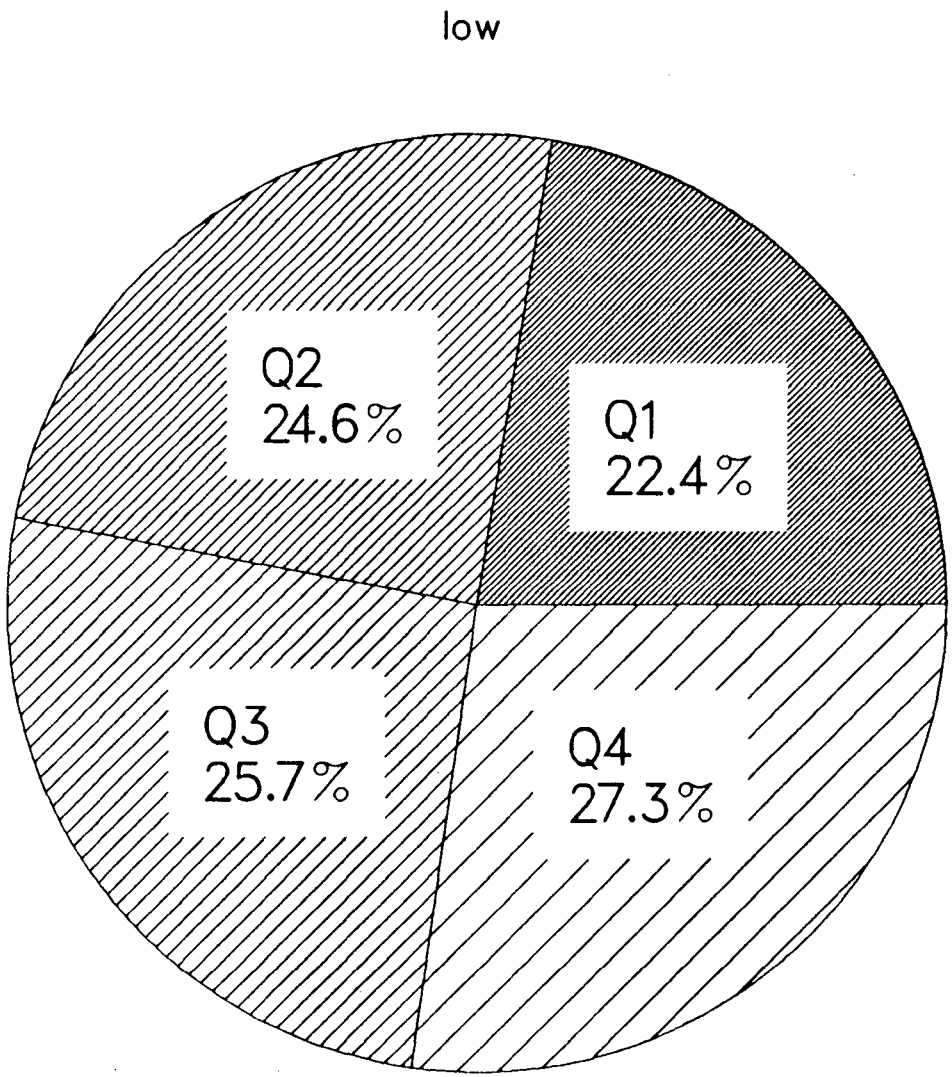

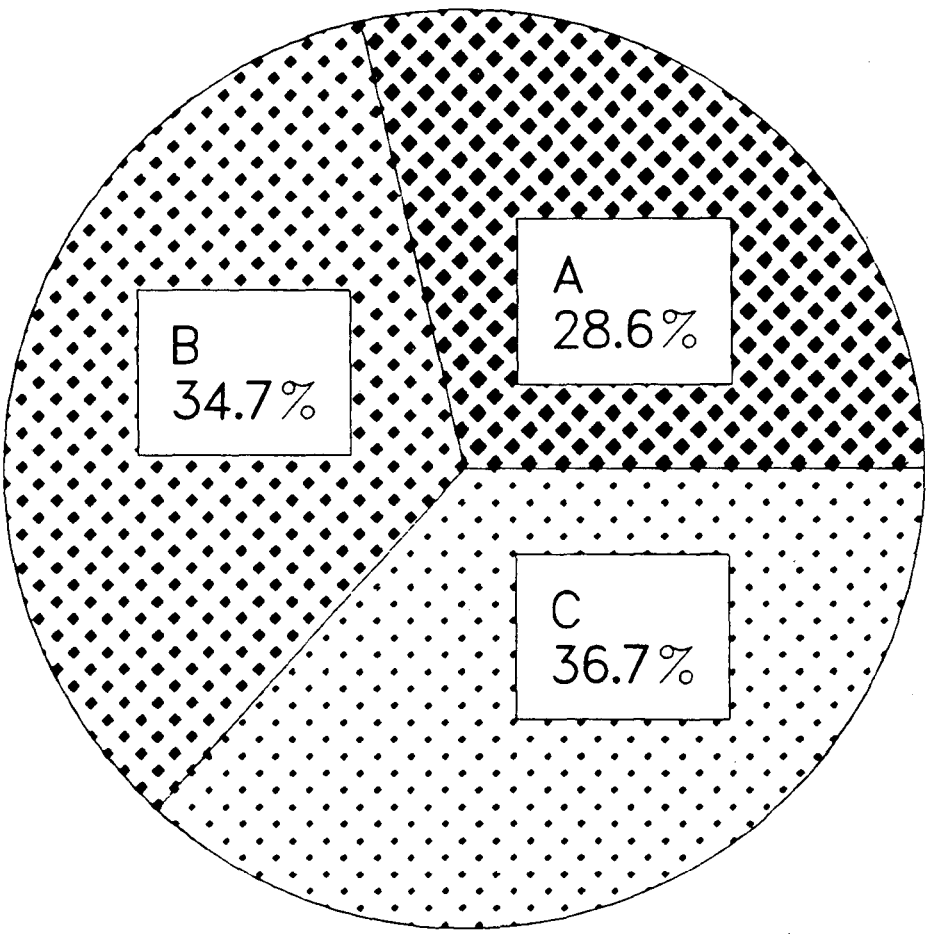
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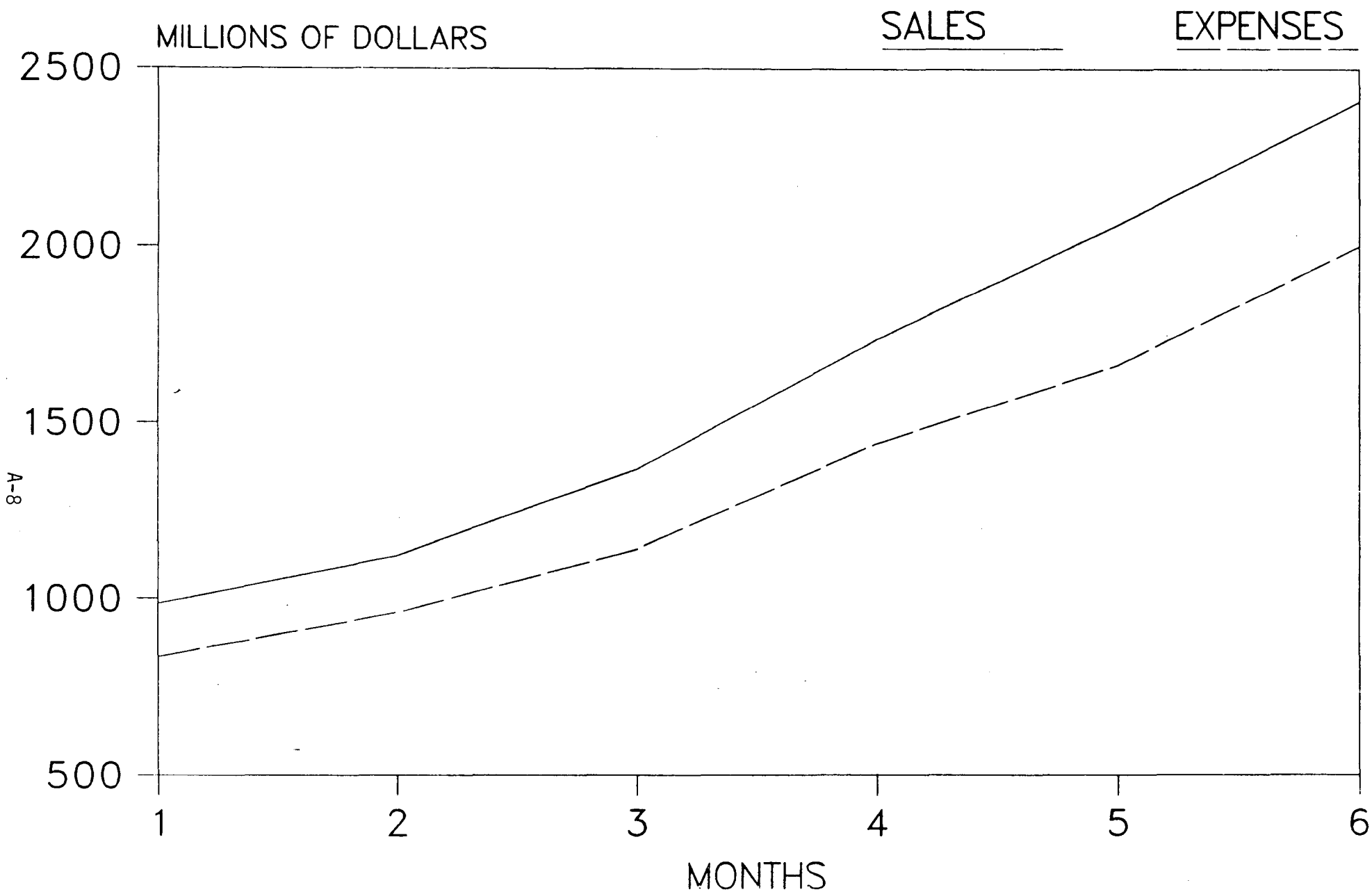

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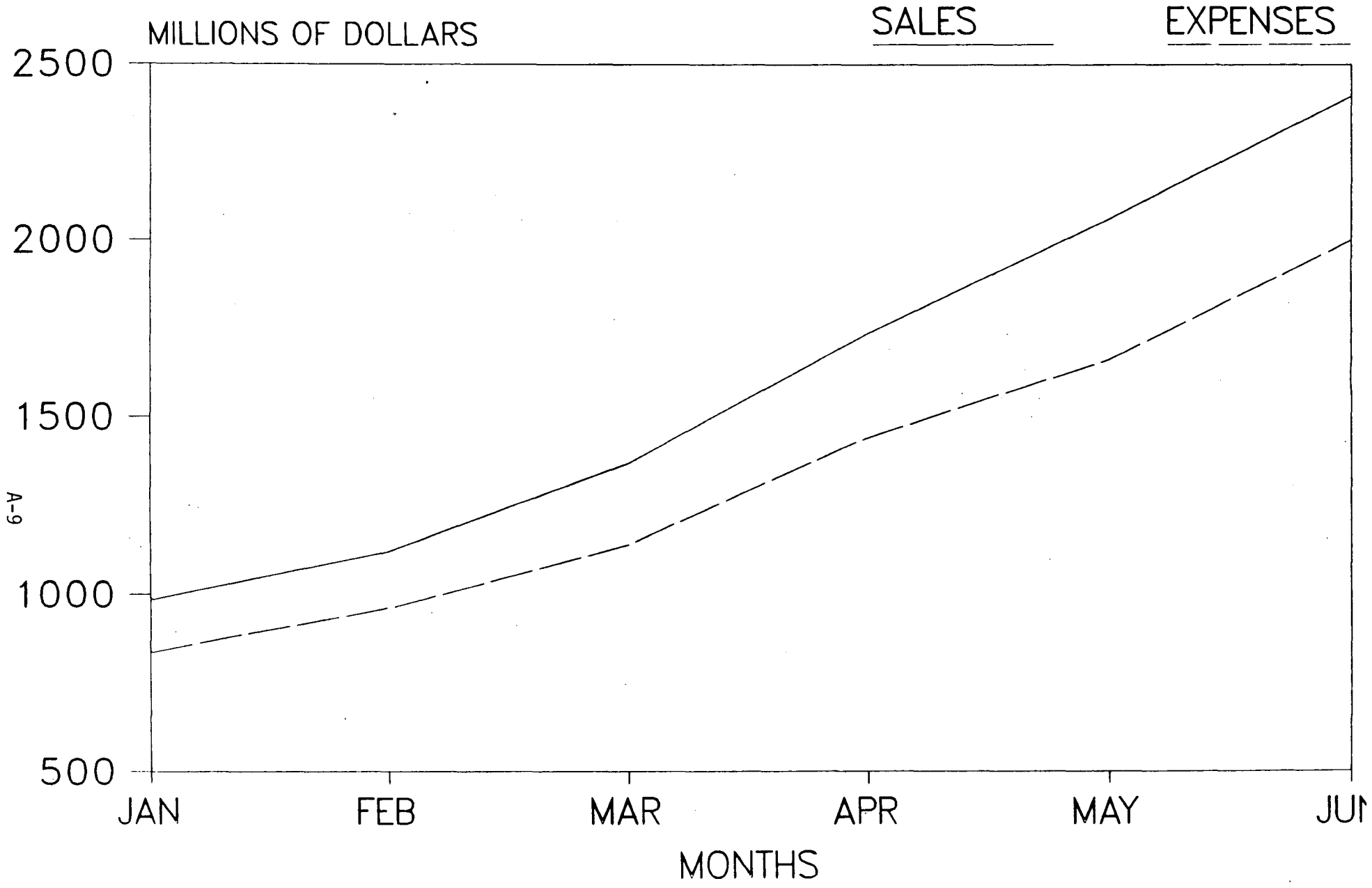

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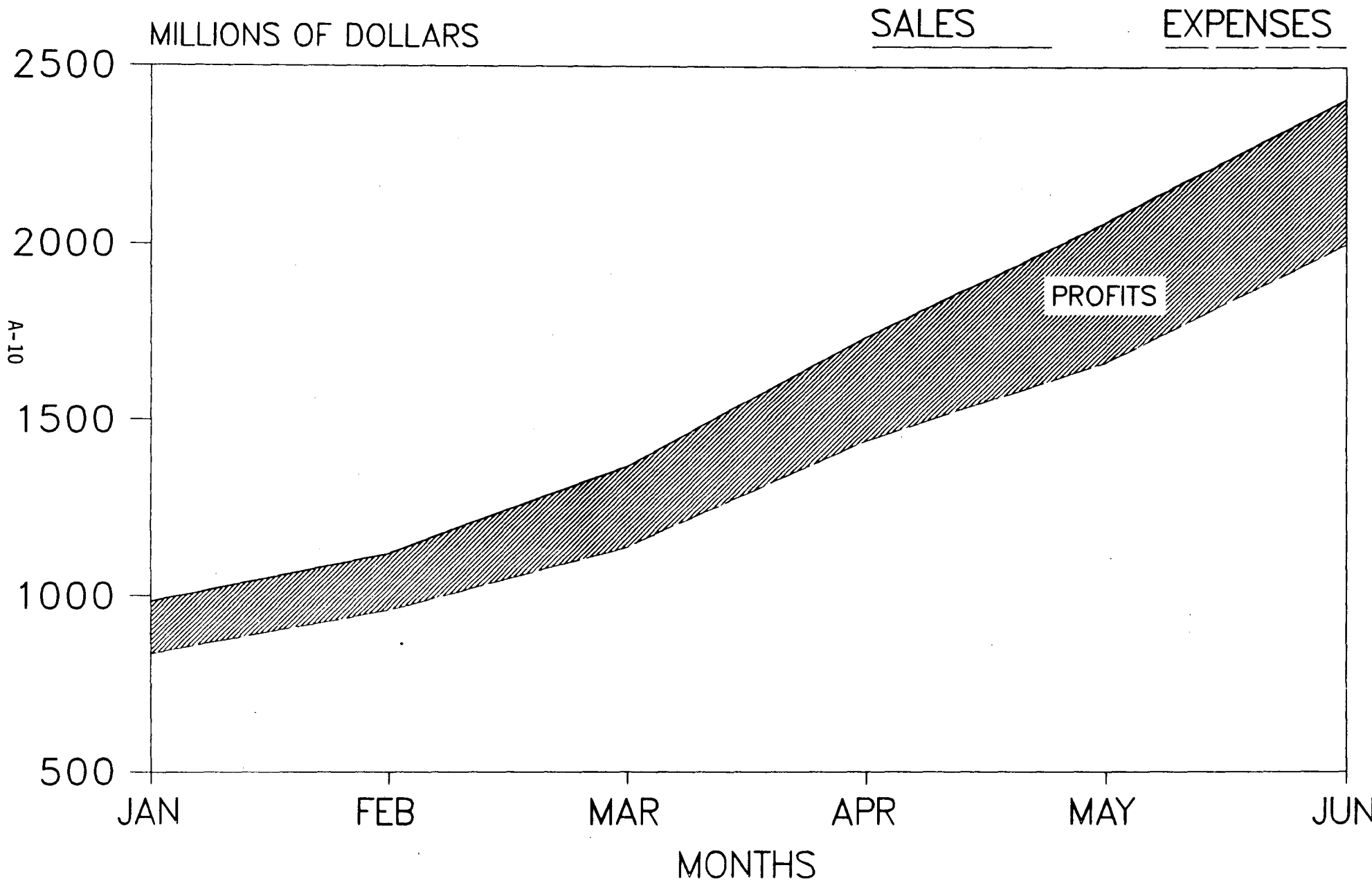

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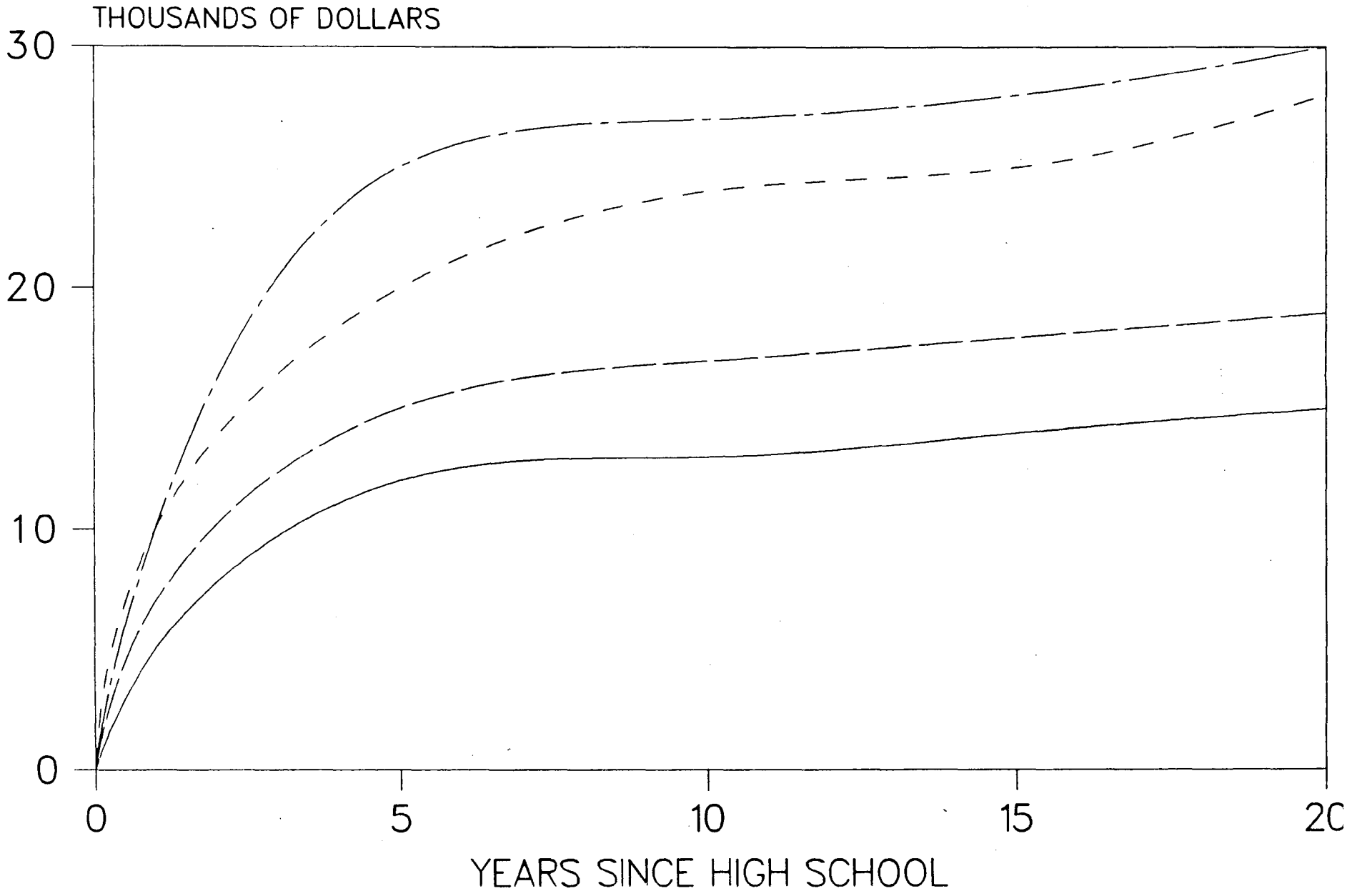

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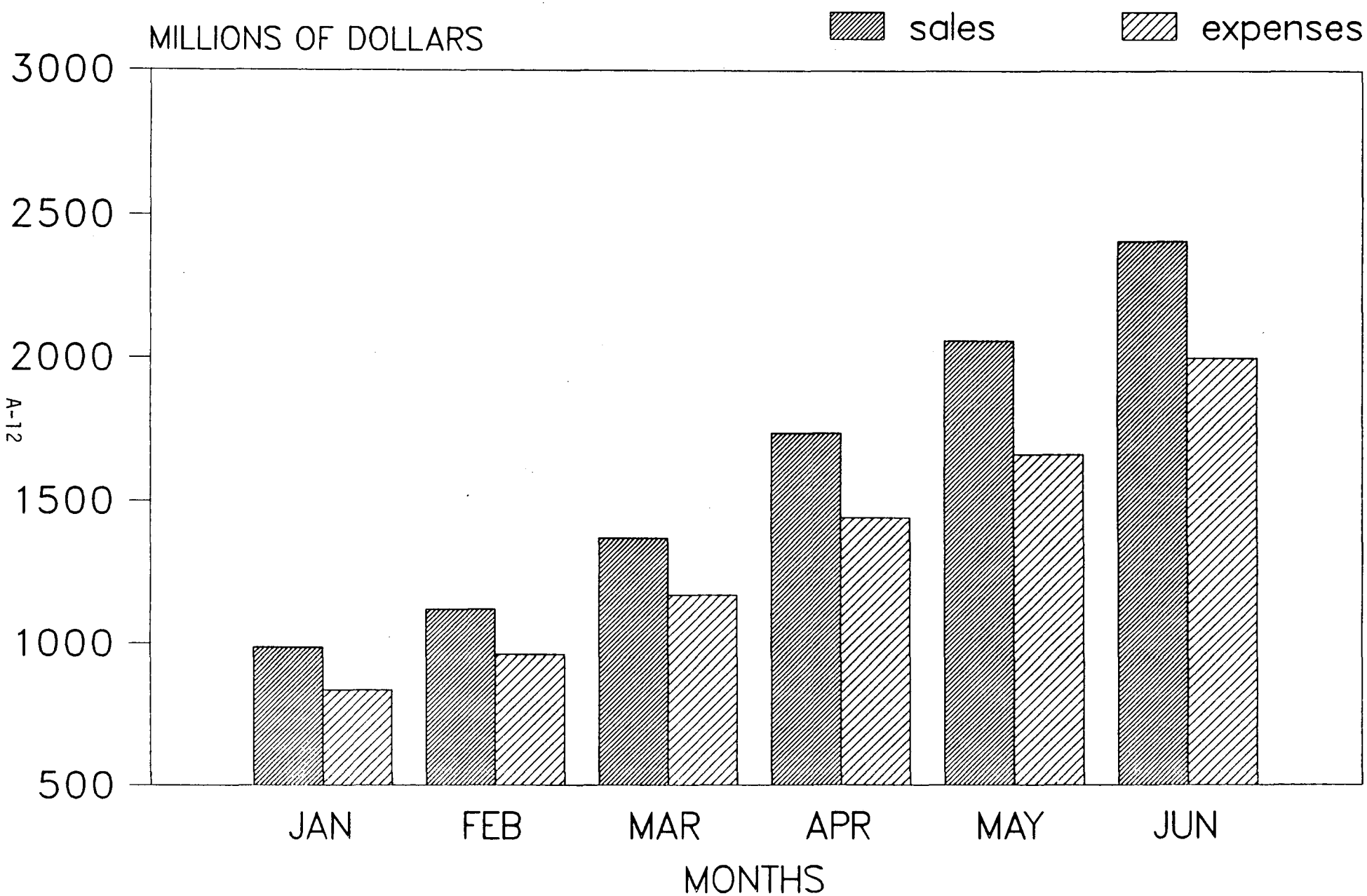

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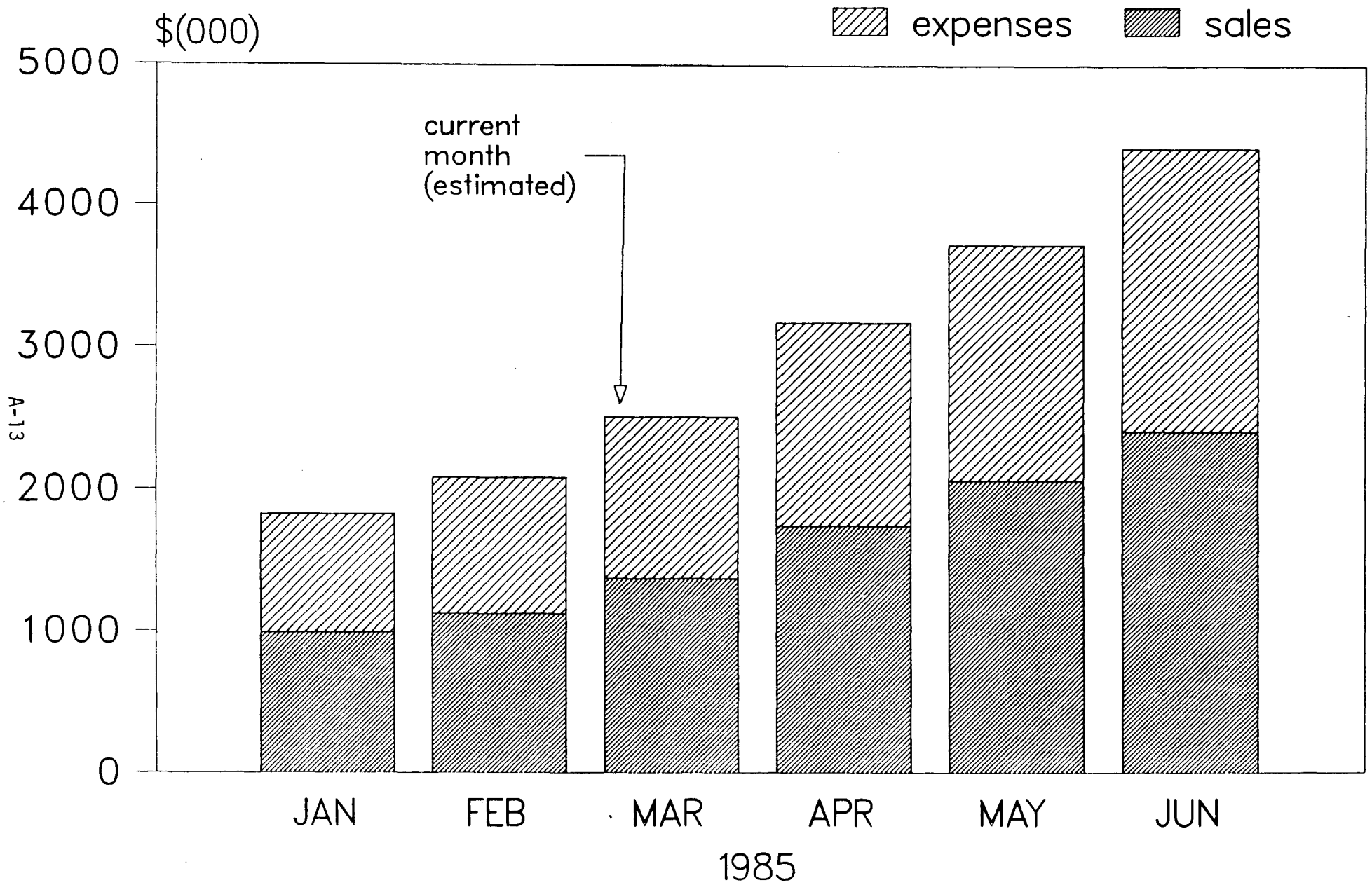

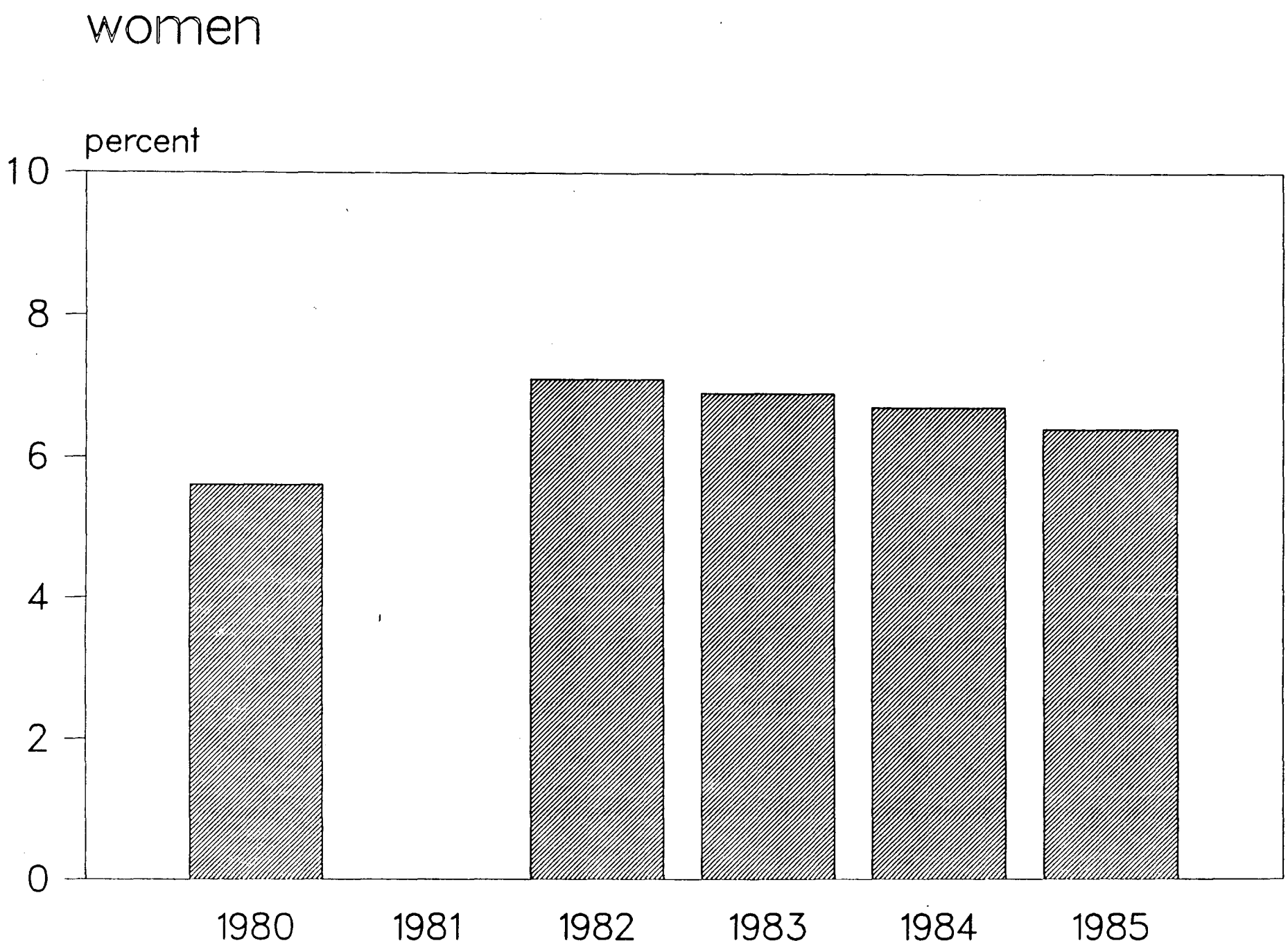
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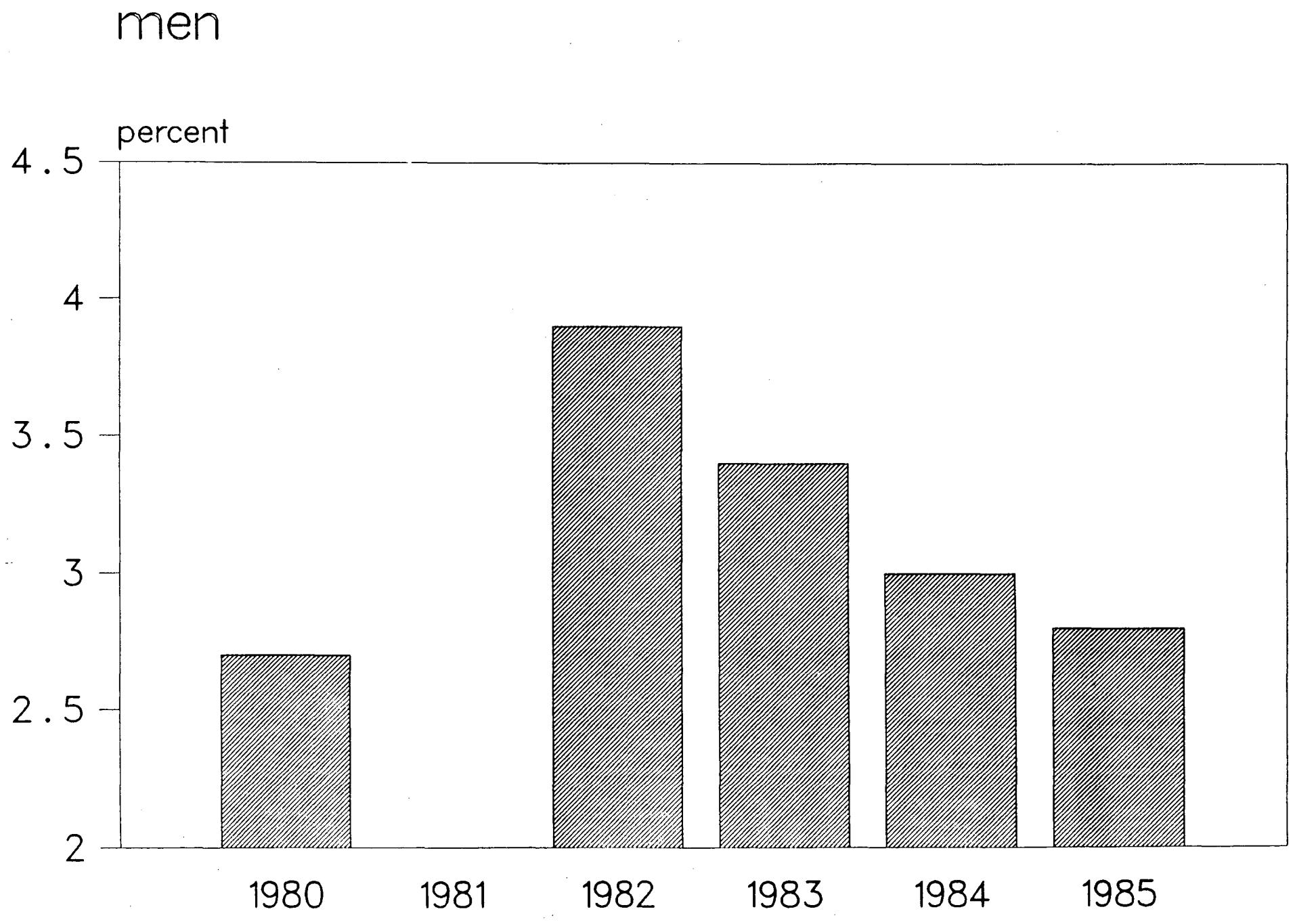
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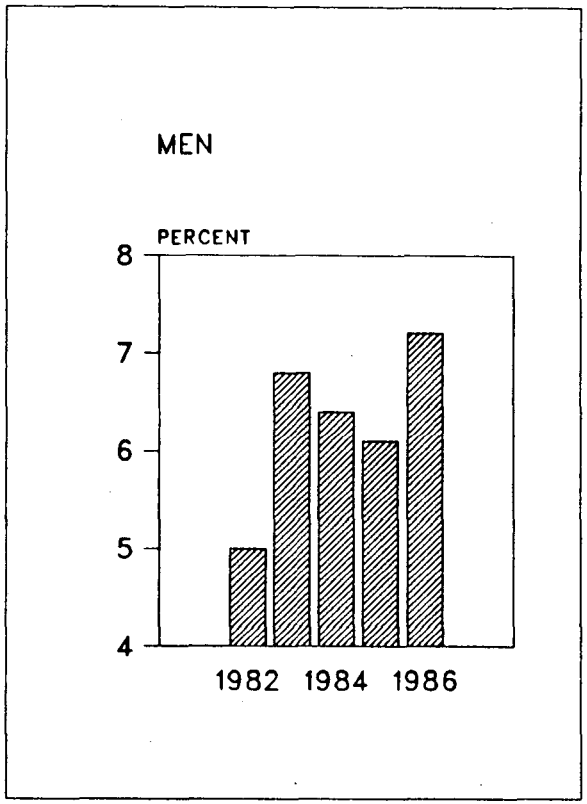
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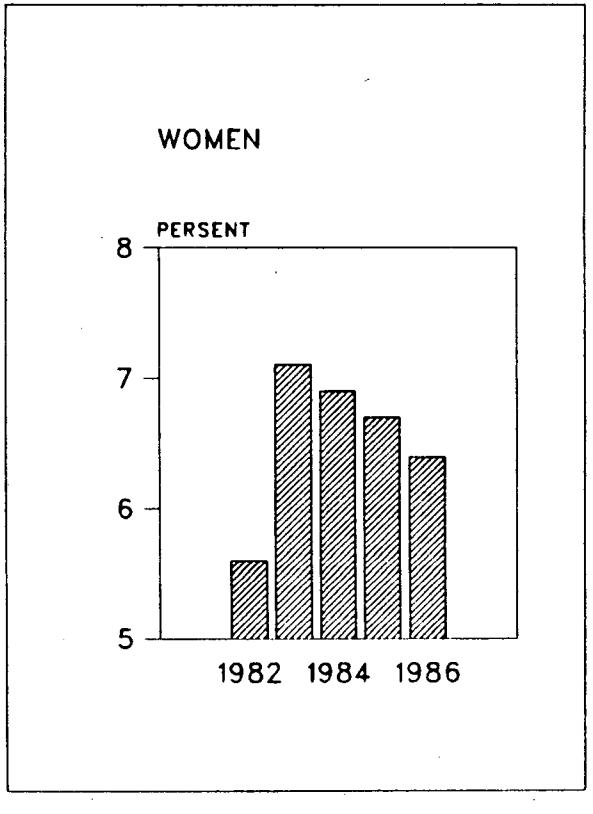
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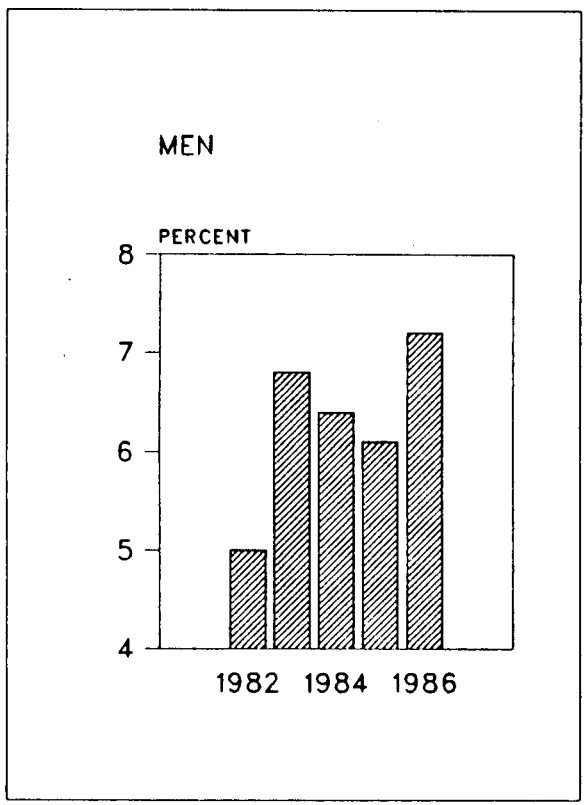
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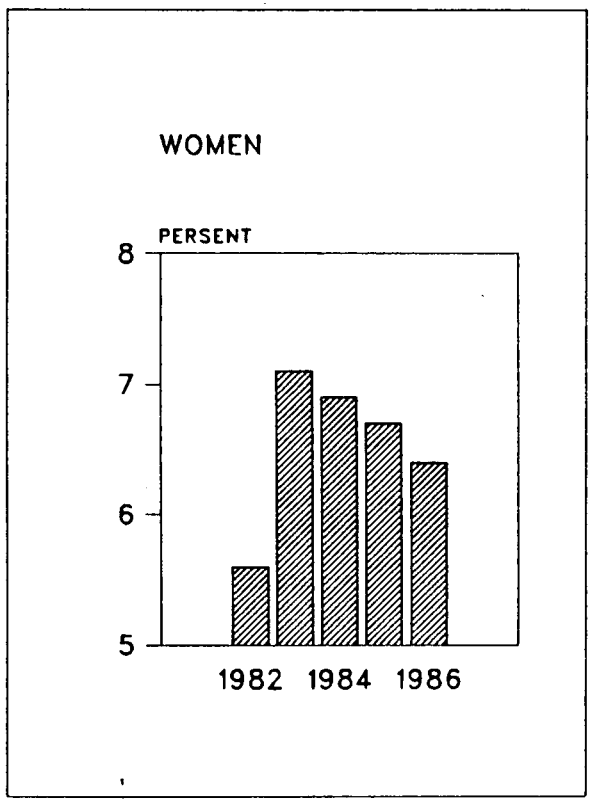
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HIGH ENERGY ELEMENTARY PARTICLE PHYSICS

Jose Luis Emmanuelli
Turabo University

Research Sponsor: Dr. Ronald Madaras
Physics Division

I'am a junior student at TURABO UNIVERSITY (affilieted to AGMEF) in Puerto Rico and a member of the Science Honor Program at there. I'am studing phisics , but I wish to complete an overall bachelor degree.

My sponsor is Dr Ronald Madaras and he is on charge of the DO EC-EM group. The initials DOrefers to the new detector to be used at the Tevatron Proton Anti-Proton collider at Fermilab (near chicago). EC-EM refers to Encap-Electromagnetic Calorimeter. The calorimeter for the DO detector have been divided in two main parts ; the Central Calorimeter (CC) and the Endcap Calorimeter (EC). The Endcap Calorimeter is divided in four modules ; Electromagnetic (EM), Inner Hadronic (IH) , Middle Hadronic (OH). The LBL phisics group has the commission to desing , prove and build the electromagnetic module.

CRYO-CYCLING TESTS OF KAPTON COVERED MULTILAYER BOARD

A special type of signal board is used in the EC-EM where the presence of gaps between adjacent boards in a layer would give unacceptable loss of sensitivity. In the EM section , multilayer signal boards are used with Cu pads on the outer surfaces and signal traces on the inner layer which bring the signals to the large radius edge of the board for ganging.

The resistive layer is made with a high resistance epoxy coating on Kapton which is laminated to the outer surface of the board. The thikness of the board is 67 mils(0.001 inch). For experimental purpose we are using prototype multilayer boards which have been prepared using a two-part graphite loaded epoxy (0.001 inch) , glassmat and a cover of Kapton.

The liquid argon calorimetry requires an efficient cryogenic system. Refrigeration for system cooldown and operation , and for argon storage and purification ,is provided by the vaporization of stored liquid nitrogen (LN). The calorimeters will be cooled from room temperature to an average of 150 K by cooling and condensing argon gas at constant cryostal pressure with special cooldown coils.

The reason for the cryo-cycling tests is to observe the behaviour and the continuity of the signal traces of each board at low temperature conditions , which is a requirement of the liquid argon calorimetry. In order to save time Robbie Smits prepared a method to cryo-cycle the multilayer boards. A ~ 4'x2'x1' container with ~ 8 cubic feet of LN. The boards are placed into the container with 3 cartoon spacers of ~1.5'', between each board.

A cord is atached to each cartoon spacer in order to be able to pull out them to have the board under LN. This is done every 10 minutes. After complete the cooldown , the boards are put out at room temperature for 30 minutes. During thi time the boards are visibles and we can check if there's some damage to the Kapton cover or to the board (fractures,warps).

After 6 cycles we check out the continuity of the traces thrugh the board by masuring the capacitance of each pad of the board. For this we use an autoranging capacitance meter. By a 12 chanel and 2 position (HI-LOW) switch selector we can take out the signal of any one of the 24 pads per conector of each side of the board. The total number of pads per side of the board is 48.

So far we haven't found any problem with the Kapton cover or with the structure of the boards. By the same way the capacitance results are of the same range and expected. We have done at least 10 og the 30 contemplated cycles , so we must wait to the end of the cycles in order to make conclution , but the pattern royects no problem in the future.

DU-238 FLATNESS MEASUREMENTS

A typical sampling cell in the calorimeter is made of absorber (depleted uranium) plates , argon gaps and multilayer boards. In order to have uranim plates and multilayer boards at the same distance inside each cell , warp considerations must be taken. For this reasson the DO EC-EM group has been doing a series of flatness measurements on the DU-238 plates (depleted uranium). By knwoing the warping condition of each plate they can desing appropiate anti-warp ways to fix the DU plates and the boards having at the same time acceptable argon gaps between them. This is the main reasson for the flatness measurements. We must know how much warping exist in those boards so we can mitigate its efects at each cell.

In the development prosess of accurate ways to measure the behaviour of the DU plates , John Taylor has been working with 2'x 2' plate making measurements of flatness. The measurements are taken by placing a load on a pre choosen point and reading it out by the METRABUS system wich is conected with the computer. The results are compared with other therical data obtained by runing a program called ANSYS where the author makes predictions about the possible behaviour of the DU plates.

The comparison of the DU plates measurements and ANSYS program is being made at the present time. By the end of July and begining of August they expect to make flatness measurements on the 3'x 7' DU-238 plates. For this the frame and the calipers mount are ready and waiting for the electronic and software work.

The obtained results of the first comparison between ANSYS and Taylor's measurements have a disagreement fluctuating only between 0.1 mils and 1.3 mils for 1 and 5 pounds load at the center of one aluminium plate. It plate is used to calibrate the system end for comparison. John Taylor found that part of this disagreement is due to a different mathematical treatment of the two sets of numbers. When both numbers are analyzed by the same way there is agreement to within about 20% at the point where the weight is applied.

In a up date about warp studies at July 25 , Philippe Eberhard point out that the residual warp of 4mm 2'x 2' DU plates obtained by calculations of flatness is to much. He bases it on ; if a model is made up with those DU plates with 9 anti-warp bolts for each one , then at 9 places the 2.3mm argon gap would be reduced to less than 0.5mm and that number doesn't seem good to the desing.

Suggested solutions given by Philippe Eberhard are : A) Build collapsable buttons on opposite side of the multilayer so that the board stays always in the midle of the gap without being broken by the buttons.

B) Decrese bolt spacing by a factor of the order of 2 or place hard spacers between the bolts.

He lets know his concern about the DU plates manipulation and possible forbidden operations. There remains the measurements of the 3'x 7' plates and by that date more conclutions can be made.

INTERFACE ELECTRONICS FOR MULTIPLEXING THE CALIPERS SIGNAL

The way to measure the flatness of the 3'x 7' DU plates will be by calipers. It was my entire responsability the construction of that board that will connect the calipers with the METRABUS system and computer.

George Gibson designed the logic to produce the interface electronics for multiplexing the signals and he supervised my work. It took 3 weeks to be done and 2 weeks more to be connected and ready to work. The requirements of the interface electronics were :

1) 2 out signal (data-clock) per caliper.
2) 28 calipers to read.

The data had to be read by the computer through the METRABUSS and codified in the RS232 transcriptor.

The electronic board desing had :

1) 19 integred circuits:

A) 14 quad analog swith/ quad multiplexer
B) 2 4-bit lath/4-to-16 line decoder
C) 2 hex inverter

D) 1 lm 337

2) 1 K resistor
3) 1 n4447 resistor
4) 1 potensiometer
5) ~233 IN and OUT conections :

A) 56 IN lines from 24 calipers
B) 2 data IN & out lines swith (1-A)
C) 2 clock IN & out lines per swith (1-A)
D) 1 IN line from RS232 (phono jack)
E) 2 out lines to the RS232 (phone jack)
F) 6 IN lines from the METRABUS

6) All the power up lines and conections.

The final product was a board with one more hex inverter and one more multiplexer with other variations concerning to the way the conections were done. They also instaled a rechargeable battery to the board , but maintaining the power up conection between the METRABUS and the board. By this if there is a electric interuption on the bilding the calipers will kepp swithed on and calibrated.

I have obtained an unmeasureable practical experience , theoretical knowledge and many personal satisfactions. I grew up here , during these few weeks,more than any other learning period in my students life. I'm proud of what I've done here , but that doesn't mean comformity. In instead of that this experience meahs challenge and stimulation to continue studing physics.

IT was a privilege have been a part of this excellent group of scientifics , workers ,friends and creative minds and hands searching for peace and knowledge.

I want to recognize the unforgetable treatment given to me by Ron Madaras , George Gibson , John Taylor and Robbie Smits. They had patience and confidence on me and will never forget it and them.

Jose L. Emmanuelli

STUDY OF THE ROLE OF MACROPHASGES IN THE INHIBITION OF ERYTHROPOIESES

Angel Malavet Rodriguez
Turabo University

Research Sponsor: Dr. John Schooley
Biology and Medicine Division

ABSTRACT

We know that Inerleukin-1 (IL-1) causes an inhibition in the formation of red blood cells. We also know that macrophages produce IL-1. We want to see if the inhibition of erythropoieses in malarial-infected mice is a result of the malarial parasite or the activation of macrophages and the resulting production of IL-1 by the malaria parasite. To study this we are going to make a spleen cell suspension from mice infected with malaria and separate the macrophages from the suspension by adherence to a plastic surface. We are then going to detach the adherent cells with lidocaine. These cells will then be used in a Krystal assay for erythropoiesis by adding varying numbers of the adherent cells (.04ml of 5.00000, 2.50000, 1.30000, .63000, .32000 cells/ml) to .04ml of a suspension of spleen cells from phenylhydrzine treated mice (12,500,000 cells/ml) with 125mU/ml of erythropoietin.

INTRODUCTION

The formation of blood in mice ocurrs in the bone marrow and in the spleen (this is not true in humans in which it only occurs in the bone marrow). This process begins with the stimulation of the progenitor cells by a substance. For example, in the formation of the red blood cells, the substance is the hormone erythropoietin.

An inhibition of this process has been seen under a number of circumstances: 1) on addition of certain compounds such as IL-1 or endotoxins or 2) after infection with a malaria parasite. What we will be trying to determine is if the inhibition of the formation of the blood cells is produced by the interaction of IL-1 between th progenitor cells and the hormone.

PURPOSE

IL-1 is a substance that causes an inhibition in the formation of red blood cells and is produced by activated macrophages. When a mouse is infected with a malaria parasite it becomes anemic. The reason is that the parasite acts on the red blood cells destroying them, but we found that the stem cells are also affected. The malaria parasite doesn't act directly on the stem cells but can stimulate the production of IL-1 by macrophages that might be the reason for the inhibition of erythropoieses.

MATERIALS

1. Mice BALB/C.
2. 3003 Falcon tissue culture dish.
3. Alpha Minimal Essential Media (MEM) containing 25% Fetal Calf Serum (FCS).
4. Lidocaine (Astra Pharmaceutical Products, Inc. Worchester, Mass. 01606).
5. Normal saline (PBS)/
6. 12x75 mm culture tubes
7. 3 100 ml sterile bottles.

PROCEDURES

1. From a mouse infected with malaria make an spleen suspension in alpha MEM 25% FCS media.

2. Take 3 3003 tissue culture dishes and add the suspension at a concentration of 20,000.000 cells/ml, 15ml in each dish. Incubate for 2 hrs at 37C and 5% CO2. Under the same conditions take 2 ml of the original suspension and place in a 12 x 75 mm culture tube in ice for 2 hrs. This will serve as the control.

3. Collect the supernatant from the 3003 dishes and transfer to a sterile 100 ml bottle. Rinse the tissue culture dish with complete media to remove the nonadherent cells. Remove the media from the dish and add to the bottle. Into another bottle pour the control cells.

4. Add 10 ml of 11 ml lidocaine to each dish and incubate for 5 minutes. Do the same with the control cells, but instead of 10 ml, add 30 ml of lidocaine in each bottle.

5. Gently scrape with a rubber policeman the bottom of the tissue culture dish. Collect the supernatant and transfer into another 100 ml sterile bottle. Jet the monolaver twice with 8 ml of normal saline expressed from a syringe with a No. 20 needle. Collect the saline and pool it with the lidocaine media. Add 48 ml of saline to the control cells and the nonadherent cells to maintain the same conditions in each one.

6. The contents of the three bottles are then centrifuged at 2000 R.P.M. for 10 minutes under refrigeration. The pellet of each is resuspended in 3 ml and counted.

7. To do the Krystal assay separate the nonadherent, the control and the adherent cells populations in concentrations of 500000, 2.50000, 1.30000, .63000, .32000 cells/ml, and add .04ml to 500000 spleen cells from a phenylhydrazine treated mouse with 125 mU/ml of erythropoietin and incubate for 22 hrs. After the 22 hrs. add 3H-Thymidine and incubate for 2 hrs. Harvest cellular contents of each well and count the radioactivity in the cells with scintillator counter.

RESULTS

From this experiment we found that there is a large inhibition when we add the adherent cell population to phenylhydrazine cells. This does not occur to the same extent with the control or the nonadherent cells populations (see fig. 1). This supports the idea that whatever causes the inhibition is in the adherent cell population.

DISCUSSION

This method is not perfect. We can't separate all the adherent cells from the original cell suspension, this is probably the reason why we can see some inhibition in the nonadherent cell population. Another problem is that we can't obtain a population of macrophages only by this method. The major contaminant is lymphocytes which also adhere to the plastic surface. One method that we can use to obtain a population of purified macrophages is by irradiation. In blood there are cells which are very sensitive to radiation such as hematopoietic cells, stromal cells and lymphocytes, and radioresistant cells like mature red cells and macrophages. If we irradiate the adherent cell population we can eliminate the lymphocytes and obtain a more purified population of macrophages (we don't have any results from such an experiment at this date).

The following is a summary of other methods which can be used to obtain purified populations of macrophages.

METHODS TO PURIFY MACROPHAGES

The formation of red blood cells is a process that takes place in the bone marrow and in the spleen of the mouse. An inhibition of this process is found with the combination of the adherent cell population from the spleen malaria mouse to the spleen cells from a mouse previously treated with PHZ. We believe that the cause of this inhibition is the present of IL-1, a compound produced by activated macrophages. To be certain of this we need to obtain populations of purified macrophages. The following is a list of methods that can be used to accomplish this:

A. Antibodies and complement
   The use of antibodies and complement is a very good method to eliminate certain sub-populations of cells from the adherent cell fraction. The antibody is used to mark a specific cell type where the complement is going to act. Complement then makes a complex called the membrane attack complex. This complex, according to the doughnut hypothesis proposed by Manfred M. Mayer in 1972, forms a hollow cylinder that possesses lipid binding regions at one end and so is able to penetrate into lipid bilaver. The cylinder projects above the outer surface of the membrane and widens at the noninserted end into an annulus. The inserted cylinder opens up transmembrane channels that connect the cell interior with the external environment, then a process of diffusion starts and water moves into the cell while proteins and high molecular weight substances will begin to leave the cell until it is reduced to an empty bag, i.e. the cell has been lysed.

If you don't want to destroy the cell you can use only antibodies against cell surface markers. Like the use of antibodies bound to a flat plastic surface of tissue culture petri dishes or flasks (e.g. the panning method which is described below). This method appears to have several advantages: cell recovery rates are high, the method is less complex than others, and it has been used with some success for positive as well as negative selection.

Affinity chromatography constitutes, in principle, an ideal method for purification of cells. It consists of immobilization in solid supports of ligands which selectively recognize membrane components. Here are some examples of affinity chromatography techniques.

1. Rosette techniques: fixation of anti-mouse immunoglobulins on ox red blood cells (by the chromic chloride method) and resetting with the cells to be purified which have previously been coated with specific monoclonal antibodies (produced by mouse hybridoma): recovery of bound cells is achieved by selective lysis of the erythrocytes.

2. Panning techniques: absorption of antibodies on polystyrene dishes (in a chemically undefined manner): most often double antibody techniques are used, plastic dishes coated with anti-mouse immunoglobulin antibodies selectively bind cells previously treated with specific monoclonal antibodies.

3. Methods involving protein A from Staphylococcus aureus. Protein A possesses the property of binding the Fc portion of IgG (Protein A is bivalent) in a theoretically reversible fashion. Cell separation can be achieved with Protein A-coated immunoglobulin-specific supports: Protein A is bound either directly to beads or to immunoglobulin-coated plastic surfaces. The immobilized Protein A can either directly interact with specific antibody-coated cells or be charged with antibodies directed against the specific antibodies used to select the desired cell population.

Here are two main types of problems with the affinity chromatography techniques.

a. Non-specific interaction between cells and affinity supports.

b. Irreversibility of cell-affinity support interactions: one reason for this is the multipoint attachment which occurs between cell and support. The second reason for irreversible bindings of cells to the affinity support may be the very high affinity between the immobilized ligand and its cell surface receptor, which often occurs when cell-ligand recognition involves antigen-antibody interactions.

It has sometimes been possible to recover cells initially bound to immobilized ligands by the use of excess ligand or ligand competitor, but this ideal situation is feasible only occasionally. In most cases alternatives must be found: melting the support, enzimatic degradation of the support or mechanical disruption. All these methods may damage the cell. Even pipetting by itself is liable to create shearing forces, which may cause loss of receptor from the cell surface.

It is clear that to date there is no single method of cell isolation by affinity chromatography that can be applied universally, mainly because the elution of intact functional cells from the affinity supports remains a troublesome step.

There is a method that tries to overcome some of the disadvantages of the above methods: immobilization of ligands has been achieved through spacer arms containing Hg-S bonds which can be cleaved with a thiol more readily than disulfide bridges. Theorganomercurial Mersalvl was covalently bound to activated trisacryl beads and this immobilized mercurial compound was reacted with thiolated ligands: the resulting Hg-S bonds can be cleaved with dithiothreitol.

B. The use of centrifuging to separate sub-populations of cells.

The sedimentation rate of cells in a liquid depends on the size and density of the cells as well as the density and viscosity of the surrounding medium. In the case of mammalian cells the osmolarity of the medium also effects the sedimentation rate and density of the cells in that, as the osolarity of the surrounding medium becomes hypertonic, the cells lose water and shrink.

Efficient separation procedures are required in order to study the function of the different cell types in blood. For white blood cells distinct density differences have provided a basis for separation of the major subgroups. Mononuclear cells monocytes and lymphocytes have a lower density than granulocytes and erythrocytes. By lysing the blood over a separation fluidof intermediate density, it is possible to separate the blood cells into two fractions by centrifugation.

Monocytes have a lower density than other cells in human blood and may be separated from other types of blood cells by density gradient centrifugation. The average monocyte purity is reported to vary from 60-90% with lymphocytes as the main contaminant. The variability in purity is due to the overlaping densities of these two cell population, and hence it would appear difficult to design an efficient separation procedure based on density difference alone. It is likely that better purity might be obtained by combining different separation techniques. For example, monocytes, partially purified by centrifugation, adhere readily to glass or plastic surfaces. Hence after incubation at 37 C. nonadhering cells can be removed by washing, and then the monocytes can be detached and recovered by various procedures.

As demonstrated in previous reports, the monocyte purity can be improved by using a hyperosmotic density gradient medium. Then the osmolarity increase, the cells expel water and shrink. The density of the cell then increases, they move faster and may even pass the density barrier that was present initially. It appears that the lymphocytes, which overlap monocytes, are more sensitive to increase in osmolarity than are monocytes. A new non-ionic iodinated gradient medium, nicodenz, has been used in these separation studies, and sodium chloride has been used to adjust the osmolarity of solutions. With the correct combination of density and osmolarity all of the cells loaded onto the gradient, except for the low density fraction of the monocytes, move to the bottom during centrifugation. A monocyte fraction of 95-98% purity can then be collected from the top of the gradient layer. This gradient medium has also been used for separation of mononuclear cells and granulocytes, in comparison with already established procedures.

1562D

EFFECT OF THROMOBOCYTOPENIA ON ARTHEROSCLEROSIS IN RABBITS WITH DIETARY HYPERCHOLESTEROLEMIA

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Research Sponsor: Dr. Shirley Ebbe
Biology & Medicine Division

Introduction:

Cardiovascular diseases have been a mayor focus of attention for many years because they account for more than half of the deaths caused by fatal disease in the United States. Artheriosclerosis affects the circulatory system directly by blocking the arteries carrying blood from the heart to the other body tissues. Patients suffering from this disease accumulate different kinds of lipids, including cholesterol, on their arterial walls.

Due to this condition, the lumen of the blood vessel decreases in diameter, and this leads to a decreased flow of blood through the vessels. It has also been observed that hypercholesterolemia leads to a decrease in the size of platelets in the blood of rabbits.

Research work at our laboratory has been undertaken to study if blood platelets can affect in some way the deposition of lipids on arteries and, therefore, their possible role in the ocurrence of artherosclerosis. The experimental design followed includes subjecting the animal model (rabbit) to a high cholesteral (1%) and peanut oil (4%) diet for eight weeks. A certain number of these animals were also treated with a guinea pig anti-rabbit serum (Aps) or normal guinea pig serum (Ngps) during the last four weeks of the eight week treatment.

Purpose:

Through this experiment, we wish to study the platelets in the rabbit's blood and the role that the platelets play in the production of artneriosclerosis disease interacting in the areas where the vessel walls are damaged, and in studying if changes occur in the amount of lipid deposited in the wall of the aorta.

Materials and methods:

Animal subjects

Ten week old New Zealand male white rabbits weighing between 2.72 kg and 4.09 kg were used for the experiments. Prior to treatment, all subjects were individually caged and provided with regular food (Purina Laboratory Chow) and water on a daily basis. A total of six experimental subjects were used for the study.

High-Cholesterol treatment

Four animals were fed a high-cholesterol diet consisting of 1% cholesterol (Dyets, Pennsylvania; USP grade) and 4% peanut oil (Planter's Co., NJ; 100% pure) for eight weeks on a daily basis. The cholesterol and oil mixture was added to high fiber rabbit pellets in the specified proportion for ingestion by the experimental animals. Two animals were used as controls and were provided regular food.

Anti-platelet serum and normal guinea pig serum

Three weeks post cholestterol treatment two of the four experimental animals subjected to high-cholesterol diets were given 1 cc of Aps diluted in 1 cc of 0.9% saline (American McGraw, American Hospital Supply Corp., Irvine, CA). The other two rabbits were injected with 1 cc of Ngps diluted in 1 cc of 0.9% saline. The control animals were given no injections. After the initial injection in the treated animals, the rabbits were continually injected at the same dosages with the respective substances.

Blood counts

Every two weeks blood cell counts were carried out. In addition to this, blood was also collected from the animals four hours past injection of the Aps and Ngps. The collected blood was placed into test tubes coated with anti-coagulant (EDTA). Platelets were counted by phase microscopy by the method of Brecher and Cronkite. Hematocrits were quantitively accounted for by using a Drummond Micronematocrit Machine. The number of red and white cell/cc blood was determined individually by using a Coulter Counter. The number of reticulocytes present in the collected blood was determined by using the method described by Brecker and Schreiderman. Leukocyte differential counts were done by microscopy of blood smears stained with Wright's and Giemsa stains.

Results and discussion:

The obtained results suggest that treatment with the anti-platelet serum (Aps) only altered the number of platelets (fig. 1) in treated subjects and not other factors such as Hemotocryte % (fig. 2), number of reticulocytes (fig. 4), number of WBC's (fig 5) and RBC's (fig. 6). The weight of the treated control animals increased gradually over the study period (fig 3). The data obtained in this study showed a high variance due to the small number of subjects. The results depend on furtner verification and will be published later on.

Comparative tables:

Figure 1: Platelet count pre- and post treatment with Aps and Ngps

		week 1	3	4	5
Diet + Aps	2	377,500	350,000	8,750	212,500
	6	269,375	267,500	13,750	12,500
Diet + Ngps	1	246,250	509,167	-------	395,000
	5	421,875	342,500	420,000	456,250
Control	3	493,750	510,625	217,250	388,300
	4	456,250	433,750	485,000	378,125

Each serum was injected into experimental subjects for the first time at week 4 and then at week 5. Analysis of the data showed that the number of platelets decreased significantly after treatment with Aps. The number of platelets generally decreased after treatment with Ngps and in control subject but the decrease was not as significant as in Aps-treated subjects.

Figure 2: Hematocrit count in blood sample

%	week	1	3	4	5
Diet + Aps	2	43	41	40	41
	6	43	39.5	39	42
Diet + Ngps	1	39	40	--	39
	5	37	36	34	34
Control	3	30	39	40	40
	4	36	39	40	40

This figure shows that there were only slight variations in hematocrit counts from blood samples collected after treatment of the animals with Aps and Ngps as compared to control subjects.

Figure 3: Weight of the animal subjects at two week intervals

Kg	week	0	1	3	5
Diet + Aps	2	3.5	3.66	3.89	4.12
	6	3.8	3.86	4.04	4.04
Diet + Ngps	1	3.66	3.75	3.86	3.86
	5	2.8	3.69	4.00	3.92
Control	3	3.5	3.5	4.04	4.37
	4	3.04	3.75	4.04	4.32

Weight measurements were taken before any treatment (week 0) and every two weeks thereon. The weight of Aps-treated subjects increased gradually over the eight week period. The weight of Ngps-treated subjects were fairly stable pre- and post-treatment with Ngps.

Figure 4: Mean number of retyculocytes in collected blood samples

%		week 1	3
Diet + Aps	2	2.30	3.75
	6	1.29	2.08
Diet + Ngps	1	1.79	2.33
	5	2.13	2.52
Control	3	11.93	3.20
	4	2.67	4.26

The data obtained shows a gradual increase in the number of immature red blood cells, suggesting normal development of retyculocytes. Subject number 3 shows great variation as compared to the other subjects. This may be due to an anemic condition present in the former at the beginning of the experiment. The data shown in this figure only includes week number one and week number 3 of the treatment.

Figure 5: White Blood Cell (WBC) count in blood samples.

10**3/mm**3	week	1	3	4	5
Diet + Aps	2	13.9	13.2	12.9	75.3
	6	90.9	11.5	86.7	71.9
Diet + Ngps	1	11.7	94.6	----	11.3
	5	91.0	96.19	12.5	96.7
Control	3	97.5	12.7	94.9	91.9
	4	12.4	11.7	11.1	71.9

The results obtained show fluctuation in the number of WBC over the time of analysis. The changes observed the number of cells are considered to be in the normal range of fluctuation. It was concluded from these results that treating rabbits with Aps and Ngps has a relatively small or no effect on the number of WBC.

Figure 6: Red Blood Cell (RBC) count in collected sample

10**6/mm**3	week	1	3	5
Diet + Aps	2	6.39	5.78	6.28
	6	6.77	5.83	7.12
Diet + Ngps	1	6.03	5.36	6.30
	5	5.68	5.12	6.36
Control	3	3.55	4.27	4.79
	4	5.49	5.15	5.69

RBC fluctuated in number during the eight-week period. The variations obtained do not deviated significantly from the normal range of fluctuation in control subjects. These results suggest that Aps and Ngps do not have significant effects on RBC numbers in treated subjects.

References:

1. Ebbe, Shirley, Phalen E., Overcash J., Howard D., Stohlman F.: Stem cell response to thrombocytopenia. The journal of laboratory and clinical medicine, 1971, p. 873-874.

2. Ebbe, Shirly, Phalen E., Stohlman F.: Abnormalities of megakaryocytes in w/wv mice. Blood, 1973, p. 858-863.

3. Mazoyer Elizabeth, Dolol K., Carpenter D., Brennan K., Yee Tamlyn, Mazoyer B., Leven R., Ebbe S.: Abnormalities of blood platelets in rabbits with dietary hypercholesterolemia and artherosclerosis. Biology and Medicine Division, 1985, p.14-15

SPSSX AND TELL-A-GRAF-LANGUAGES

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Research Sponsor: Dr. Elon Close
Information and Computer Science Division

During this summer I was tasked with the integration of two software packages. A software package is a program or a group of programs that have a common origin. These programs work together to perform a certain task for which they had been written. In this case the two software packages that I had to connect together were SPSSX ,which is a statistical software package written in COBOL, and with TELL-A-GRAF, which is a software package written in FORTRAN.

The problem that was presented to me was that there were no software packages from SPSSX to draw graphics available. The only software package available was TELL-A-GRAF graphics package for which we have to adapt the output of SPSSX. To be able to do this I had to learn both languages plus the mastering of the VISUAL editor.

To get an acceptable SPSSX output any commands may be used. The specific command to use depends on what your need is. You may use the list,report or frecuency commands to get the output that you need or any other suitable command as far as it is accepted by the language. After you get the output of the run you shall edit the output to be accepted by TELL-A-GRAF. In way to edit the output you have to be very knowledgeable of the visual editor.

The use of the REPORT COMMAND was presented, but the formatted output was not as expected. It can not be use to write a separate output file. This is a restriction on this command, so in way to be able to write an output file acceptable for TELL-A-GRAF input, there is a lot of file editing to be performed by the programmer. The data has to be gathered and then there could be the need for calling the old file in a couple of times so as to get the data in a way that you do not have to write so much.

The following commands may be used for the vi editing session :

{ } indicates the reserved words or commands.

1 {:} to enter the command mode for special instructions.

2 {:r}<filename> to read files into the editing file for gathering of data.

3 {dw} delete word

4 {:#,#w!}<filename>
where # is the line numbers to be included and written in the new file.

The mastering of the vi editor will make it possible for you to get the output that you want.

SPSSX OUTPUT GENERATOR PROGRAM.

```
TITLE 'REPORTE DE DATA DEL SURVEY DE GUITARRA'
FILE HANDLE GUITAR/
 NAME = 'GUITAR.FIL'
GET FILE = GUITAR
IF (STAR EQ 3) CASH=1
SORT CASES BY STAR (D)
REPORT FORMAT=MARGIN(1,55) BRKSPACE(-1)
 /VARIABLES=AGE 'AGE' PLAYED 'YEARS' 'PLAYED'  (OFFSET(1))
 QSEX 'sex'  (OFFSET(1))
 /CTITLE='REPORTE de data del SURVEY DE GUITARRA'
         'EDAD MEDIA, ANOS TOCANDO GUITARRA, y'
         'RATING de JOHNNY CASH COMO ESTRELLA FAVORITA'
 /BREAK=STAR(LABEL) 'STAR PLAYER' (18)  (TOTAL) (SKIP(2))
 /SUMMARY=MEAN
BREAKDOWN AGE BY STAR BY SEX
TEMPORARY
RECODE SEX ('F'=0) ('M'=1) into sex1
VARIABLE LABELS SEX1 'SEX OF RESPONDENT'
VALUE LABELS SEX1 0 'FEMALE' 1 'MALE'
BREAKDOWN VARIABLES=AGE (15,34) STAR (1,5) SEX1 (0,1)
 /CROSSBREAK=AGE BY STAR BY SEX1
OPTION 6 7
```

SPSSX OUTPUT

```
 5-AUG-86   SPSS-X RELEASE 2.1 FOR VAX/VMS
13:41:55    Lawrence Berkeley Laboratory     DEC VAX-8600   VMS V4.4


For VMS V4.4            Lawrence Berkeley Laboratory      License Number 19090

   1  0          TITLE 'REPORTE DE DATA DEL SURVEY DE GUITARRA'
   2  0          FILE HANDLE GUITAR/
   3  0           NAME = 'GUITAR.FIL'
   4  0          GET FILE = GUITAR

FILE CALLED GUITAR  :
  LABEL: GUITAR BRAND PREFERENCE SURVEY
  CREATED 27-MAY-85 21:15:50      16 VARIABLES


   5  0          IF (STAR EQ 3) CASH=1
   6  0          SORT CASES BY STAR (D)

SIZE OF FILE TO BE SORTED:       202 CASES OF    128 BYTES EACH.
SORT COMPLETED SUCCESSFULLY.  FILE SIZE:         51 BLOCKS.
```

PRECEDING TASK REQUIRED 0.35 SECONDS CPU TIME; 0.69 SECONDS ELAPSED.

```
  7  0          REPORT FORMAT=MARGIN(1,55) BRKSPACE(-1)
  8  0           /VARIABLES=AGE 'AGE' PLAYED 'YEARS' 'PLAYED'  (OFFSET(1))
  9  0           QSEX 'sex'  (OFFSET(1))
 10  0           /CTITLE='REPORTE de data del SURVEY DE GUITARRA'
 11  0                   'EDAD MEDIA, ANOS TOCANDO GUITARRA, y'
 12  0                   'RATING de JOHNNY CASH COMO ESTRELLA FAVORITA'
 13  0           /BREAK=STAR(LABEL) 'STAR PLAYER' (18)  (TOTAL) (SKIP(2))
 14  0           /SUMMARY=MEAN
```

REPORT REQUIRES 1717 BYTES FOR THIS TASK

REPORTE de data del SURVEY DE GUITARRA
EDAD MEDIA, ANOS TOCANDO GUITARRA, y
RATING de JOHNNY CASH COMO ESTRELLA FAVORITA

STAR PLAYER	AGE	YEARS PLAYED	sex
GORDON LIGHTFOOT	22.44	2.58	
GLEN CAMPBELL	22.23	2.54	
JOHNNY CASH	22.00	2.60	
ROY CLARK	22.09	2.64	
JOHN DENVER	21.31	2.21	
TOTAL	22.00	2.51	

```
 5-AUG-86   REPORTE DE DATA DEL SURVEY DE GUITARRA
13:41:57    Lawrence Berkeley Laboratory     DEC VAX-8600   VMS V4.4

PRECEDING TASK REQUIRED       0.30 SECONDS CPU TIME;       0.45 SECONDS ELAPSED.

  15  0          BREAKDOWN AGE BY STAR BY SEX

THERE ARE   202448 BYTES OF MEMORY AVAILABLE.
THE LARGEST CONTIGUOUS AREA HAS   161958 BYTES.

***** GIVEN WORKSPACE ALLOWS FOR  3564 CELLS WITH  2 DIMENSIONS FOR BREAKDOWN.
```

FILE: GUITAR BRAND PREFERENCE SURVEY

- - - - - - - - - - - - - - - - - D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE AGE AGE OF RESPONDENT
BROKEN DOWN BY STAR TRY NAME BRAND GUITAR FROM WHICH PLAYER
BY SEX SEX OF RESPONDENT

VARIABLE	VALUE	LABEL	MEAN	STD DEV	CASES
FOR ENTIRE POPULATION			21.9950	4.5880	202
STAR	1	JOHN DENVER	21.3125	3.5976	48
SEX	F	FEMALE	20.7917	3.6829	24
SEX	M	MALE	21.8333	3.5098	24
STAR	2	ROY CLARK	22.0864	5.0995	81
SEX	F	FEMALE	21.8462	5.3048	13
SEX	M	MALE	22.1324	5.0987	68
STAR	3	JOHNNY CASH	22.0000	5.6372	10
SEX	F	FEMALE	19.0000	.0000	1
SEX	M	MALE	22.3333	5.8737	9
STAR	4	GLEN CAMPBELL	22.2308	4.5672	13
SEX	F	FEMALE	23.4000	4.7223	5
SEX	M	MALE	21.5000	4.6291	8
STAR	5	GORDON LIGHTFOOT	22.4400	4.4546	50
SEX	F	FEMALE	21.4706	4.0330	17
SEX	M	MALE	22.9394	4.6364	33

TOTAL CASES = 202

PRECEDING TASK REQUIRED 0.18 SECONDS CPU TIME; 0.23 SECONDS ELAPSED.

```
 16  0          TEMPORARY
 17  0          RECODE SEX ('F'=0) ('M'=1) into sex1
 18  0          VARIABLE LABELS SEX1 'SEX OF RESPONDENT'
 19  0          VALUE LABELS SEX1 0 'FEMALE' 1 'MALE'
 20  0          BREAKDOWN VARIABLES=AGE (15,34) STAR (1,5) SEX1 (0,1)
 21  0           /CROSSBREAK=AGE BY STAR BY SEX1
 22  0          OPTION 6 7
 23  0
 24  0
```

INTEGER BREAKDOWN NEEDS 408 BYTES OF MEMORY.

THERE ARE 202240 BYTES OF MEMORY AVAILABLE.
THE LARGEST CONTIGUOUS AREA HAS 161792 BYTES.

5-AUG-86 REPORTE DE DATA DEL SURVEY DE GUITARRA
13:41:57 Lawrence Berkeley Laboratory DEC VAX-8600 VMS V4.4

FILE: GUITAR BRAND PREFERENCE SURVEY

- C R O S S---B R E A K D O W N O F - - - -

STAR TRY NAME BRAND GUITAR FROM WHICH PLAYER BY SEX1 SEX OF RESPONDENT

VARIABLE AVERAGED... AGE AGE OF RESPONDENT

- -

STAR	SEX1 MEAN / COUNT / SUM	FEMALE 0	MALE 1	ROW TOTAL
JOHN DENVER	1	20.79 24 499.00	21.83 24 524.00	21.31 48 1023.00
ROY CLARK	2	21.85 13 284.00	22.13 68 1505.00	22.09 81 1789.00
JOHNNY CASH	3	19.00 1 19.00	22.33 9 201.00	22.00 10 220.00
GLEN CAMPBELL	4	23.40 5 117.00	21.50 8 172.00	22.23 13 289.00
GORDON LIGHTFOOT	5	21.47 17 365.00	22.94 33 757.00	22.44 50 1122.00
COLUMN TOTAL		21.40 60 1284.00	22.25 142 3159.00	22.00 202 4443.00

```
 5-AUG-86   REPORTE DE DATA DEL SURVEY DE GUITARRA
13:41:57    Lawrence Berkeley Laboratory     DEC VAX-8600   VMS V4.4

PRECEDING TASK REQUIRED       0.18 SECONDS CPU TIME;       0.21 SECONDS ELAPSED.

          25 COMMAND LINES READ.
           0 ERRORS DETECTED.
           0 WARNINGS ISSUED.
           1 SECONDS CPU TIME.
     1468803 SECONDS ELAPSED TIME.
             END OF JOB.
```

SPSSX OUTPUT EDITED AND TRANSFORMED INTO

TELL-A-GRAF INPUT.

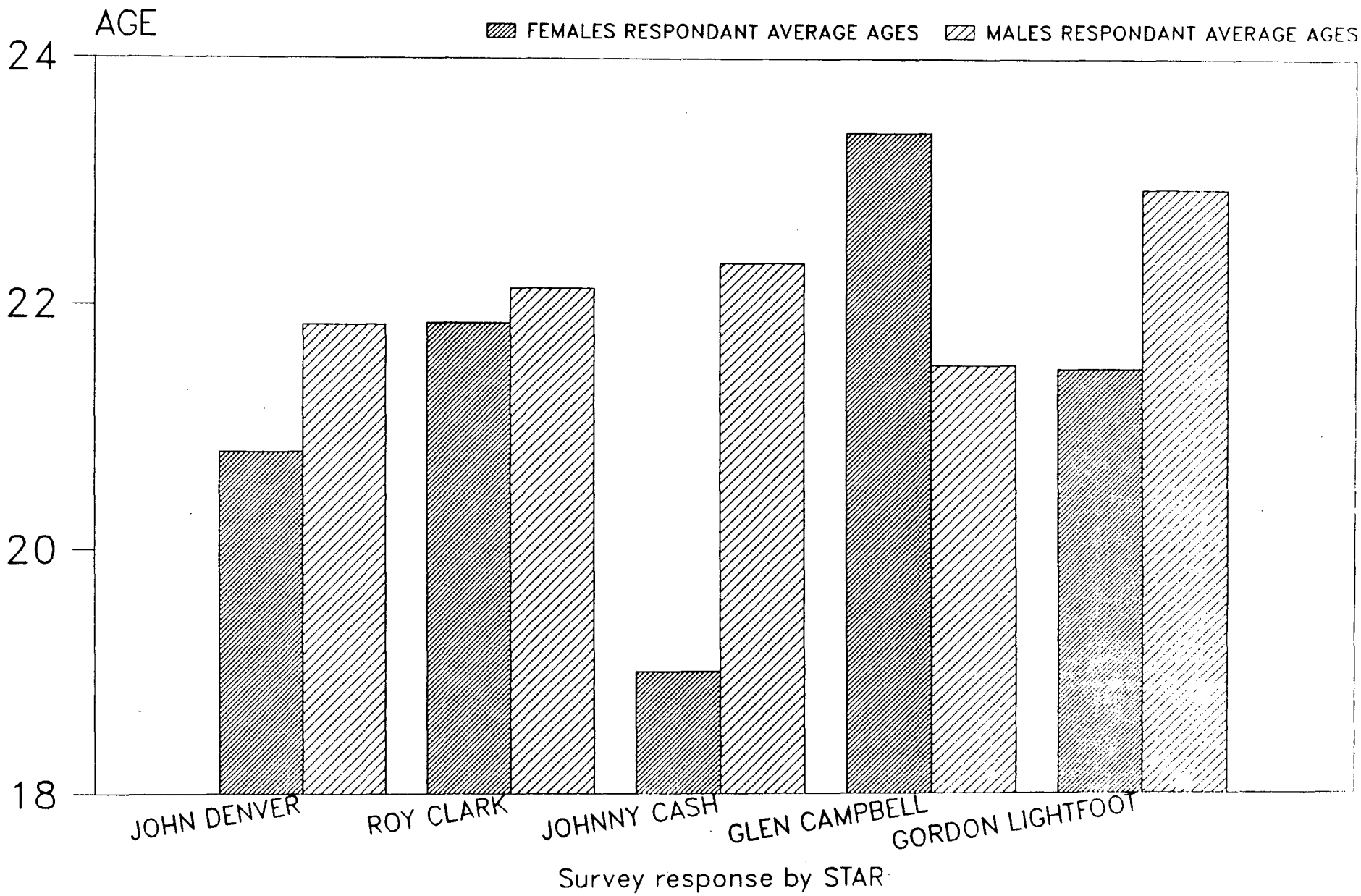
```
GENERATE A BARCHART.
SEQUENCE DATA.
"FEMALES RESPONDANT AVERAGE AGES".
20.7917  21.8462  19.0000    23.4000    21.4706
"MALES RESPONDANT AVERAGE AGES".
21.8333  22.1324  22.3333    21.5000    22.9394
END OF DATA.
INDEPENDENT DIVISION LABELS IS "JOHN DENVER" "ROY CLARK" "JOHNNY CASH" "GLEN CAMPBELL" "GORDON
TITLE IS "AGE AVERAGE SURVEY RESPONSE BARCHART".
X AXIS LABEL IS "Survey response by STAR".
Y AXIS LABEL IS "AGE".
go.
```

TELL-A-GRAF INPUT CONVERSION OF FILE EDITED.

```
GENERATE A VERTICAL BAR CHART .
INDEPENDENT DIVISION-LABELS IS "JOHN DENVER" "ROY CLARK"          --
 "JOHNNY CASH" "GLEN CAMPBELL" "GORDON LIGHTFOOT" .
INDEPENDENT LABEL TEXT IS "Survey response by STAR" .
DEPENDENT LABEL TEXT IS "AGE" .
TITLE TEXT IS "AGE AVERAGE SURVEY RESPONSE BARCHART" .
INPUT DATA.
"FEMALES RESPONDANT AVERAGE AGES"
 1 20.7917 2 21.846201 3 19 4 23.4 5 21.4706
"MALES RESPONDANT AVERAGE AGES"
 1 21.8333 2 22.132401 3 22.3333 4 21.5 5 22.9394
END OF DATA.
GO.
**FILE**
```

TELL-A-GRAF OUTPUT.

AGE AVERAGE SURVEY RESPONSE BARCHART



ATMOSPHERIC AEROSOL CHEMICAL SAMPLING

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Turabo University

Research Sponsor: Dr. Tihomar Novakov
Applied Sciences Division

Introduction

Puerto Rico is a relatively small island, suitable for environmental studies on the formation and transformation of acidifying chemical species. It has two distinctly different meteorological conditions: The northern part of the island has a high index of humidity with relatively large amounts of precipitation, and the southern part is almost arid. One of the outstanding environmental problems in Puerto Rico is the deposition of an acidic form of rain, in addition to particulate sulfate and nitrate. Local and distant sources of pollution have different effects on atmospheric chemical species and, therefore, on the deposition of the acidic form of rain. Local sources of pollution such as automobiles, fuel oil, and coal combustion products from local industries and pollutants carried in air currents from distant pollution sources have high concentrations of SO_2.

Interest in the amounts, origin, and fate of certain elemental species has quickened in recent years for several reasons: Trace metals such as mercury, barium, and lead are known to have implications for public health and ecology. Other elemental species such as iodine may, in compound form, serve as condensation nuclei and lead to the artificial stimulation of rain (Schaefer, 1966).

It has been demonstrated that the chemical reaction involved in the formation of acid rain is the oxidation of SO_2 to S(IV). When SO_2 and nitrogen oxides (NO_x) are released into the air, they often combine with water to form sulfuric and nitric acids. The chemical soup travels with the winds and eventually falls to earth as acidic rain, snow, and even fog.

Purpose

The specific aim of this study is to develop the procedures for differentiating between the concentrations and effects of distant sources and those of

local sources on atmospheric chemical species. Because of the unique geographical and climatological factors of Puerto Rico, the island is appropriate for these studies.

Aerosol characterization studies based on trace element analysis and the established analysis methods will be performed.

Experimental Procedures

Seventy-one samples were collected in Puerto Rico, place in petri dishes, labelled, and shipped to Lawrence Berkeley Laboratory. Aerosol samples were collected in two filter substrates (47-mm diameter quartz and Teflon), and the sampler is a high-volume sampler for particle collection on an 8"x10" quartz filter. The 47-mm Teflon Millipore filters were used for trace element analysis by x-ray fluorescence spectrometry (Dzubay et al., 1977).

The exposed quartz filters were analyzed for total carbon concentrations collected by burning the carbonaceous material in the samples and quantitatively accounting for the evolved carbon dioxide gas (CO_2 coulometer). The quartz filters were also used for water extraction by ion chromatography (IC). Three punches of 1.19 cm^2 area were taken from the quartz filters and extracted in 15 ml of water with sonication for 10 minutes. The extract was filtered and analyzed for anions and cations using a Dionex System 12 ion chromatograph. We detected anions (Cl^-, $SO_4^=$, NO_3^-), and we also detected cations (NH_4^+, K^+, Na^+). For ions we had to prepare standards to calibrate the instrument. The obtained concentrations of anion standards are: 2.8×10^{-5} M to 1.4×10^{-4} M. For cations we used CS-1 separator column, eluant of 5 mM HCl, and regenerant for CSF of 40 mM KOH. The concentrations of cation standards are: 10 ppm to .5 ppm.

Discussion and Results

Figure 1 shows the 24-hr total mercury concentration in $\mu g/m^3$ for each sampling day. High concentrations of mercury have been found in the samples. The levels are found during the day (max. value: 80 ng/m^3) and low levels during night hours (min. value: 10 ng/m^3). The source of mercury producing this cyclicity is unknown at the moment.

Figure 2 shows the Pb vs. Hg graph. There is no correlation between the two elements because the origin of both is not the same. The Pb is from the exhaust of automobiles and the Hg is not present in gasoline.

We also found the presence of V. It is suggested that the V present in the atmosphere, part of which was collected with the experimental filters, is derived from the burning of oil in thermoelectrical industries.

Carbonaceous aerosols have several components: soot, an indicator and tracer for incomplete combustion, and organic carbon, which may be combustion generated, produced in the atmosphere by photochemical processes.

Acknowledgments

I thank AGMEF and Dr. T. Novakov for providing me the opportunity to realize this work at Lawrence Berkeley Laboratory. I also want to thank W.H. Benner for his theoretical and experimental support for this project.

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APPENDIX

4/24/86
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5/29/86
Date

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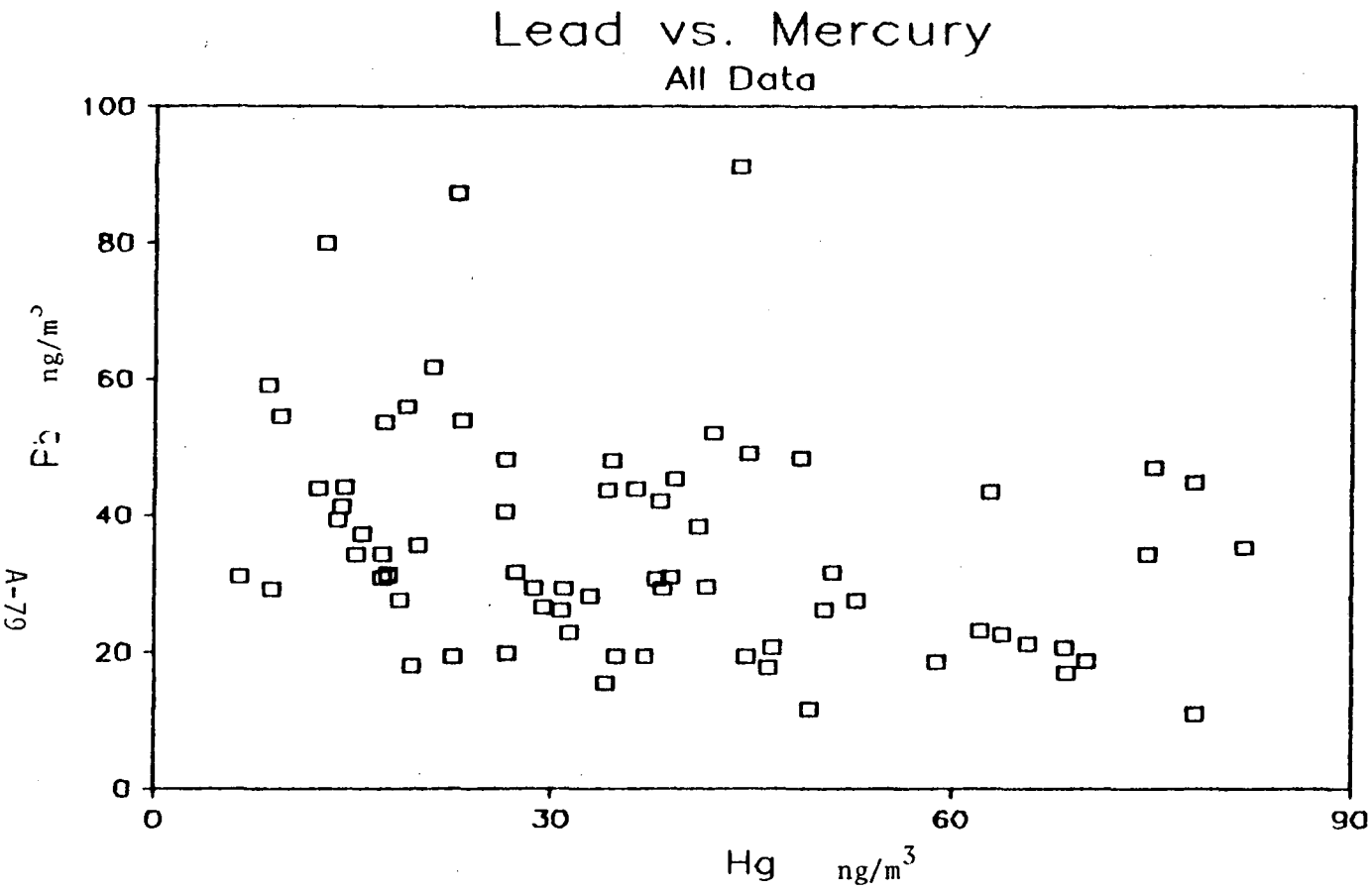

Figure 2.

NITROUS OXIDE FORMATION IN COMBUSTION

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Turabo University

Research Sponsor: Dr. Nancy Brown
Applied Sciences Division

The concentration of nitrous oxide, a greenhouse gas present in the atmosphere with strong absorptions in the 4 and 8 micron regions of the spectrum, has been increasing at a rate of 0.20% per year since 1962.[1] Nitrous oxide emanates from the troposphere and reacts in th stratosphere with oxygen atoms to produce nitric oxide, which in run, is a major scavenger of stratospheric ozone. The latter plays an important role in shielding the earth's surface from biologically detrimental ultra violet radiation. Ozone concentration determines the stratospheric temperature structure, and its modification will effect transport mechanisms in the stratosphere which may induce change in weather and climate.

It is known that nitrous oxide is present in the exhaust of gas and oil power plants. Concentrations as high as 100 ppm have been detected in the exhaust of coal-fired plants by Weiss.[2] However, its formation has not been confirmed by studies on hydrocarbon combustion.

The purpose of this study was to determine the conditions that lead to the formation and destruction of nitrous oxide during the combustion of hydrocarbon fuels.

The following question was answered. Is N_2O formed in well-controlled environment of laboratory scale combustion experiments? In order to do so, Gas Chromatography was used. The Gas Chromatograph [(G.C.) Model 5880A] used is a micro-processor based instrument designed to provide the maximum flexibility in user-defined configurations. It consists of a mainframe and two terminals. Each terminal contains a print-plot mechanism and a functional keyboard. The mainframe houses a column oven; injection and detection systems, gas flow systems and necessary electronics. The rear of the mainframe provides a means to connect the G.C. with power, gases and terminals or other external devices. The G.C. contains an Electron Capture Detector (ECD) as well as a Thermal Conductivity Detector (TCD). These are connected in parallel and are operated with Helium as a carrier gas and Argon methane as makeup gas. The TCD is a single filament ceramic cartridge which examines samples and reference gas streams alternately. Since it

recognizes almost any gas, any gas different from Helium is detected. The TCD can't measure very small concentrations therefore the ECD was included. This device operates by sensing a decrease in the number of free electrons in the detector cavity due to their capture by electrophilic sample molecules. The signal depends on the capture of electrons by various substances being sensed which causes a reduction in the ion current.

The combustion system consisted of a flat-flame burner using methane, air and ammonia. The flowrates of the gases passing through the burner were determined by settling various rotometers at fixed positions during the sampling process. Just above the burner we had a probe through which the gases flowed and then passed through a desiccant to remove the water that was present in the sample. The desiccant used was Aquasorb. Gaseous samples were withdrawn from the burner and injected into the sample loop of the G.C.

The combustion parameters that were varied to study their effects of n_2O were: nitrogen concentration and fuel/air ratio. By varying the methane flowrate, a general combustion sample was made to check that the burner and the sampling system were in normal conditions.

Figure 1 represents the CO_2% as a function of radius. The squares represent z's position at 10.0 mm and the diamonds represent z's position at 0.5 mm as shown on the graph were z represents height. The graph shows a high concentration of CO_2 at the low edge of the burner and a high CO_2 concentration at the center of the burner. It also shows a greater amount of scatter in the position where z = 10.0 mm elevation rises faster and stays constant for a longer period of time.

Figure 2 represents the O_2% as a function of radius. The positions are the same as those in Figure 1. The graph shows a high concentration of O_2 at eh edge of the burner and low concentration of O_2 at the center of the burner. The scatter is less than that of CO_2%/Radius but it is still present.

Figure 3 represents the N_2O parts per million as a function of Radius. The z positions are the same as those in Figures 1 and 2. At 10.0 mm there is a low concentration of N_2O at the edge of the burner. The concentration seems to increase but it eventually decreases. At the edge

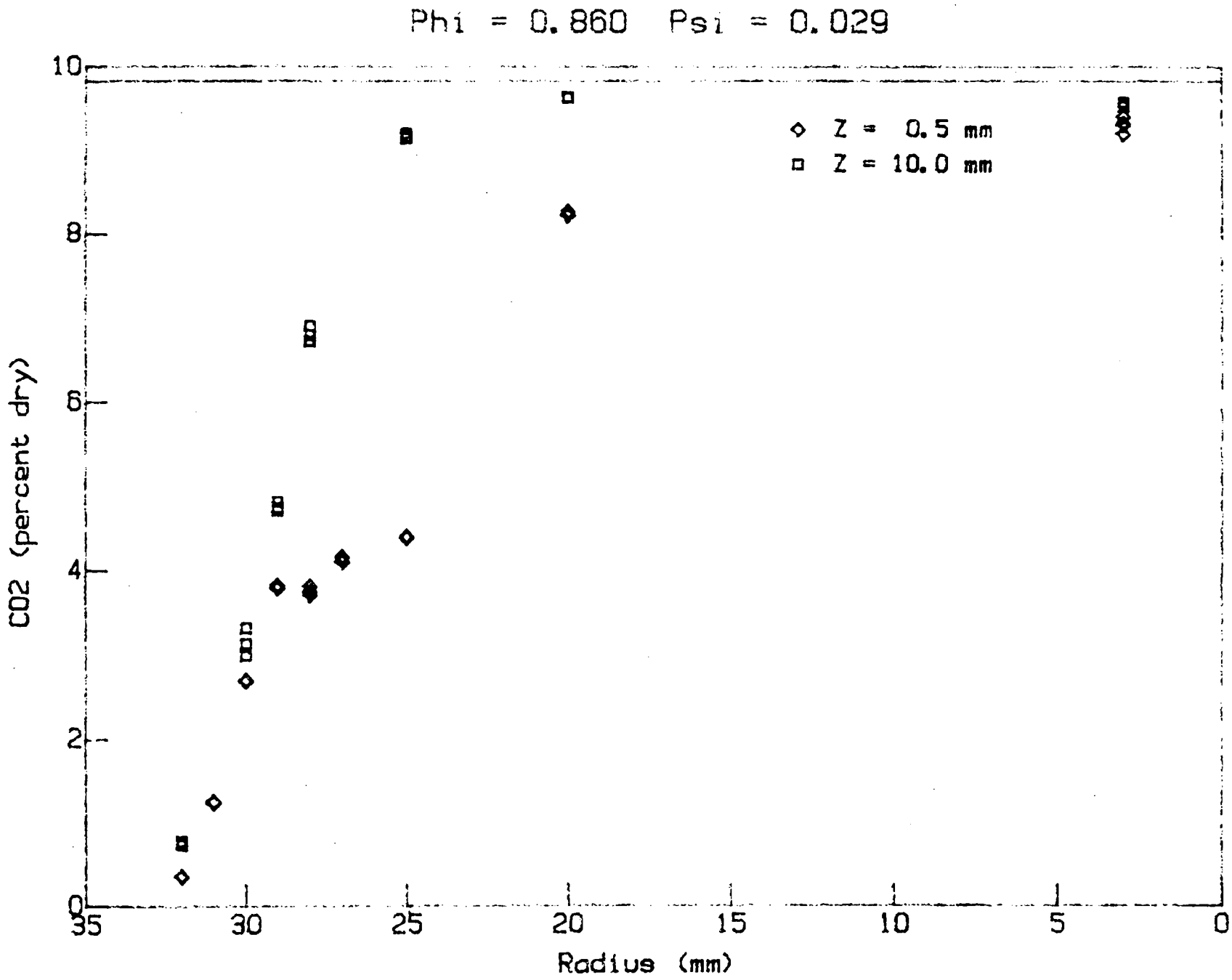
of the burner there is a low concentration of N_2O. As the sampling is done further away from the burner there is a higher concentration of N_2O but as the sampling gets nearer to the center of the burner it has a low concentration.

Figure 4 represent CO_2% versus height. At the edge of the burner there is a low concentration of CO_2 which gradually increase and then decreases. At the center of the burner the concentration of CO_2 is rather high and stays constant for the rest of the samples.

Figure 5 represents O_2% versus height. At the edge of the burner the graph shows a high concentration of O_2 which decreases a little but gradually continues to increase. Near the center of the burner there is constant low concentration of O_2.

Figure 6 represents N_2O parts per million versus height. The graph shows a high concentration of N_2O near the edge of the burner and a low concentration at the center of the burner.

From the results that were obtained we can conclude that N_2O is formed in a well-controlled environment of laboratory - scale combustion. The results show that approximately 14 to 20 ppm of N_2O were formed. As the probe is farther away from the burner the detection of N_2O is less. Although the temperature wasn't taken into consideration it can be an influential factor in the behavior that has been seen. Due to dilution, the gratest amount of heat concentrates at the center of the burner. This explains why CO_2 has a greater concentration at the center of the burner. We can suggest that N_2O is a fragile molecule and doesn't exist where there is high temperature as in the center of the burner. The causes of the formation a destruction of N_2O in the entire process is yet unknown. Studies are being conducted to determine why and under what conditions this occurs.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Z = 0.5 mm
Z = 10.0 mm
CO2 (percent dry)
Radius (mm)
0
2
4
6
8
10
35
30
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10
5
0

Figure 1

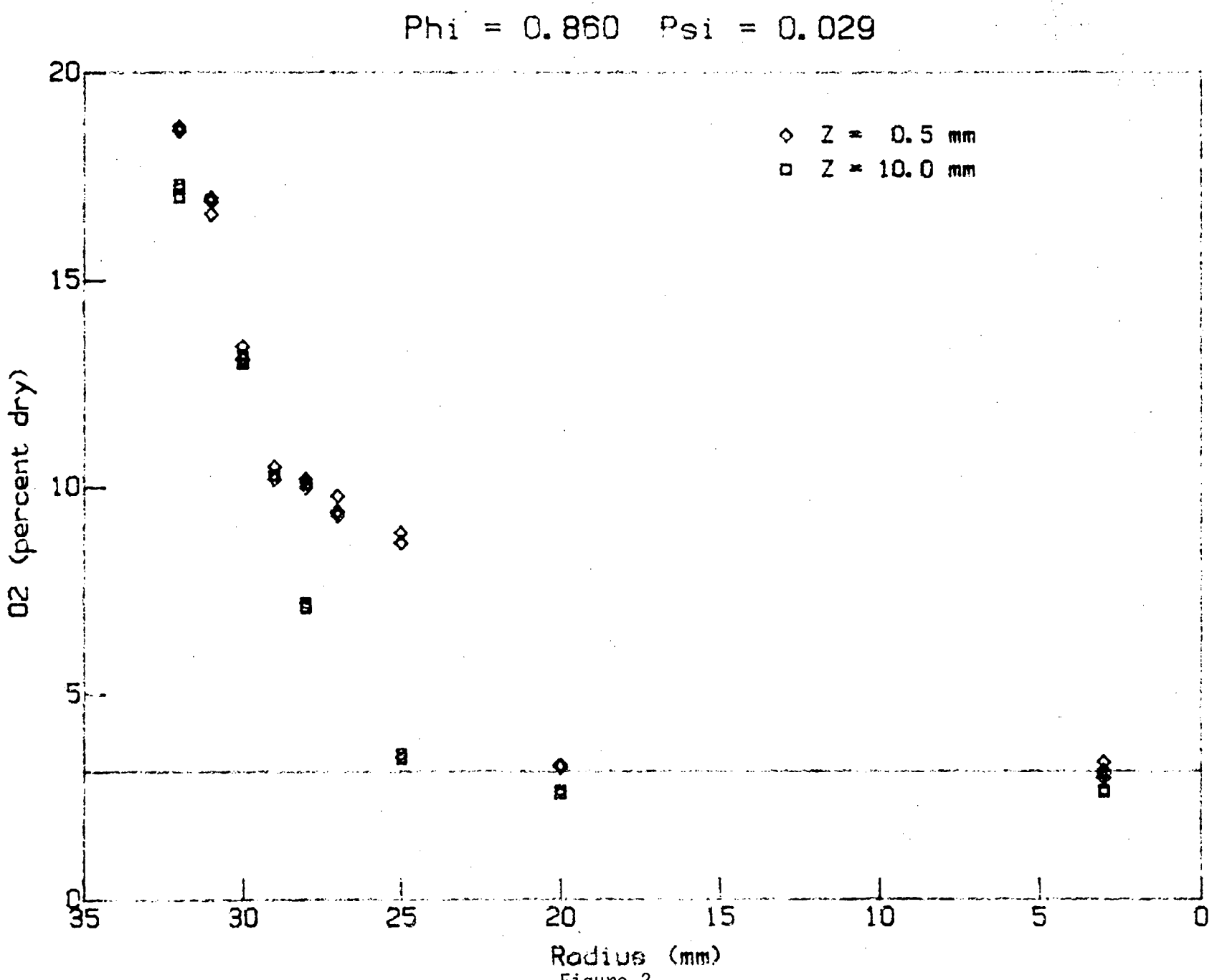
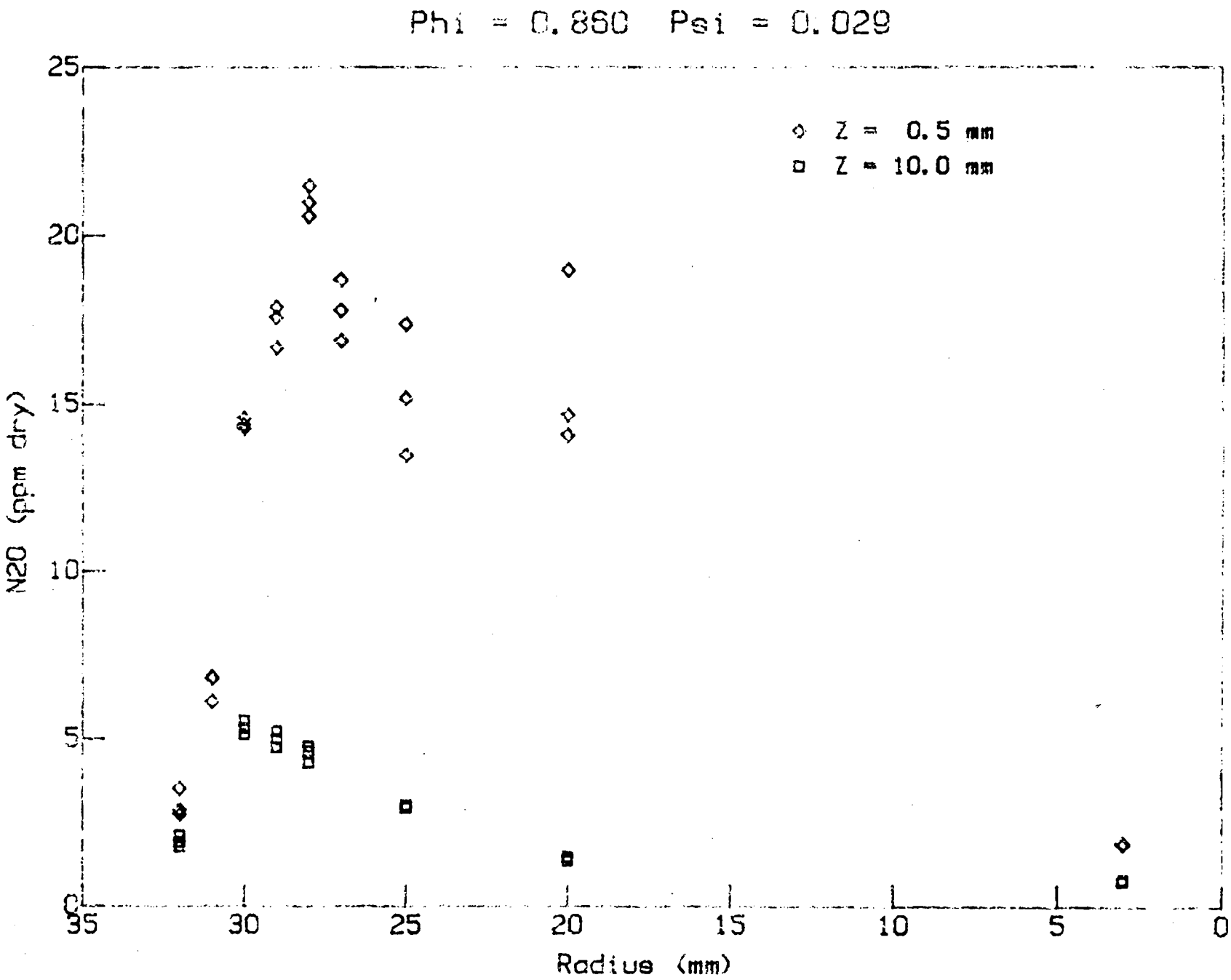
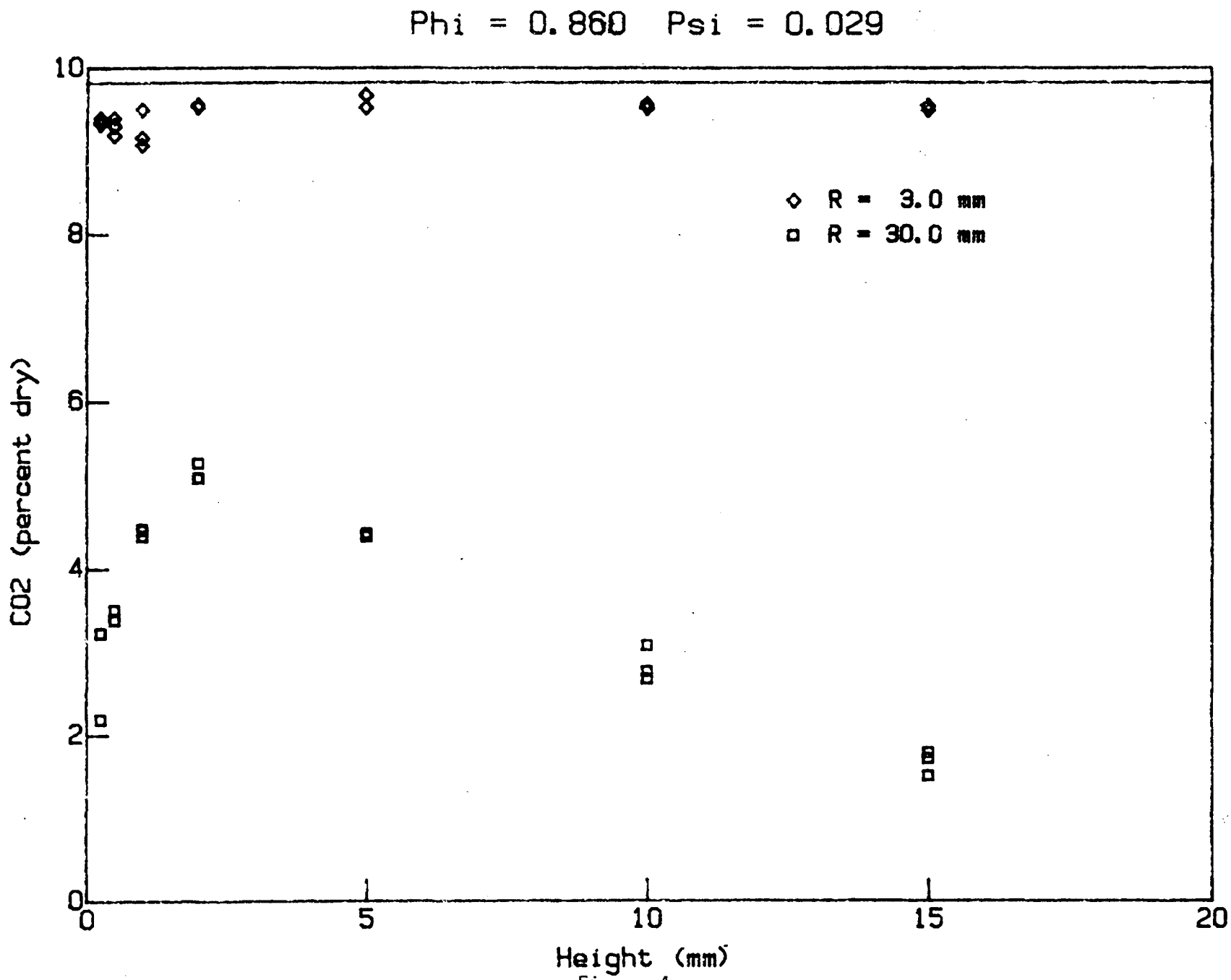
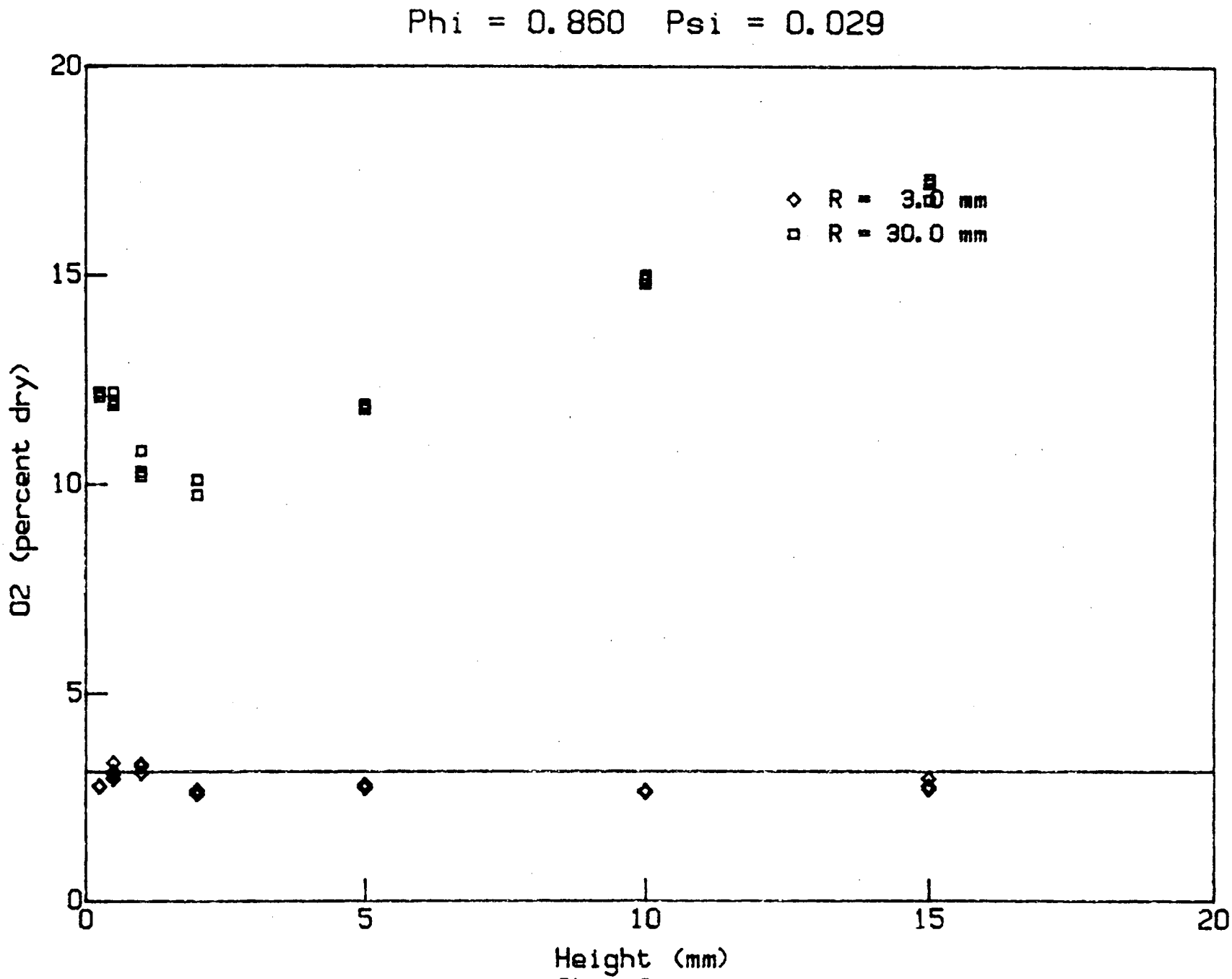

Phi = 0.860 Psi = 0.029
Z = 0.5 mm
Z = 10.0 mm
O2 (percent dry)
Radius (mm)
20
15
10
5
0
35
30
25
20
15
10
5
0

Figure 2


Phi = 0.860 Psi = 0.029
Z = 0.5 mm
Z = 10.0 mm
N2O (ppm dry)
Radius (mm)
0
5
10
15
20
25
35
30
25
20
15
10
5
0
Figure 3


Phi = 0.860 Psi = 0.029
R = 3.0 mm
R = 30.0 mm
CO2 (percent dry)
Height (mm)
0
2
4
6
8
10
0
5
10
15
20
Figure 4


Phi = 0.860 Psi = 0.029
R = 3.0 mm
R = 30.0 mm
O2 (percent dry)
Height (mm)
0
5
10
15
20
Figure 5

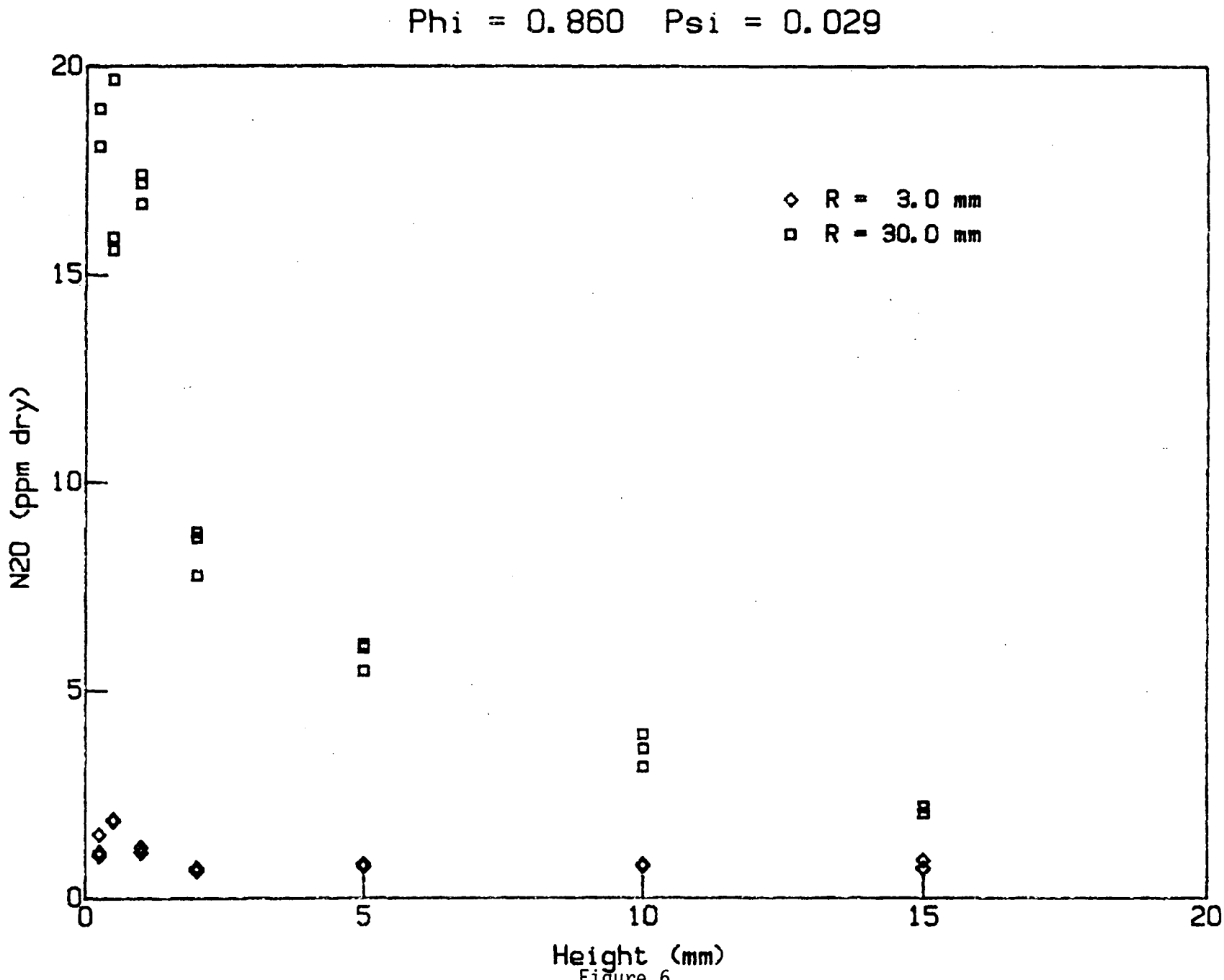

Phi = 0.860 Psi = 0.029
R = 3.0 mm
R = 30.0 mm
N2O (ppm dry)
Height (mm)
0
5
10
15
20

Figure 6

References

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LANGUAGE TRANSLATION AND SOFTWARE MAINTANENCE

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Metropolitan University

Research Sponsor: Dr. Vern Paxson
Engineering Division
(Real Time Systems Group)

While at the Lawrence Berkeley Laboratories, I worked with the Real Time Systems Group under the supervision of Vern Paxson. My project was to convert a program called WORD2PS.c into a Lex program called WORD2PS.l (figure 1). In addition to this, I was also able to work in software maintenance (fixing and debugging programs).

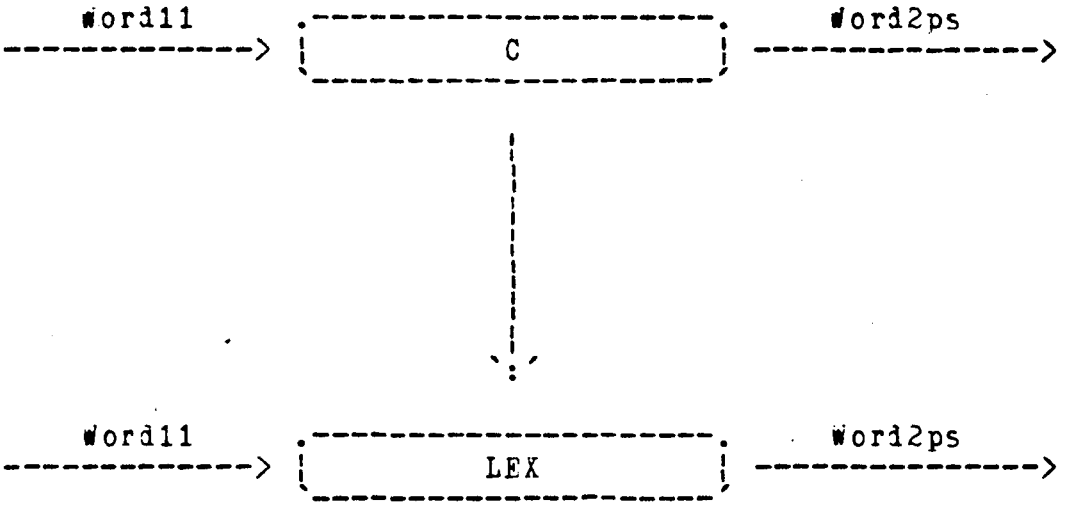

figure 1: Diagram of project. The top section represents how Word2ps was and the bottom section represents how Word2ps is currently.

WORD2PS is a program which translates documents written in the Word-11 language into the Postscript language. The translation is made up of three phases:

first, relevant macro files are determined and read; second, the document is processed, all word-11 commands are recognized and the appropriate postscript commands are output; third, all literal text are output as Postscript strings.

My task was to rewrite the WORD2ps program written entirely in C language into a program which has sections written in Lex. Lex is not a complete programing language. It is a generator that represents a language feature that can be added to "host languages" (different programing languages) that is designed for lexical analysis of a text. It utilizes input files that contain regular expressions to be searched for and actions written in C or in ratfor (a Fortran preprocessor) to be executed when the expressions are found. In other words, a lex program will pattern match a given input and then execute corresponding actions written in C or in ratfor. In lex, WORD2PS or any other program is easier to maintain because extension may be added, sections may be changed and bugs may be fixed without much trouble.

In order to work on the project, I had to learn various things (figure 2). My first week here I learned learned to work with eunice, the visual editor vi

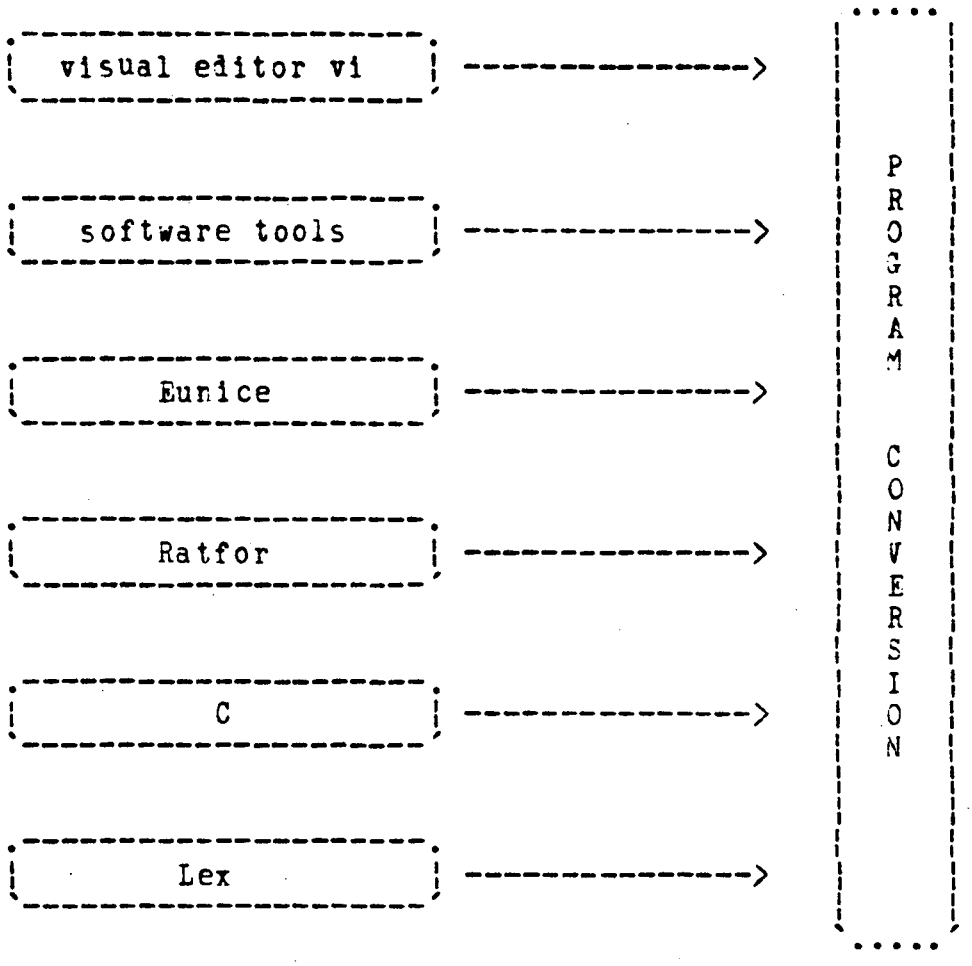

figure 2: Languages and editors needed to be learned in order to be able to convert tne program.

and with software tool. Eunice is a hybrid of Unix (which contains an operating sistem and application or utility software) built for VMS. The visual editor vi is a screen oriented display editor which eunice contains. It is a very powerful user friendly editor. Software tools is a virtual operating system that can be implemented on top of a variety of host operating systems.

In addition to software tools, vi and eunice, I had to learn programming in C (a general purpose language that features modern commands), in ratfor and in lex. I learned to program in these languages by writing a simple program that counts the foo, bar and bletch (the words mentioned are only dummy words, any other three words could have been picked to be counted.) that are entered in each of the different programing in languages (appendix A contains these programs).

After learning all that was essential, I then began the conversion of WORD2PS.c (written entirely in C) to WORD2PS.l (written in lex). The routine "doescape" contained a switch statement that could be considered the most important part of the program. The switch statement ,switch case, takes an entered character and searches the "case" statements to find a match. When the match is

found, the desired action is done. Lex does pattern matching so this section was the first part that was converted to lex. An input routine was added to be used by the lex section being that lex accepts entered data using an input routine.

The doescape routine was called only once in word2ps.c. This was found in the routine named morepages. The next step then was to interline doescape in morepages, and convert that into a lex program section. The conversion was done using a "start condition" (see appendix A, program 6 for an example of a lex start condition). The doescape routine that was lex was turned into the start condition "DE" for morepages().

When the conversion was finished, the two programs were then run using a sample program. The output that each program generated was then compared to determine the difference between these. This was done twice, once after the first change was made and once after the second change was made. The differences were only minor being that more than 90% of the output was equivalent. With these results it can be determined that the change from WORD2PS.c to WORD2PS.l is successful (A copy of word2ps.c and word2ps.l can be found in appendix B and C. Compare the programs and note the changes made that were made.)

Ideally, the conversion of Word2ps to a lex program should not have affected its output at all. The fact that there were minor differences between the two programs' output means that there is some type of bug in the program. This bug could also have been in the original program and only uncovered by the changes I made. Since it is a small bug it may be due to an error in the translation but at the completion the bug my be fixed.

Another aspect of my work while at LBL was software maintenance. A meeting is held in the RTSG conference room once a week. Bugs that have been fixed or new bugs that have been found are discussed. The new bugs are distributed to different members of the group so that they can be fixed. A bug is a problem that has been found in a software. Once assigned to fix a bug, the program that contains it is copied into your own directory and then locked. Locking a program insures that you are the only person working on it. The bug is then searched for and once found is fixed. When this has been accomplished, the program is compiled and run to assure that the bug has indeed been fixed and that no other part of the program has been affected. A change request form is then filed. If the change is successful, the program can then be unlocked.

My work experience at LBL helped me to get interested in computer research. Not only did I receive the opportunity to learn various languages and operating systems but I was able to work with a very talented group of people who were kind and considerate. And with a very patient supervisor who would explain things until I understood them making me feel at ease and comfortable. My summer experience at RTSG has been very gratifying.

APPENDIX A

Example programs 1 through 6

Program #1: This program is written in ratfor. It, like
---------- all of the sample programs will count how many foo, bar and bletch (these are sample words) are entered.

```
DRIVER(try)

                NOIMPLICIT

character       str1(MAXLINE)           # word  entered
integer         cnta, cntb, cntc        # counter variables
integer         equal, getlin           # functions used

# counter variables are cleared

cnta = 0
cntb = 0
cntc = 0


# a statement will be printed to the user

call putlin(" This program will count how many foo,
                bar @n", STDOUT)
call putlin(" and bletch are encountered.  Return must
                be  @n",STDOUT)
call putlin(" pressed after each word is entered.  @n",
                STDOUT)
call putlin("@n Please begin entering the words.   @n",
                STDOUT)


# the foo, bar and bletch entered, will now be counted

while (getlin(str1,STDIN) != EOF)
{

if (equal(str1, "foo@n") == YES)
    cnta = cnta + 1
else if (equal(str1, "bar@n") == YES)
    cntb = cntb + 1
else if (equal(str1, "bletch@n") == YES)
    cntc = cntc + 1
}
```

```
# the answers will be printed on the screen

call putlin(" foo found were", STDOUT)
call putdec(cnta, STDOUT)
call putlin("@n bar found were", STDOUT)
call putdec(cntb, STDOUT)
call putlin("@n bletch found were", STDOUT)
call putdec(cntc, STDOUT)

DRETURN
END
```

Program #2: This program is also written in ratfor but
---------- the RTSG group.

```
# try3 - counts foo, bar and bletch

# Ver  Date     Who  Remarks
# --- -------- --- ------------------------------------
# Ø2a Ø3JulyB6 RT  .Written

# This program will count how many foo, bar and bletch
# are found in a file entered manually.  The file will be
# read and all the foo, bar and bletch in it will be
# counted.

DRIVER(try3)
NOIMPLICIT

character str1(MAXLINE)          # line that is entered
character str2(MAXLINE)          # word taken from file
integer cnta, cntb, cntc         # counter variables
integer i                        # variable for the getwrd
integer equal, getlin, getwrd    # functions used

cnta = Ø
cntb = Ø
cntc = Ø

while ( getlin( str2, STDIN ) != EOF )
{
i = 1

    while ( getwrd( str2, i ,str1 ) != Ø )
    {
    if ( equal( str1, "foo" ) == YES )
        cnta = cnta + 1
    else if ( equal( str1, "bar" ) == YES )
        cntb = cntb + 1
    else if ( equal( str1, "bletch" ) == YES )
        cntc = cntc + 1
    }
}

call printf ( "foo = %d, bar = %d, bletch = %d@n",
              cnta, cntb, cntc )

DRETURN
end
```

Program #3: This program is written in C.

```
/* tryc1 - counts foo, bar and bletch */

/*  Ver  Date     Who  Remarks
 *  --- -------- --- ----------------------------------
 *  02a 03July86 RT  .written
 */

/*  This program will count how many foo, bar and bletch
 *  are found in a file entered manually.  The file will
 *  be read and all the  foo, bar and bletch  in it will
 *  be counted.
*/

#include <stdio.h>
#include <ctype.h>

main()
    {
    char str1[BUFSIZ];          /* line that is entered */
    char str2[BUFSIZ];          /* word taken from file */
                                /* counter variables    */
    int cnta = 0, cntb = 0, cntc = 0;
    int i;

    while ( gets( str2 ) != NULL )
        {
        i = 0;

        while ( getwrd( str2, &i ,str1 ) != 0 )
            {

            if ( strcmp( str1, "foo" ) == 0 )
                ++cnta;
            else if ( strcmp( str1, "bar" ) == 0 )
                ++cntb;
            else if ( strcmp( str1, "bletch" ) == 0 )
                ++cntc;
            }
        }
```

```
    printf ( "foo = %d, bar = %d, bletch = %d\n",
             cnta, cntb, cntc );
    }


int getwrd( in, i, out )
char in[], out[];
int *i;

    {
    int j = 0;

    while ( in[*i] != '\0' && isspace( in[*i] ) != 0 )
        ++*i;

    while ( in[*i] != '\0' && isspace( in[*i] ) == 0 )
        out[j++] = in[(*i)++];

    out[j] = '\0';
    return(j);
    }
```

Program #4: This program is written in Lex.

```
int cnta = 0, cntb = 0, cntc = 0;
%%
foo       ++cnta;
bar       ++cntb;
bletch    ++cntc;
.|\n      ;
%%
main()
{
yylex();
printf( "# of foos = %d, # of bars = %d, # of bletchs
         = %d\n", cnta, cntb, cntc );
}
```

Program #5: This program is also written in Lex but it will write the word magic when it finds a foo, a bar or a bletch.

```
%%
foo|bar|bletch      printf( "magic" );
```

Program #6: This program is written in lex using a start condition.

```
%{
int cnta = 0, cntb = 0, cntc = 0;
%}
%START AA

%%
    int mcnt = 0;

magic   {
        ++mcnt;
        if (( mcnt % 2) == 0)
            BEGIN 0;
        else
            BEGIN AA;
        }

<AA>foo --cnta;
<AA>bar --cntb;
<AA>bletch  --cntc;
foo     ++cnta;
bar     ++cntb;
bletch  ++cntc;

%%
main()
    {
    yylex();
    printf( "# of foos = %d, # of bars = %d, # of
            bletches = %d\n", cnta, cntb, cntc );
    }
```

APPENDIX B

This is the original WORD2PS program (word2ps.c). Take special not of the doescape and morepages routines.

```
#include <stdio.h>
#include <signal.h>
#include <setjmp.h>
#include <sgtty.h>
#include <sys/file.h>

#define False Ø
#define True ØxFF
#define PPI 72.2

#ifdef  petvax
#define DEFAULT_W11PS_MAC "disk$tools:[tex.laserwrt]"
#else   petvax
#define DEFAULT_W11PS_MAC "disk$user1:[tab.src]"
#endif  petvax

#define RADICAL 326
#define RADICALEX 14Ø

#define LF                  '\Ø12'
#define FF                  '\Ø14'
#define ESC                 '\Ø33'
#define START_DOC           'S'
#define END_DOC             'E'
#define MANUAL_FEED         'M'
#define HALF_LINE           'D'
#define ON_BOLD             'Q'
#define OFF_BOLD            'R'
#define ON_UNDERLINE        'I'
#define OFF_UNDERLINE       'J'
#define H_SPACE             'H'
#define ON_SUPER            'T'
#define OFF_SUPER           'U'
#define ON_SUB              'V'
#define OFF_SUB             'W'
#define DOWN                'D'
#define OFF_ALTCHAR         '#'
#define ON_ALTCHAR          'X'

#define MAXLINE 8Ø
#define MAXBUF 512
#define MAXBUF2 1Ø24

char buf[MAXBUF2], lastchar=Ø;
int hnspace = False;
char lastcommand = OFF_ALTCHAR;
char psstring[MAXBUF];
char psbuf[MAXLINE];
char macdir_array[MAXLINE];
char *macdir = macdir_array;
double width[128];
int altch[128];
int bufind = MAXBUF2, bufcount = MAXBUF2, psi = Ø;
```

```
int scanind = MAXBUF, scancount = MAXBUF, curmax = MAXBUF2;
double cpi;
double fontsize;
FILE *fp;
double lasthscale=0, deltac, totalwidth,  hscale, ptsize;

int underline = False;
int proportional = False;
int scanning = False;
int italics = False;
int boldface = False;
int superscript = False;
int subscript = False;
int altchar = False;

char usage[] = "usage: \"word2ps [-i] filename\"\n";

main(ac, av)
char **av;
{
        char *name, morepages(), header();

        name = "";
        ac--; av++;
        while (ac) {
                if (av[0][0] == '-')
                        {
                        switch (av[0][1])
                                {
                                case '\0':
                                        name = av[0];
                                        break;
                                case 'i':
                                        italics = True;
                                        break;
                                default:
                                        fprintf(stderr, usage);
                                        exit(1);
                                }
                        }
                else
                        {
                        name = av[0];
                        }
                ac--; av++;
                }

        fp = fopen(name, "r+");
        if (fp == NULL)
                {
                fprintf( stderr, "word2ps: can't open w11 file %s\n", name);
                exit(1);
                }
```

```
        if ( ! header() )
                fprintf( stderr,
                "word2ps: bad header in word11 print image file\n");
        else
                {
                reset();
                while ( morepages() != EOF )
                        printps( "showpage" );
                /* end the document */
                printps( "ed" );
                if ( psbuf[0] != '\0' )
                        puts( psbuf );
                }

        fclose(fp);

}

char doescape()
{
        char c, getch();
        char *escerr = "word2ps: no char following escape char\n";
        char *boldofferr = "word2ps: bold, off found when not on\n";
        char *boldonerr = "word2ps: bold, on found when already on\n";
        char *undofferr = "word2ps: underline, off found without on\n";
        char *supofferr = "word2ps: off-super, not superscripting\n";
        char *subofferr = "word2ps: off-sub, not subscripting\n";
        char *badescerr = "%% word2ps: bad escape char";

        if ( getch() < 0 )
                {
                fprintf(stderr, escerr );
                printf( "\n%% %s", escerr );
                }
        else
                {
                /* check if string needs to be finished */
                if ( buf[bufind] != H_SPACE  )
                        finishstring();

                switch( buf[bufind] )
                        {

                        case MANUAL_FEED:
                                printps( "mf" );
                                break;

                        case ON_BOLD:
                                /* start a for loop to be repeated once */
                                if ( boldface )         /* already boldfacing */
                                        {
                                        fprintf( stderr, boldonerr );
                                        printf( "\n%% %s", boldonerr );
```

```
                }
        else if ( altchar )
                printps( "SBfont bd" );
        else if ( italics )
                printps( "IBfont bd" );
        else
                printps( "Bfont bd" );

        boldface = True;
        break;

case OFF_BOLD:
        /* end for-loop to do bold face */
        if ( ! boldface )
                {
                fprintf( stderr, boldofferr );
                printf( "\n%% %s", boldofferr );
                }
        else
                {
                if ( lastcommand == ON_ALTCHAR )
                        printps( "/prealtfont preboldfont d
                printps( "obd" );
                boldface = False;
                }
        break;

case HALF_LINE:
        doreturn( "sr" );
        /* get rid of next line feed */
        if ( getch() != LF )
                bufind--;
        break;

case END_DOC:
        /* last char in file */
        return EOF;

case ON_UNDERLINE:
        underline = True;
        if ( italics )
                {
                if ( boldface )
                        printps( "IBfont itf" );
                else
                        printps( "Ifont itf" );
                }
        else
                printps( "u" );
        break;
```

```
case OFF_UNDERLINE:
        if ( ! underline )
                {
                fprintf( stderr, undofferr );
                printf( "\n%% %s", undofferr );
                }
        else if ( italics )
                printps( "oitf" );
        else
                printps("ou" );
        underline = False;
        break;

case H_SPACE:
        /* next 3 chars are the amount to horiz move by  */
        hhspace = True;
        getscale();
        break;

case ON_SUPER:
        /* turn superscript toggle on */
        if ( ! superscript )
                {
                superscript = True;
                printps( "p" );
                }
        break;
case OFF_SUPER:
        /* turn superscript off */
        if ( ! superscript )
                {
                fprintf( stderr, supofferr );
                printf( "\n%% %s", supofferr );
                }
        else
                {
                if ( lastcommand == ON_ALTCHAR )
                        printps( "/prealtfont presmfont def
                printps( "op" );
                superscript = False;
                }
        break;
case ON_SUB:
        /* turn subscript toggle on */
        if ( ! subscript )
                {
                subscript = True;
                printps( "b" );
                }
        break;
case OFF_SUB:
        /* subscript off */
        if ( ! subscript )
```

```
                                        {
                                        fprintf( stderr, subofferr );
                                        printf( "\n%% %s", subofferr );
                                        }
                                else
                                        {
                                        if ( lastcommand == ON_ALTCHAR )
                                                {
                                                printps( "/prealtfont presmfont ds
                                                }
                                        printps( "ob" );
                                        subscript = False;
                                        }
                                break;
                        case OFF_ALTCHAR:
                                if ( altchar )
                                        {
                                        printps( "oaf" );
                                        altchar = False;
                                        }
                                break;

                        case ON_ALTCHAR:
                                /* change print wheel, greek = symbol font? */
                                if ( ! altchar )
                                        {
                                        if ( boldface )
                                                printps( "SBfont af" );
                                        else
                                                printps( "Sfont af" );
                                        altchar = True;
                                        }
                                break;

                        default:
                                fprintf( stderr, "%s %c\n", badescerr, buf[bufind]
                                printf( "\n%% %s\n", baiescerr );

                        }
                if ( ! hhspace )
                        lastcommand = buf[bufind];
                else
                        hhspace = False;
                }
        return 'g';                                     /* anything other than EOF
}

doreturn( tmp )
char *tmp;
{
```

```
        if ( underline )
                {
                printps( "ou" );
                printps( tmp );
                printps( "u" );
                }
        else
                printps( tmp );
}

escapeit( ch )
char ch;
{

        /* check to see if char already added to string */
        if ( psi < 0 )
                {
                psi = 0;
                return;
                }

        /* escape any special postscript chars */
        if ( ch == '(' || ch == ')' || ch == '\\' )
                {
                psstring[psi] = '\\';
                psi++;
                }
        psstring[psi] = ch;
        psi++;
}

finishstring()
{
        static char tmp[MAXLINE];
        int i;
        double spacewidth;
        static double oldwidth=0;

    /* construct postscript character string */
        if ( psi > 0 )
                {
                psstring[psi] = '\0';
                psi = 0;
                sprintf( tmp, "(%s) s", psstring );
                printps( tmp );
                oldwidth = 0;
                }
        else if ( oldwidth != totalwidth )
                {
                if ( proportional )
                        spacewidth = (totalwidth/cpi)*PPI;
                else
                        spacewidth = totalwidth*fontsize;
```

```
                sprintf( tmp, "%.1f sp", spacewidth );
                printps( tmp );
                oldwidth = totalwidth;
                }
}

get_alt( ch )
char ch;
{

        /* check to see if char already added to string */
        if ( psi < 0 )
                {
                psi = 0;
                return;
                }

        /* fill in alternate character array */
        if ( altch[0] == 0 )
                {
                init_alt();
                }

        /* get octal Symbol-font value for character */
        if ( altch[ch] == 0 )
                fprintf( stderr,
                "getalt: char '%d' has no symbol value\n", ch );
        else
                {
                /* add to current string */
                sprintf( &(psstring[psi]), "\\%03d", altch[ch] );
                psi = psi + 4;
                }
}

char getch()
{
        int *ptr, *max, fread();

        if ( scanning )
                {
                ptr = &scanind;
                max = &scancount;
                }
        else
                {
                ptr = &bufind;
                max = &bufcount;
                }

        (*ptr)++;
        if ( *ptr >= *max )
```

```
                {
                if ( curmax > MAXBUF )
                        {
                        if ( *max > MAXBUF )
                                {
                                /* read into 1st buffer */
                                curmax = fread( buf, 1, MAXBUF, fp );
                                if ( curmax == 0 )
                                        return -1;
                                *ptr = 0;
                                }
                        }
                else
                        {
                        if ( *max > MAXBUF )
                                {
                                /* section 1 already read in */
                                *ptr = 0;
                                }
                        else
                                {
                                /* read in 2nd buffer */
                                curmax = MAXBUF + fread( &buf[MAXBUF], 1, MAXBUF,
                                if ( curmax == MAXBUF )
                                        return -1;
                                }
                        }
                *max = curmax;
                }

        return buf[*ptr];
}

getscale()
{
        int j, k, atoi();
        char hspace[4];

        /* next 3 chars are the amount to horiz move by  */
        hscale = 0;
        k = 0;
        for ( j = 0; j < 3; j++ )
                {
                hspace[k] = getch();
                if ( hspace[k] == -1 )
                        break;

                if ( ( k == 0 && hspace[k] != '0' ) || ( k != 0 ) )
                        k++;
                }
```

```
        if ( k != 0 && j == 3 )
                {
                hspace[k] = '\0';
                hscale = atoi( hspace );
                }
}

char header()
{
        FILE *mfp;
        char getch(), c, tmp[MAXLINE];
        int atoi(), ic;
        double atof();
        char *getenv(), *macfile=tmp;
        int i, ii, value, fscanf(), strlen();

        /* initialize the postscript output buffer */
        psbuf[0] = '\0';

        /* try to get the starting sequence of the document, ctrl-S */
        if ( ((c = getch()) < 0)  || (c != ESC) )
                return False;

        if ( ((c = getch()) < 0)  || (c != START_DOC) )
                return False;

        if ( getch() < 0 )
                {
                fprintf(stderr, "header: cpi needs to follow start char\n" );
                return False;
                }

        /* get character per inch number from buf, the format is
         * the ascii rep of the number followed by an excl point */
         fontsize = atoi( &buf[bufind] );
         while ( (c = getch()) > 0 && c != ' ' && c != '!' ) ;
         if ( c == ' ' )
                {
                cpi = atoi( &buf[bufind] );
                while ( (c = getch()) > 0 && c != '!' );
                }
         if ( c != '!' )
                {
                fprintf(stderr, "header: exclam pt needs to follow cpi in header\
                return False;
                }

        /* output the fontsize */
        printf( "/fontsize %.0f def\n", fontsize );

        /* find out if document is proportionally spaced */
        if ( ((c = getch()) < 0) || (c == '!') )
                {
```

```
        fprintf( stderr,
        "header: proportional flag needs to follow cpi in header\n" );
        return False;
        }

switch ( c )
        {
        case '0':
                proportional = False;
                if ( ((c = getch()) < 0) || (c != '!') )
                        {
                        fprintf( stderr,
                        "header: excl pt doesn't follow proport flag in he
                        return False;
                        }
                if ( ((c = getch()) > 0)  )
                        {
                        ptsize = atof( &buf[bufind] );
                        while ( (c = getch()) > 0 && c != '!' );
                        }
                if ( c != '!' )
                        {
                        fprintf(stderr,
                        "header: exclam pt needs to follow ptsize in heade
                        return False;
                        }
                break;
        case '1':
                proportional = True;
                if ( ((c = getch()) < 0) || (c != '!') )
                        {
                        fprintf( stderr,
                        "header: excl pt needs to follow prop flag in heade
                        return False;
                        }
                break;
        default:
                fprintf( stderr,
                "header: proportional flag needs to follow cpi in header\n"
                return False;
        }


/* get word11-postscript macro filename from header */
macdir = getenv( "W11PS_MAC");
if ( macdir == '\0' )
        macdir = DEFAULT_W11PS_MAC;

strcpy( macfile, macdir );
ii = strlen( macfile );
```

```
/* copy common macros */
strcpy( macfile+ii, "mac11.all" );
mfp = fopen(macfile, "r");
if (mfp == NULL)
        {
        fprintf( stderr, "word2ps: can't open w11 macro file %s\n", macfil
        exit(1);
        }
while ( (ic=getc(mfp)) != EOF )
        putchar( ic );
fclose(mfp);

/* copy font macros */
i = ii + 6;
while ( (c = getch()) > 0 && c != '!' )
        {
        macfile[i] = c;
        i++;
        }
macfile[i] = '\0';
mfp = fopen(macfile, "r");
if (mfp == NULL)
        {
        fprintf( stderr, "word2ps: can't open w11 macro file %s\n", macfil
        exit(1);
        }
while ( (ic=getc(mfp)) != EOF )
        putchar( ic );
fclose(mfp);

printps( "id" );

/* if prop font then read in char width table */
if ( proportional )
        {
        strcpy( macfile+ii, "wid11.");
        i = strlen( macfile );
        while ( (c = getch()) > 0 && c != '!' )
                {
                macfile[i] = c;
                i++;
                }
        macfile[i] = '\0';
        mfp = fopen(macfile, "r");
        if (mfp == NULL)
                {
                fprintf(stderr, "word2ps: can't open width file %s\n", mac
                exit(1);
                }

        /* fill in alternate character array */
        while ( fscanf( mfp, "%d %d", &i, &value ) != EOF )
                width[i] = value;
```

```
                fclose(mfp);
                }

        return True;
}

init_alt()
{
        FILE *mfp;
        char tmp[MAXLINE];
        int i, value, fscanf();
        char *macfile=tmp;

        strcpy( macfile, macdir );
        strcat( macfile, "symbol.oct" );
        mfp = fopen(macfile, "r");
        if (mfp == NULL)
                {
                fprintf(stderr, "word2ps: can't open symbol oct file %s\n", macfi
                exit(1);
                }

        /* fill in alternate character array */
        while ( fscanf( mfp, "%d %d %s", &i, &value, macfile ) != EOF )
                altch[i] = value;

        fclose(mfp);
}

char nextch()
{
        char ch, getch();
        int i;


        scanind = bufind;
        scancount = bufcount;
        scanning = True;

        /* search ahead for next true char, ignoring all else */
        while ( scanning )
                {
                ch = getch();
                switch( ch )
                        {
                        case -1:
                                scanning = False;
                                break;

                        case ESC:
                                if ( getch() == B_SPACE )
                                        for ( i=0; i<3; i++ )
                                                getch();
                                break;
```

```
                        case FF:
                        case LF:
                                cn = -1;
                                scanning = False;
                                break;

                        default:
                                if ( ch <= 126 && ch >= 32 )
                                        scanning = False;
                                break;
                        }
                }

        return ch;
}


printps( tmp )
char *tmp;
{
        int strlen();

        if ( strlen(psbuf) + strlen(tmp) + 1 >= MAXLINE )
                {
                puts( psbuf );
                psbuf[0] = '\0';
                }
        else
                strcat( psbuf, " " );

        strcat( psbuf, tmp );
}

char morepages()
{

        char ch, nextch(), getch(), doescape();
        char *strerr = "word2ps: premature string end\n";
        static char tmp[MAXLINE];
        int j;

        printps( "ip" );
        for(;;)
                {
                /* get next char from word11 file */
                if ( getch() < 0 )
                        return EOF;
```

```
switch( buf[bufind] )
        {
        case ESC:
                if ( doescape() == EOF )
                        return EOF;
                break;

        case LF:
                reset();
                doreturn( "r" );
                break;

        case FF:
                reset();
                return FF;

        default:
                {
                if ( buf[bufind] > 126 || buf[bufind] < 32 )
                        break;

                if ( buf[bufind] != ' ' )
                        {
                        /* print out spacing  */
                        if ( psi <= 0 && lastchar == ' ' )
                                finishstring();

                        /* add char to string */
                        if ( altchar )
                                get_alt( buf[bufind] );
                        else
                                escapeit( buf[bufind] );
                        }

                /* if first space then output previous string */
                else
                        {
                        if ( psi > 0 )
                                finishstring();
                        }

                /* find next char and it's scale */
                lasthscale = hscale;
                lastchar = buf[bufind];
                ch = nextch();
                if ( ch != -1 )         /* at end of line */
                        {
                        if ( proportional )
                                deltac = lasthscale +
                                        (width[lastchar] - width[cr
                        else
                                deltac = ptsize;
                        totalwidth = totalwidth + deltac;
                        }
```

```
                                        /* DEBUG:
                                         *printf(" lastchar:'%c', ch:'%d', hscale:%.0f,
                                         *total:%.1f, ltotal:%.1f\n", lastchar, ch, last
                                         *totalwidth, (totalwidth/cpi)*PPI);
                                         */
                                        }

                                }
                        }

}

reset()
{
        /* if there are chars, print them */
        if ( psi > 0 )
                finishstring();

        totalwidth = 0;
        lasthscale = 0;
        psi = 0;
}
```

APPENDIX C

This is the changed WORD2PS program (word2ps.l). Notice the changes that have been made in morepages() and the added routines, input and cleanup. In the first section you can find the lex program.

```
%{
#include word2ps.h
%}

LF                  \Ø12
FF                  \Ø14
ESC                 \Ø33
START_DOC           S
END_DOC             E
MANUAL_FEED         M
HALF_LINE           D
ON_BOLD             Q
OFF_BOLD            R
ON_UNDERLINE        I
OFF_UNDERLINE       J
H_SPACE             H
ON_SUPER            T
OFF_SUPER           U
ON_SUB              V
OFF_SUB             W
DOWN                D
OFF_ALTCHAR         #
ON_ALTCHAR          X

%START DE
%%

    char getch(), nextch(), ch;
    char *boldofferr = "word2ps: bold, off found when not on\n";
    char *boldonerr = "word2ps: bold, on found when already on\n";
    char *undofferr = "word2ps: underline, off found without on\n";
    char *supofferr = "word2ps: off-super, not superscripting\n";
    char *subofferr = "word2ps: off-sub, not subscripting\n";
    char *badescerr = "% word2ps: bad escape char";
    char *strerr = "word2ps:  premature string end\n";
    static char tmp[MAXLINE];
    int j;


{ESC}   {
        BEGIN ( DE );
        }

{LF}    {
        reset();
        doreturn( "r" );
        return ( NOT_EOF );
        }

{FF}    {
        reset();
        return ( FF );
        }
```

```
.|\n    {
        if ( buf[bufind] > 126 || buf[bufind] < 32 )
            break;

        if ( buf[bufind] != ' ' )
            {
            /* print out spacing  */
            if ( psi <= 0 && lastchar == ' ' )
                finishstring();

            /* add char to string */
            if ( altchar )
                get_alt( buf[bufind] );
            else
                escapeit( buf[bufind] );
            }

        /* if first space then output previous string */
        else
            {
            if ( psi > 0 )
                finishstring();
            }

        /* find next char and it's scale */

        lastnscale = nscale;
        lastchar = buf[bufind];
        ch = nextch();

        if ( ch != -1 )          /* at end of line */
            {

            if ( proportional )
                deltac = lasthscale + (widtn[lastchar] - widtn[ch]) / 2.0;

            else
                deltac = ptsize;
                totalwidth = totalwidth + deltac;
            }

        }

<DE>{MANUAL_FEED}    {
                     printps( "mf" );
                     cleanup();
                     BEGIN ( 0 );
                     }

<DE>{ON_BOLD}        {
                     /* start a for loop to be repeated once */
                     if ( boldface )              /* already boldfacing */
```

```
                    {
                    fprintf( stderr, boldonerr );
                    printf( "\n%% %s", boldonerr );
                    }

                else if ( altchar )
                    printps( "SBfont bd" );

                else if ( italics )
                    printps( "IBfont bd" );

                else
                    printps( "Bfont bd" );

                boldface = True;
                cleanup();
                BEGIN ( 0 );
                }

<DE>{OFF_BOLD}  {
                /* end for-loop to do bold face */
                if ( ! boldface )
                    {
                    fprintf( stderr, boldofferr );
                    printf( "\n%% %s", boldofferr );
                    }

                else
                    {
                    if ( lastcommand == ON_ALTCHAR )
                        printps( "/prealtfont preboldfont def" );

                    printps( "obd" );
                    boldface = False;
                    }

                cleanup();
                BEGIN ( 0 );
                }

<DE>{HALF_LINE} {
                doreturn( "sr" );

                /* get rid of next line feed */
                if ( getch() != LF )
                   bufind--;

                cleanup();
                BEGIN ( 0 );
                }
```

```
<DE>{END_DOC}          {
                       /* last char in file */
                       return (EOF);
                       }

<DE>{ON_UNDERLINE}     {
                       underline = True;

                       if ( italics )
                           printps( boliface ? "IBfont itf" : "Ifont itf" );

                       else
                           printps( "u" );

                       cleanup();
                       BEGIN ( 0 );
                       }

<DE>{OFF_UNDERLINE}    {
                       if ( ! underline )
                           {
                           fprintf( stderr, undofferr );
                           printf( "\n%% %s", undofferr );
                           }

                       else if ( italics )
                           printps( "oitf" );

                       else
                           printps("ou" );

                       underline = False;
                       cleanup();
                       BEGIN ( 0 );
                       }

<DE>{H_SPACE}          {
                       /* next 3 chars are the amount to horiz move by  */
                       nhspace = True;
                       getscale();
                       cleanup();
                       BEGIN ( 0 );
                       }

<DE>{ON_SUPER}         {
                       /* turn superscript toggle on */
                       if ( ! superscript )
                           {
                           superscript = True;
                           printps( "p" );
                           }
```

```
                    cleanup();
                    BEGIN ( Ø );
                    }

<DE>{OFF_SUPER}     {
                    /* turn superscript off */
                    if ( ! superscript )
                        {
                        fprintf( stderr, supofferr );
                        printf( "\n%% %s", supofferr );
                        }

                    else
                        {
                        if ( lastcommand == ON_ALTCHAR )
                            printps( "/prealtfont presmfont def" );
                        printps( "op" );
                        superscript = False;
                        }

                    cleanup();
                    BEGIN ( Ø );
                    }

<DE>{ON_SUB}        {
                    /* turn subscript toggle on */
                    if ( ! subscript )
                        {
                        subscript = True;
                        printps( "b" );
                        }

                    cleanup();
                    BEGIN ( Ø );
                    }

<DE>{OFF_SUB}       {
                    /* subscript off */
                    if ( ! subscript )
                        {
                        fprintf( stderr, subofferr );
                        printf( "\n%% %s", subofferr );
                        }

                    else
                        {
                        if ( lastcommand == ON_ALTCHAR )
                            printps( "/prealtfont presmfont def" );

                        printps( "ob" );
                        subscript = False;
                        }
```

```
                        cleanup();
                        BEGIN ( 0 );
                        }

<DE>{OFF_ALTCHAR}       {
                        if ( altchar )
                            {
                            printps( "oaf" );
                            altchar = False;
                            }

                        cleanup();
                        BEGIN ( 0 );
                        }

<DE>{ON_ALTCHAR}        {
                        /* change print wheel, greek = symbol font? */
                        if ( ! altchar )
                            {
                            printps( boldface ? "SBfont af" : "Sfont af" );
                            altchar = True;
                            }

                        cleanup();
                        BEGIN ( 0 );
                        }

<DE>.|\n                {
                        fprintf( stderr, "%s %c\n", badescerr, buf[bufind] );
                        printf( "\n%% %s\n", badescerr );

                        cleanup();
                        BEGIN ( 0 );
                        }

%%

main(ac, av)
char **av;
    {
    char *name, morepages(), header();

    name = "";
    ac--; av++;
    while (ac) {
        if (av[0][0] == '-')
            {
            switch (av[0][1])
                {
                case '\0':
                    name = av[0];
                    break;
```

```
                case 'i':
                    italics = True;
                    break;
                default:
                    fprintf(stderr, usage);
                    exit(1);
                }
            }

        else
            {
            name = av[0];
            }
        ac--; av++;
        }

    fp = fopen(name, "r+");
    if (fp == NULL)
        {
        fprintf( stderr, "word2ps: can't open w11 file %s\n", name);
        exit(1);
        }

    if ( ! header() )
        fprintf( stderr, "word2ps: bad header in word11 print image file\n");
    else
        {
        reset();
        while ( morepages() != EOF )
            printps( "showpage" );

        /* end the document */
        printps( "ed" );
        if ( psbuf[0] != '\0' )
            puts( psbuf );
        }

    fclose(fp);
    }


doreturn( tmp )
char *tmp;
    {

    if ( underline )
        {
        printps( "ou" );
        printps( tmp );
        printps( "u" );
        }
    else
        printps( tmp );
    }
```

```
escapeit( ch )
cnar ch;
    {

    /* cneck to see if char already added to string */
    if ( psi < 0 )
        {
        psi = 0;
        return;
        }

    /* escape any special postscript chars */
    if ( ch == '(' || ch == ')' || ch == '\\' )
        {
        psstring[psi] = '\\';
        psi++;
        }
    psstring[psi] = ch;
    psi++;
    }


rinisnstring()
    {
    static cnar tmp[MAXLINE];
    int i;
    double spacewidtn;
    static double oldwidth=0;

    /* construct postscript character string */
    if ( psi > 0 )
        {
        psstring[psi] = '\0';
        psi = 0;
        sprintf( tmp, "(%s) s", psstring );
        printps( tmp );
        oldwidth = 0;
        }
    else if ( oldwidth != totalwidth )
        {
        if ( proportional )
            spacewidth = (totalwidth/cpi)*PPI;
        else
            spacewidth = totalwidth*fontsize;
        sprintf( tmp, "%.1f sp", spacewidth );
        printps( tmp );
        oldwidth = totalwidth;
        }
    }
```

```
get_alt( ch )
char ch;
    {

    /* check to see if char already added to string */
    if ( psi < 0 )
        {
        psi = 0;
        return;
        }

    /* fill in alternate character array */
    if ( altch[0] == 0 )
        {
        init_alt();
        }

    /* get octal Symbol-font value for character */
    if ( altch[ch] == 0 )
        fprintf( stderr, "getalt: char '%d' has no symbol value\n", ch );
    else
        {
        /* add to current string */
        sprintf( &(psstring[psi]), "\\%03d", altch[ch] );
        psi = psi + 4;
        }
    }


char getch()
    {
    int *ptr, *max, fread();

    if ( scanning )
        {
        ptr = &scanind;
        max = &scancount;
        }
    else
        {
        ptr = &bufind;
        max = &bufcount;
        }

    (*ptr)++;
    if ( *ptr >= *max )
        {
        if ( curmax > MAXBUF )
            {
            if ( *max > MAXBUF )
                {
                /* read into 1st buffer */
                curmax = fread( buf, 1, MAXBUF, fp );
```

```
                if ( curmax == 0 )
                    return -1;
                *ptr = 0;
                }
            }
        else
            {
            if ( *max > MAXBUF )
                {
                /* section 1 already read in */
                *ptr = 0;
                }
            else
                {
                /* read in 2nd buffer */
                curmax = MAXBUF + fread( &buf[MAXBUF], 1, MAXBUF, fp );
                if ( curmax == MAXBUF )
                    return -1;
                }
            }
        *max = curmax;
        }

    return buf[*ptr];
    }


getscale()
    {
    int j, k, atoi();
    char hspace[4];

    /* next 3 chars are the amount to horiz move by  */
    hscale = 0;
    k = 0;
    for ( j = 0; j < 3; j++ )
        {
        hspace[k] = getch();
        if ( hspace[k] == -1 )
            break;

        if ( ( k == 0 && hspace[k] != '0' ) || ( k != 0 ) )
            k++;
        }

    if ( k != 0 && j == 3 )
        {
        hspace[k] = '\0';
        hscale = atoi( hspace );
        }
    }
```

```
char header()
    {
    FILE *mfp;
    char getch(), c, tmp[MAXLINE];
    int atoi(), ic;
    double atof();
    char *getenv(), *macfile=tmp;
    int i, ii, value, fscanf(), strlen();

    /* initialize the postscript output buffer */
    psbuf[0] = '\0';

    /* try to get the starting sequence of the document, ctrl-S */
    if ( ((c = getch()) < 0)  || (c != ESC) )
        return False;

    if ( ((c = getch()) < 0)  || (c != START_DOC) )
        return False;

    if ( getch() < 0 )
        {
        fprintf(stderr, "header: cpi needs to follow start char\n" );
        return False;
        }

    /* get character per inch number from buf, the format is
     * the ascii rep of the number followed by an excl point */

    fontsize = atoi( &buf[bufind] );
    while ( (c = getch()) > 0 && c != ' ' && c != '!' ) ;
    if ( c == ' ' )
        {
        cpi = atoi( &buf[bufind] );
        while ( (c = getch()) > 0 && c != '!' );
        }

    if ( c != '!' )
        {
        fprintf(stderr, "header: exclam pt needs to follow cpi in header\n" );
        return False;
        }

    /* output the fontsize */
    printf( "/fontsize %.0f def\n", fontsize );

    /* find out if document is proportionally spaced */
    if ( ((c = getch()) < 0) || (c == '!') )
        {
        fprintf( stderr,
        "header: proportional flag needs to follow cpi in header\n" );
        return False;
        }
```

```
switch ( c )
    {
    case '0':
        proportional = False;
        if ( ((c = getch()) < 0) || (c != '!') )
            {
            fprintf( stderr,
            "header: excl pt doesn't follow proport flag in header\n" );
            return False;
            }

        if ( ((c = getch()) > 0)  )
            {
            ptsize = atof( &buf[bufini] );
            while ( (c = getch()) > 0 && c != '!' );
            }

        if ( c != '!' )
            {
            fprintf(stderr,
            "header: exclam pt needs to follow ptsize in header\n" );
            return False;
            }
        break;

    case '1':
        proportional = True;
        if ( ((c = getch()) < 0) || (c != '!') )
            {
            fprintf( stderr,
            "header: excl pt needs to follow prop flag in header\n" );
            return False;
            }
        break;

    default:
        fprintf( stderr,
        "header: proportional flag needs to follow cpi in header\n" );
        return False;
    }

/* get word11-postscript macro filename from header */
macdir = getenv( "W11PS_MAC");
if ( macdir == '\0' )
    macdir = DEFAULT_W11PS_MAC;

strcpy( macfile, macdir );
ii = strlen( macfile );

/* copy common macros */
strcpy( macfile+ii, "mac11.all" );
mfp = fopen(macfile, "r");
```

```
if (mfp == NULL)
    {
    fprintf( stderr, "word2ps: can't open wi1 macro file %s\n", macfile );
    exit(1);
    }

while ( (ic=getc(mfp)) != EOF )
    putchar( ic );

fclose(mfp);

/* copy font macros */
i = ii + 6;
while ( (c = getch()) > 0 && c != '!' )
    {
    macfile[i] = c;
    i++;
    }

macfile[i] = '\0';
mfp = fopen(macfile, "r");
if (mfp == NULL)
    {
    fprintf( stderr, "word2ps: can't open wi1 macro file %s\n", macfile );
    exit(1);
    }
while ( (ic=getc(mfp)) != EOF )
    putchar( ic );
fclose(mfp);

printps( "id" );

/* if prop font then read in char width table */
if ( proportional )
    {
    strcpy( macfile+ii, "wid11.");
    i = strlen( macfile );
    while ( (c = getch()) > 0 && c != '!' )
        {
        macfile[i] = c;
        i++;
        }
    macfile[i] = '\0';
    mfp = fopen(macfile, "r");
    if (mfp == NULL)
        {
        fprintf(stderr, "word2ps: can't open width file %s\n", macfile);
        exit(1);
        }
```

```
        /* fill in alternate character array */
        while ( fscanf( mfp, "%d %d", &i, &value ) != EOF )
            width[i] = value;
        fclose(mfp);
        }

    return True;
    }


init_alt()
    {
    FILE *mfp;
    char tmp[MAXLINE];
    int i, value, fscanf();
    char *macfile=tmp;

    strcpy( macfile, macdir );
    strcat( macfile, "symbol.oct" );
    mfp = fopen(macfile, "r");
    if (mfp == NULL)
        {
        fprintf(stderr, "word2ps: can't open symbol oct file %s\n", macfile);
        exit(1);
        }

    /* fill in alternate character array */
    while ( fscanf( mfp, "%d %d %s", &i, &value, macfile ) != EOF )
        altch[i] = value;

    fclose(mfp);
    }


input()

    {
    char *escerr = "word2ps: no char following escape char\n";

    if ( getch() < 0 )
        {
        fprintf(stderr, escerr );
        printf( "\n%% %s", escerr );
        return ( EOF );
        }

    else
        {
        /* check if string needs to be finished */
        if ( buf[bufind] != H_SPACE  )
            finishstring();
```

```
        return ( buf[bufind] );
        }
    }


cnar nextch()
    {
    cnar ch, getch();
    int i;

    scanind = bufind;
    scancount = bufcount;
    scanning = True;

    /* search ahead for next true char, ignoring all else */
    wnile ( scanning )
        {
        ch = getch();
        switch( ch )
            {
            case -1:
                scanning = False;
                break;

            case ESC:
                if ( getch() == H_SPACE )                    /* skip the 3 di
                    for ( i=0; i<3; i++ )
                        getch();
                break;

            case FF:

            case LF:
                ch = -1;
                scanning = False;
                break;

            default:
                if ( ch <= 126 && ch >= 32 )
                    scanning = False;
                break;
            }
        }

    return ch;
    }


printps( tmp )
cnar *tmp;
    {
    int strlen();
```

```
    if ( strlen(psbuf) + strlen(tmp) + 1 >= MAXLINE )
        {
        puts( psbuf );
        psbuf[0] = '\0';
        }
    else
        strcat( psbuf, " " );

    strcat( psbuf, tmp );
    }


char morepages()
    {
    char getch()

    printps( "ip" );
    for(;;)
        {
        /* get next char from wordll file */
        if ( getch() < 0 )
            return EOF;

        yylex();
        }
    }


cleanup()
    {
    if ( ! nnspace )
        lastcommand = buf[bufind];
    else
        nnspace = False;
    }


reset()
    {
    /* if there are chars, print them */
    if ( psi > 0 )
        finishstring();

    totalwidth = 0;
    lastnscale = 0;
    psi = 0;
    }
```

```
%i
#include <stdio.h>
#include <signal.h>
#include <setjmp.h>
#include <sgtty.h>
#include <sys/file.h>

#define False 0
#define True 0xFF
#define PPI 72.2

#ifdef  petvax
#define DEFAULT_W11PS_MAC "disk$tools:[tex.laserwrt]"
#else   petvax
#define DEFAULT_W11PS_MAC "disk$user1:[tab.src]"
#endif  petvax

#define RADICAL 326
#define RADICALEX 140

#define LF              '\012'
#define FF              '\014'
#define ESC             '\033'
#define START_DOC       'S'
#define END_DOC         'E'
#define MANUAL_FEED     'M'
#define HALF_LINE       'D'
#define ON_BOLD         'Q'
#define OFF_BOLD        'R'
#define ON_UNDERLINE    'I'
#define OFF_UNDERLINE   'J'
#define H_SPACE         'H'
#define ON_SUPER        'T'
#define OFF_SUPER       'U'
#define ON_SUB          'V'
#define OFF_SUB         'W'
#define DOWN            'D'
#define OFF_ALTCHAR     '#'
#define ON_ALTCHAR      'X'

#define MAXLINE 80
#define MAXBUF 512
#define MAXBUF2 1024

#define NOT_EOF (! EOF)
#undef input

char buf[MAXBUF2], lastchar=0;
int nnspace = False;
char lastcommand = OFF_ALTCHAR;
char psstring[MAXBUF];
char psbuf[MAXLINE];
char macdir_array[MAXLINE];
```

```
char *macdir = macdir_array;
double width[128];
int altch[128];
int bufind = MAXBUF2, bufcount = MAXBUF2, psi = 0;
int scanind = MAXBUF, scancount = MAXBUF, curmax = MAXBUF2;
double cpi;
double fontsize;
FILE *fp;
double lasthscale=0, deltac, totalwidth,  hscale, ptsize;

int underline = False;
int proportional = False;
int scanning = False;
int italics = False;
int boldface = False;
int superscript = False;
int subscript = False;
int altchar = False;

char usage[] = "usage: \"word2ps [-i] filename\"\n";
```

EFFECT OF THROMBOPOETIC SUBSTANCES ON MEGAKARYOCYTE METABOLISM

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Research Sponsor: Dr. Robert Leven
Biology and Medicine Division

One of the primary objectives of the current work at our laboratory is to investigate the effect of thrombopoetic substances on megakaryocyte metabolisim.

Megakaryocytes are large cells produced in the bone marrow which eventually mature into platelets. Platelets are circulating anucleate cells involved in thrombosis and hemostasis. In order to understand the control of platelet production, studies will be done with a source rich in thrombopoetin. Thrombopoetin is a poorly defined substance which is known to induce megakaryocytopoiesis. It has been shown that a fraction of plasma from thrombocytopenic rabbits (rabbits with a low platelet count) can stimulate thrombopoiesis in vivo. No direct effect of thrombopoetin on mature megakaryocytes has been previously demonstrated, therefore the experiments and data to follow are a significant contribution to basic knowledge in the mechanisms of platelet formation through their precursors: megakaryocytes.

There is little known about megakaryocytes due to the difficulty in obtaining sufficient quantities of cells for biochemical analysis. As this obstacle is overcome, the following areas are under investigation: megakaryocyte maturation, morphology, and metabolism. I have studied two major metabolic activities of megakaryocytes: Lactic Acid output by the cells and glucose uptake, both in presence of normal and thrombocytopenic rabbit plasma.

METHODS

The approach used to be able to analyse these metabolic activities involves the isolation of megakaryocytes by a process developed by Levine and Fedorko. The femur, tibia, and humerus are removed from a guinea pig, then cracked and opened to extract the bone marrow. The bone marrow is suspended in a balanced salt solution, ultracentrifuged on a density gradient and allowed to sit on unit velocity gradients which finally isolate megakaryocytes that are purified up to a 900 fold compared to the starting material. The cells are maintained in a liquid culture with Dublecos Modified Eagle Medium, antibiotics and calf serum for up to 48 hours with little loss of viability. After the cell suspension is prepared, throbocytopenic rabbit plasma (TRP) or normal rabbit plasma (NRP) are added to megakaryocyte cultures with an equal numbers of cells.

Lactic Acid Assay

This procedure is based on the production of NADH from NAD as lactate is converted into pyruvate by lactate dehydrogenase.

PYRUVIC ACID + NADH ---- LACTIC ACID + NAD

(high A340) (low A340)

To measure lactic acid, the reaction is carried out from right to left with excess NAD. To force the reaction to completion in this direction, it is necessary to trap formed pyruvic acid with hydrazine. The increased Absorbance at 340nm due to NADH formation becomes a measure of lactid acid orginally present.

Samples of megakaryocytes in culture media with TRP (5ul/ml) as well as a sample with NRP (5ul/ml), and a sample with no plasma (as control) are incubated for approximately 18 hours. These samples are then centrifuged to collect the supernatant without cells. Protein in the supernatant is removed by addition of twice the supernatant volume of 8% perchloric acid. The protein free sample (0.2ml) was added to 2.8ml of reaction mixture containing lactate dehydrogenase and NAD in glicine buffer. Samples are then incubated at 37.1^{o}C for 30 minutes followed by an OD340 absorbance reading with a narrow bandwidth spectrofotometor. Lactic acid values are expressed in mg/100 ml.

Protein Assay

The Bradford Protein assay is based on a change in OD reading due to the binding of coomasie blue dye to proteins present in the sample.

Pelleted cells from the lactic acid assay sample collection were diluted 10 to 50 fold to fall within the range of the protein standard curve. A dilution of the samples up to .8ml plus a blank with .8ml of water were vortexed with .2ml of the Dye Reagent Concentrate. After a period of 5 minutes the OD595 of samples were read. Results were expressed in ug protein/ml.

Glucose uptake

Isolated megakaryocytes are incubated at 10^5 cells/ml of liquid culture medium, plus 25ug of TRP or NRP. A control sample with no plasma is also incubated for the same time as the respective TRP or NRP samples. Just before incubation at 37.1^{o}C, 5uCi of ^{14}C glucose was added to each sample. At a given time point ranging from 30 min. up to 18 hours, the samples are filtered and rinsed with a balanced salt solution. Finally, the filter is prepared for scintillation counting. Results were given in counts/min.

RESULTS

The results of the assays were expressed as follows:

a) mglactate/ml media
b) mglactate/10^5 megakariocytes
c) mglactate/ug protein
d) uMol/min/mg protein

TEST 1

Megakaryocyte isolation: June 25, 1986
3 guinea pigs
Yield: 1.13 10^5 cells/ml; total of sample: 6.44 10^5 cells
2 dishes with 2 ml each (322,00 cells/dish)
Dish 1: 4ul NRP Dish 2: 4 ul TRP

Lactic acid assay:	Protein assay:
TRP: 19.46 mg/100ml	TRP: 32.57 ug
NRP: 17.77 mg/100ml	NRP: 24.55 ug
MEIDA:13.80 mg/100ml	

TEST 2

Megakaryocyte isolation: July 1, 1986
3 guinea pigs
Yield: total sample: 1,060,000 cells
3 dishes with 2ml each (360,000 cells/dish)
Dish 1: 5ulTRP Dish 2: 5ulNRP Dish 3: no plasma

Lactic acid assay	Protein assay
TRP: -	TRP: 60.95 ug
NRP: 36.00mg	NRP: 86.66 ug
MEDIA: 13.80 mg/100ml	

TEST 3

Megakaryocyte isolation: July 21, 1986
2 guinea pigs
Yield: Total sample: 993,600 cells
2 dishes with 1ml each (496,800 cells/dish)
Dish 1: 5ul TRP Dish 2: 5ul NRP

Lactic acid assay	Protein assay
TRP: 18.09 mg/100ml	TRP: 77.14 ug
NRP: 19.53 mg/100ml	NRP: 55.00 ug
MEDIA: 9.77 mg/100ml	

TEST 4

Megakaryocyte isolation: July 23, 1986
2 guinea pigs
Yield: Total sample: 1,038,800 cells
2 dishes with 1ml each (519,400 cells/dish)
Dish 1: 5 ul TRP Dish 2: 5ul NRP

Lactic acid assay	Protein assay
TRP: 48.23 mg/100ml	TRP: 13.51 ug
NRP: 46.22 mg/100ml	NRP: 28.60 ug

NOTE: Tests 1-3 were with 17 hour incubations and test 4 was incubated for 41 hours.

TABLE 1

mg lactate/ml media

SAMPLE	TEST 1	TEST 2	TEST 3	TEST 4	AVERAGE
TRP	.061	-	.083	.41	.072
NRP	.040	.222	.098	.39	.120

TABLE 2

mg/ lactate/10^5 Megakaryocytes

SAMPLE	TEST 1	TEST 2	TEST 3	TEST 4	AVERAGE
TRP	.0037	-	.0160	.0790	.0102
NRP	.0024	.0062	.0197	.0750	.0094

NOTE: Averages are calculated with tests 1 through 3 since test 4 was incubated 41 hours while the others were incubated 17 hours.

TABLE 3

mg lactate/mg protein

SAMPLE	TEST 1	TEST 2	TEST 3	TEST 4	AVERAGE
TRP	6.8	-	1.07	30.35	3.46
NRP	3.2	.51	1.78	13.68	1.63

THE EFFECTS OF IRRADIATION ON NORMAL KIDNEY TISSUE

Corliss Austin
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Research Sponsor: Dr. Adrian Rodriquez
Biology & Medicine Division

I. INTRODUCTION

In human cancer radiotherapy, the radiation dose administered is usually limited by the tolerance of normal tissue. The accelerated charged particles from the Berkeley Bevelac are being used for cancer radiotherapy.

Presently, I am contributing to various related projects in the area of radiobiology. We induce negative responses in the kidney, spinal cord, testes, and lung following irradiation. For example, irradiating the spinal cord of Sprague Dawley rats results in paralysis; mouse testes lose weight at approximately twenty-eight days following irradiation due to the death of spermatogonia; and fibrosis becomes apparent in the lungs. Tumor induction was enhanced in mice by implanting pituitary glands into their spleens.

While participating in the previously mentioned projects, I mastered specific surgical techniques. However, my major project deals with the normal tissue tolerance of the kidney. The kidney is and ideal organ to study as a test system for the evolution of the late effects of both single high doses and low repeated doses of ionizing radiation. It is particularly radiosensitive and has been found to limit radiation therapy in the abdomen (Hall, 1978). Information about the time course and severity of renal damage following heavy ion irradiation is of clinical and radio-biological importance.

Three functional elements can be separately examined in this

organ: (a) the functional status of both secretory and absorptive activity in the epithelial cells of the tubule; (b) The ultrafiltration of the plasma in the glomerular apparatus, which is ultimately measured in the filtration rate; and finally (c) The rate of perfusion of the kidney by plasma presenting itself for filtration in the glomerular apparatus.

We are studying the late effects in the mouse kidney after irradiation with single and fractionated doses of x-rays or heavy charged particles. Our methods for determining tissue damage and repair in the kidney are similar to those performed at the Grey Laboratory of Cancer Research at Middlesex, England. The data obtained may be extrapolated to human tissues and be of use to radiotherapists in the treatment of cancer patients. We will compare the relative biological effectiveness (RBE) of x-ray to heavy particles and observe the repair of radiation damage after fractionated doses.

II. Materials and Methods

LAF_1 female mice were irradiated at 11-13 weeks of age (weight range 18-25g). The Bevelac beams used were 670MeV/amu for neon and silicon plateau. The RBE's of these beams were compared to 250kVp x-rays for various weeks after irradiation. Collimation of the field was necessary to limit irradiation specifically to the kidney area.

The methods, which were developed by Williams and Kenekamp, Stewart et. al., and Alpen and Stewart include those for the

measurement of glomerular filtration rate and tubular secretory function. In addition, hematocrit values will be a measure of the anemia of renal insufficiency. The functional tests are run at eight to ten week intervals during the year following bilateral kidney irradiation.

III. ASSAYS

A. Isotope Clearance

1. ^{51}Cr–EDTA Clearance from Plasma. The method to estimate glomerular filtration rate is based on the rate of clearance of ^{51}Cr–EDTA (Ethylene–diamine–tetraacetate). EDTA is a physically inert substance which can be used as an inulin substitute for the estimation of glomerular filtration rate from the plasma. This label acts as inulin does in that it is filtered at the glomerular surface and is neither secreted nor reabsorbed in the tubules. Therfore, its rate of disappearance from the plasma is an estimate of glomerular filtration rate (GFR).

Twenty microcuries of ^{51}Cr–EDTA are injected intraperitoneally into the mouse, and forty minutes later a blood sample of approximately 0.7 ml is taken from the retro–orbital sinus. This sample is drawn into a heparinized capillary tube, and centrifuged for five minutes. The radioactivity in 20 ul of plasma was counted for ten minutes with a TM Analytic Gamma Counter. High residual ^{51}Cr levels in the plasma indicate a decrease in GFR

2. ^{131}I–Hippuran Clearance from Plasma. ^{131}I–Hippuran

is being used to measure the effective renal plasma flow (ERPF) in mice. Hippuran is actively secreted into the tubular fluid and is removed during single passage through the kidney. The renal plasma flow itself is an important measure of the vascular state of the kidney.

B. Uninary Frequency

A modification of the urinary frequency apparatus developed by Stewart, et. al., is being used to assess tubular function. Mice were placed in an apparatus consisting of five individual parallel cages with wire bar floors and separate food and water containers. Absorbent paper is slowly drawn beneath the cages at a constant speed. The number of urine spots in twenty-four hours correlates with total urine output. Urine output indicates the secretory and concentrating abilities of the kidney. The total number of urine spots per mouse were counted and the area of the spots determined. Ultraviolet light, which causes a bright yellow fluorescence of urine pigments, was used to enhance the visibility of the spots. The following parameters were used to analyze the urinary frequency data.

a. Frequency index, which is the mean number of spots for each dose group.
b. Mean frequency index as a function of dose.
c. Standard deviation.
d. Standard error of the mean.

C. Hematocrit

The hematocrit, which is the volume of red blood cells to plasma, has been shown to be more sensitive than the ^{51}Cr-EDTA clearance in detecting renal radiation damage. This assay is useful in detecting anemia. The effects of anemia probably arise due to changes outside of the vascular or glomerular endothelium. The ultimate purpose of this assay is to demonstrate the time relationship between the endothelial changes of glomerular damage and the onset of anemia.

IV. CONCLUSION

My major research project deals with the normal tissue tolerance of the kidney. The kidney response to irradiation is dose dependent. Urine frequency increases after irradiation; the red blood cell level decreases, resulting in anemia and, an increase in ^{51}Cr-EDTA clearance indicates the loss of glomerular filtration.

The RBE for x-rays is being compared to heavier particles, e.g., neon, silicon, etc. According to recent data, neon shows a higher RBE than silicon. The reason for the low RBE of silicon is unclear at this time.

The repair of the kidney is also dose-dependent. A decrease in urine output and an increase in ^{51}Cr-EDTA as irradiation doses decreases denotes renal repair.

During my CO-OP experience, I contributed to various projects which related to my major project. I have helped

irradiate the spinal cords of Sprague Dawley rats at the Bevelac. See figure III for a view of the apparatus used for dose administration. This irradiation resulted in paralysis.

While participating in various research projects, I have learned specific surgical techniques for the removal of vertebrae, kidney, liver, lungs, and testes.

In addition to the previous surgical techniques, I have assisted in the perfusing and suturing of mouse tissues, learned radiation safety techniques, general laboratory procedures, proper handling and care of laboratory animals, literature searching skills, cell colony staining techniques, and data storage and analysis procedures.

The Lawrence Berkeley Laboratory/Jackson State University CO-OP program has proven to be very rewarding. I was allowed the opportunity to work at a national laboratory with Patty Powers-Risius, Adrian Rodriguez, Kristina Kavanau, and Randy DeGuzman. My work was stimulating and I enjoyed the challenge of developing a greater awareness of radiobiology. I also gained a greater awareness of the roles of researchers and my desire to work in a health related field has become even stronger. Deepest gratitudes are rendered to God and to those persons who made it possible for me to gain such an exceptionally educational and self-rewarding experience.

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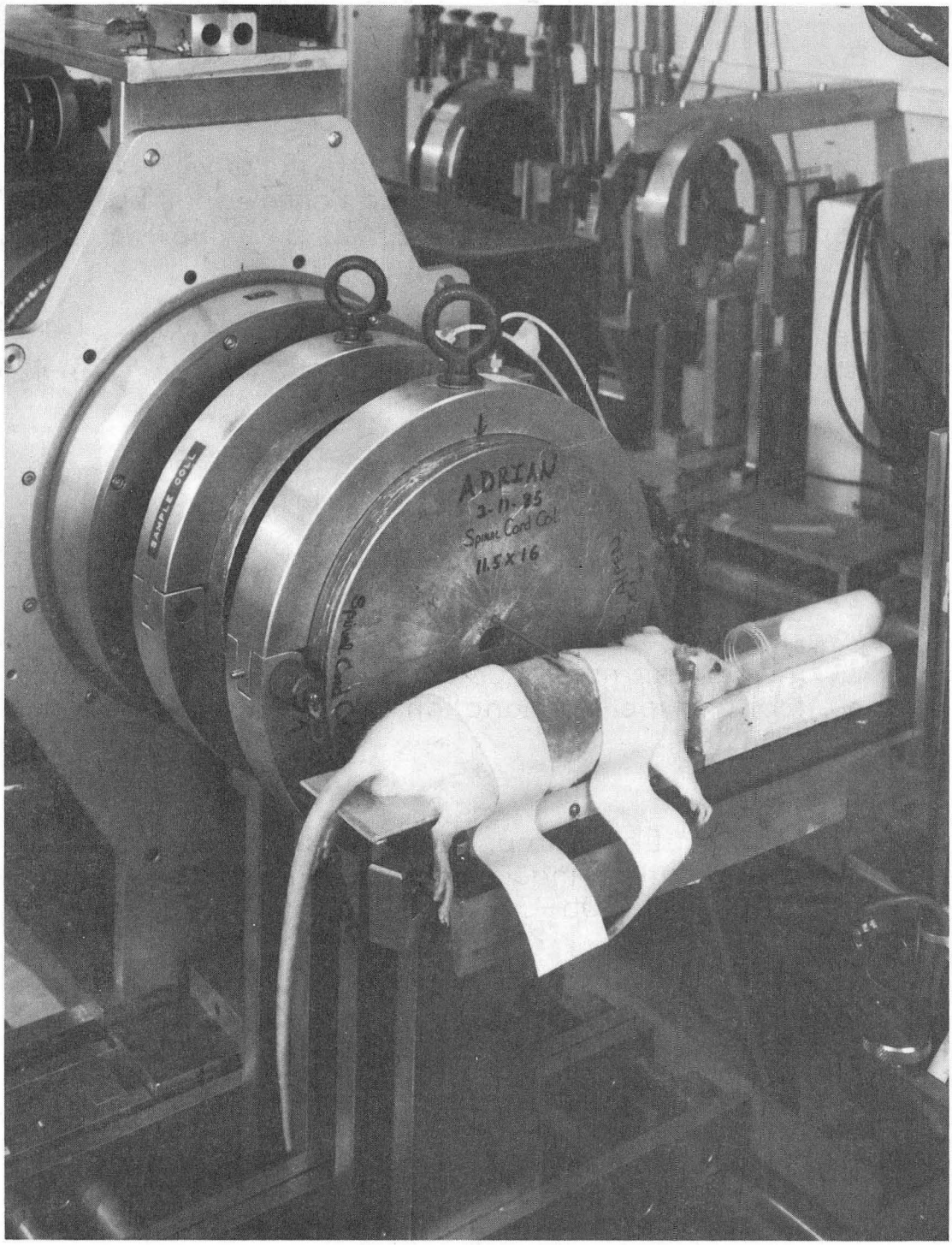

CBB 853-1819

Fig. III-9

ASPECTS OF RADIATION RESEARCH

Raquel B. Cobbins
Jackson State University

Research Sponsor: Dr. E. John Ainsworth
Biology & Medicine Division

ASPECTS OF RADIATION RESEARCH

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Biology & Medicine Division

Efforts to understand the beneficial and harmful effects of ionizing radiations have continuously increased, since the discovery of X-rays by William C. Roentgen in 1895. This discovery stimulated the scientific minds of many research scientists.Their efforts to predict the approximate hazards of exposure to radiation have, with time, been even more persistent. Due to increase in the world's interest in nuclear energy and nuclear devices, the need for these efforts has,also, expanded. The Radiation Biophysics Group of the Bio-Medical Division of Lawrence Berkeley Laboratory has been instrumental in the effort to satisfy this need. I have been able to be a part of this group through the Jackson State University/Lawrence Berkeley Laboratory Co-Op Program. This program has allowed me to participate in various research activities of this research group.

The Radiation Biophysics Group of the Laboratory is involved in an approach to understand the harmful effects of a wide array of ionizing radiations. The BEVALAC accelerator is the sole source of accelerated heavy particles, from protons to uranium ions. This group is the core of the worldwide effort to discover the undiscovered aspects of Ionizing Radiation.

Their research is,primarily, separated into five subjects of concentration: Physics of Interactions Between Fast Charged Particles and Matter, Molecular Studies, Cellular and Tumor Radiobiology, Tissue Effects of Heavy Charged Particle Beams, and Heavy-Ion Therapy and Related Physics and Biology.These subjects are of great concern to Bio-Medical Research today.The study of physical interactions between fast charged particles and matter is needed for the measurement and understanding of particle fields. On the molecular level, the manner in which ionizing radiations produce alterations in DNA is being investigated by using diffusion controlled free radical interactions. Through studies of cellular and tumor biology, it has been shown that heavy ions are more efficient than x-rays in producing cell transformation as well as cell lethality. Also, the study of tissue effects of heavy charged particle beams has been in demand. Due to the possibility of normal tissues not being able to be rectified after being in the path of the radiation therapy beam,dose that can be delivered during cancer radiation therapy is limited.Heavy-ion therapy, along with related physics and biology, has,also, achieved several awarding results through its' intense research.Treatment of ocular tumors and tumors adjacent to the spinal cord have,now, been improved. Radioactive beams have been used to accurately localize the Bragg peak of beams used in therapy. This method guarantees a significant improvement of our abilities to protect sensitive structures in patients that would be in the adjoining position of the beam. Each of these groups have yielded results that, presently, seem to be important to the advancement of bio-medical science.

The majority of my research exposure has been with the study of tissue effects of heavy charged particle beams. I have been under the direction of Dr. E.J. Ainsworth. Dr. Ainsworth has done intense studies on the consequences of acute heavy-ion injury to stem cells in bone marrow. He has reported that repair of heavy-ion injury seems to leave some permanent damage in the hemopoietic system. This is due to the lack of total recovery of the red corpuscle series. He is, also, in the process of finding short- and long-term lethal effects caused by exposures to charged heavy-ion particles. This project is called SKYHOOK.SKYHOOK was initiated in order to test the hypothesis that LET(linear energy transfer) was a sufficient predictor for life shortening and that low-energy charged particles with an LET approximately the same as that for fission neutrons would produce quantitatively similar life-shortening results after single or multi-fractionated doses. Another purpose was to study life shortening, using high doses, over a LET range from less than one to approximately two hundred keV's per micrometer. In pursuit of these goals, CB6F1 mice were given total-body irradiation of various charged particles. These exposures were done at the BEVELAC.

At the beginning of these studies, it was obvious that the effectiveness of the various particles was not known. The experiments were designed with the understanding that LET would have predictive value. The ultimate goal was to produce a data base that would permit the ultimate design of a minimum number of low-dose studies where the

goal is to determine the slope of the dose-response curve over a range of low doses that are relevant for radiation protection in terrestrial as well as space environments. The principal endpoint in the SKYHOOK experiment is excess mortality rate, days of life-span loss, and percent life shortening in relation to dose and other physical properties of the radiations studied. Mortality will be complete in all groups currently under study in approximately two years.This includes all unirradiated control animals.

I feel that this project has contributed greatly to the pursuit of understanding the effects of various ionizing radiations. The SKYHOOK project will achieve its' original goal of providing at least qualitative estimates of RBE(relative biological effectiveness) for life shortening after single and fractionated doses over a wide range of dose-averaged LETs. Upon the achievement of this goal, there will, hopefully, be new studies on leukemogenesis. The mouse model will be used to explore the effects of dose fractionation over a selected range of dose-averaged LETs. These topics of concern to the radiation researcher should be explored even more to ensure that any risks in the space environment will not be seriously underestimated.

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PERFORMANCE ANALYSIS OF ETHERNET

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Research Sponsor: Dr. Van Jacobson
Engineering Division
(Real Time Systems Group)

The Ethernet local area network provides a communication facility for high speed data exchange (e.g., electronic mail, file transfers, remote log-on) among computers and others digital devices located within a moderate-sized geographic area (a radius of 2.5 kilometers). The computers are physically connected by a shielded coaxial cable. Data is transmitted at a rate of 10 million bits per second. This kind of network can support up to 1024 computing devices.

Ethernet has been used extensively at Lawrence Berkeley Laboratory (LBL) to interconnect all of its major computing machines. LBL has two large incompatible local networks. One is DECNET, which comprises mostly Vaxes running the VMS operating system. These speak to each other via DECNET communication procedures (or protocol). The other is a network comprising mainly of Vaxes running UNIX, and they communicate with each other via the TCP/IP protocol. These two networks are connected via gateways and are incorporated into the Internetwork environment.

The basic LBL Ethernet has over 150 nodes and is growing rapidly. This makes it one of the larger local area networks. Although this kind of network is designed to support up to 1024 nodes, it has been found that as the number of devices increase there tends to be a significant decrease in performance. For example, as more traffic enters the network, the throughput decreases and users experience slower response times. It is this fact that gives rise to the need for an investigation into the characteristics of the network.

The object of this research is to study the characteristics of the network with a view to identifying those factors which tend to have a negative effect on performance. It is hoped that the results will serve as a basis for making recommendations to allow for more efficient utilization of the network.

Methodology

The data for this analysis is collected by a PDP-11/34 monitor. Data transmitted across the network is collected every tenth of a millisecond and stored on tape. The tape data is divide into several files. At the beginning of each hour the current file is closed and a new one is opened to record data for the next hour. The raw data is in binary form and has to be converted to ASCII before any analysis can be done. The information in each file is comprised of seven-field records with the following format :

(i) Destination address
(ii) Source address
(iii) Length of packet sent
(iv) Protocol type used
(v) Time since the hour
(vi) Time interval between end of packets
(vii) Status of the received packet

The data is then analysed in various ways with the aid of several Eunice data manipulating tools. These include :

(i) AWK - A programming language whose basic operation is to search files for patterns and to perform specified actions upon those lines or fields which contain instances of those patterns.

(ii) DM - A powerful data manipulating program which allows one to extract columns of data from a file, possibly based on conditions, and produce algebraic combinations of columns.

(iii) MKHIST - A tool which produces a histogram of any given data.

(iv) GRAPH - A tool which produces various graphical representations of data.

The task of analysing data may appear simple with the use of the above tools, but great care has to be taken when one is dealing with such large volumes of data. Factors such as storage space and CPU time are of major importance; therefore, one has to apply methods that will efficiently utilize these resources.

Among the various analyses performed on the data are frequency distribution of inter-arrival time (i.e.,the time interval between the arrival of one packet and the next) and packet length, quantile plot (a plot of the sorted data from which the median can be easily identified), and Fourier transform (a measure of the fluctuations in a time series by representing the data in terms of periodic functions). Much of the analysis at this stage is centered on conversations between hosts using the same protocol (e.g., communication between two DECNET hosts). Hosts are computers attached to a network. The purpose is to examine the effect of each protocol on network traffic

behavior. Conversations between pairs of DECNET hosts were extracted and studied. Likewise conversations between TCP/IP hosts. The following are some preliminary results.

The data is transmitted in packets of sizes ranging from 64 bytes to 1 kilobyte. Typically, the sender will transmit a packet of data and wait for a response from the receiver before sending another packet. Upon receiving the packet the receiver will send an acknowledgement (usually a 64 byte packet) indicating receipt of the sender's packet. If no acknowledgement is received after a fixed time interval, the sender will retransmit the data. The following are some interesting behavior exhibited by some of the network's hosts. These findings are based on various graphical interpretations of the data.

DECNET hosts tend to transmit data in very small packets, mostly between 64 and 120 bytes. According to the DECNET protocol, the maximum packet size that can be transmitted is 1.5 kilobytes.

TCP/IP hosts transmit significantly larger packets than their DECNET counterparts (packet size range from 64 to 930 bytes). The IP protocol allows a maximum packet size of 1.5 kilobytes. It appears that some of the IP hosts transmit two 1 kilobyte packets successively. The acknowledgement following seems to be one for each set of two data packets received. This is a feature of all the conversations involving printers. Conversations between two computers using this protocol show packet sizes similar to those used by DECNET hosts.

One particular interesting feature which occurred in several DECNET graphs was uni-directional data flow. There were a number of cases where data was being transmitted continously over a period of time and there was no apparent response from the destination node. Closer examination revealed that all these one-way conversations could be paired in such a way to show communication via intermediate nodes. The majority of these conversations involved the cluster of Vax 8600 computers. DECNET uses a point-to-point routing mechanism in which messages destined for a particular node, will be accepted by intermediate nodes along the network and then forwarded directly or indirectly to the final destination. This system is not evident in all the DECNET conversations as it is also possible to send messages directly to any node. The feature displayed by the 8600's is just a clear example of DECNET's routing method (store-and-forward technique).

Another interesting feature is the inter-arrival time of packets. The pattern shown by DECNET hosts indicates that the majority of packets are transmitted between 5ms and 20ms intervals. The arrival rate of IP packets seems to be more widely distributed (5 - 148ms).

Discussion

At this stage of the analysis some of our conclusions might be premature, however, some fair deductions can be made when the observed behavior is compared with our knowledge of

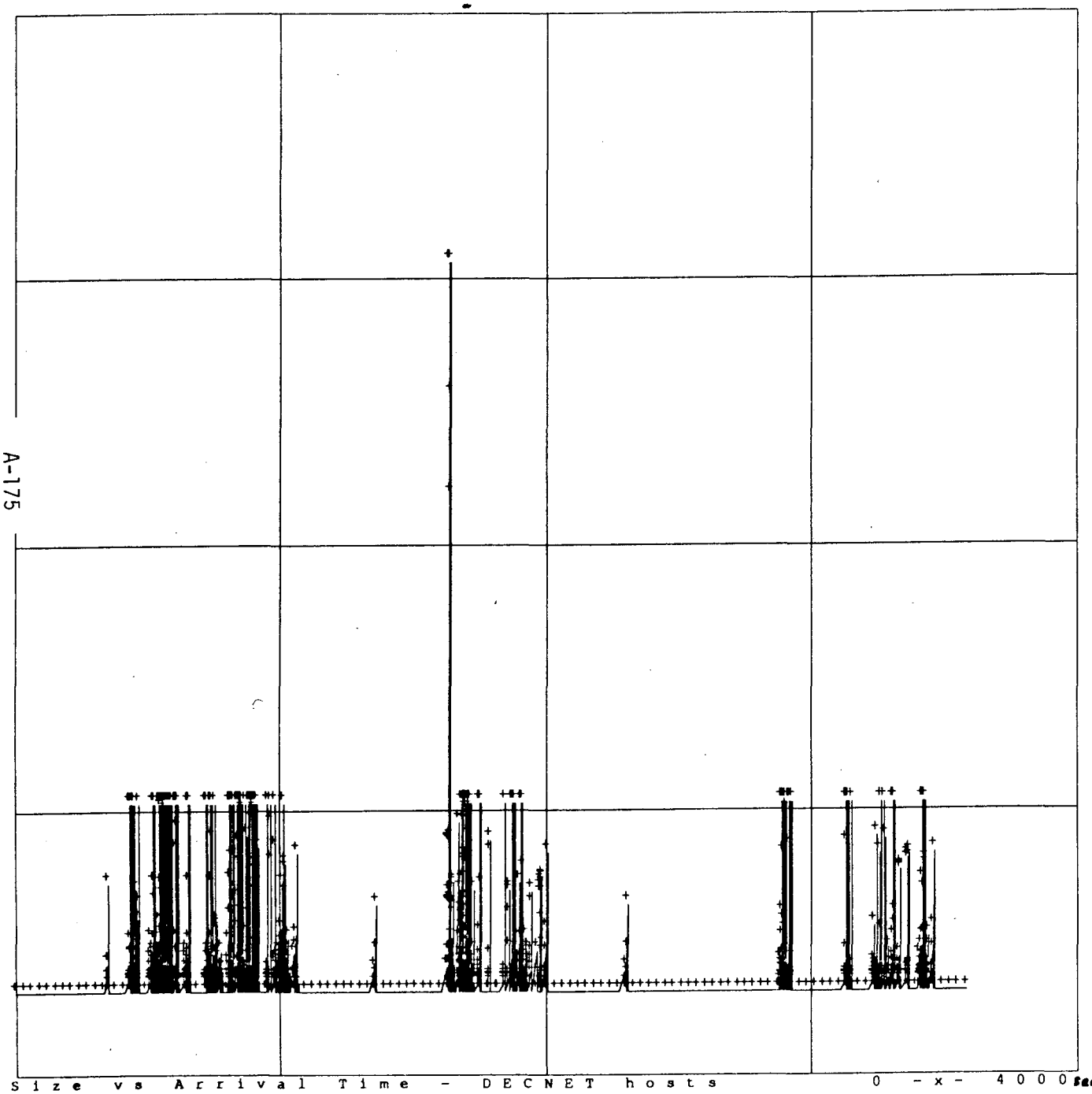
queuing theory and expected network traffic behavior. It is a fact that the larger the packet size, the greater the throughput as larger packets would result in less traffic along the network. It would therefore appear that the IP protocol, in some cases, allows for more efficient use of the network than does DECNET. However, the overhead incurred in breaking the message up into chunks might be an important contributing factor to the apparent slower arrival rate of IP packets.

As previously mentioned, the vast majority of packets sent by computers using either protocol was in the range of 64 to 120 bytes. This small packet size might be due to the fact that most of the network traffic data at the Laboratory is electronic mail data, which is usually sent in packets less than or equal to 120 bytes.The incidents of large packets are probably file transfers (as evident in the printer conversations).

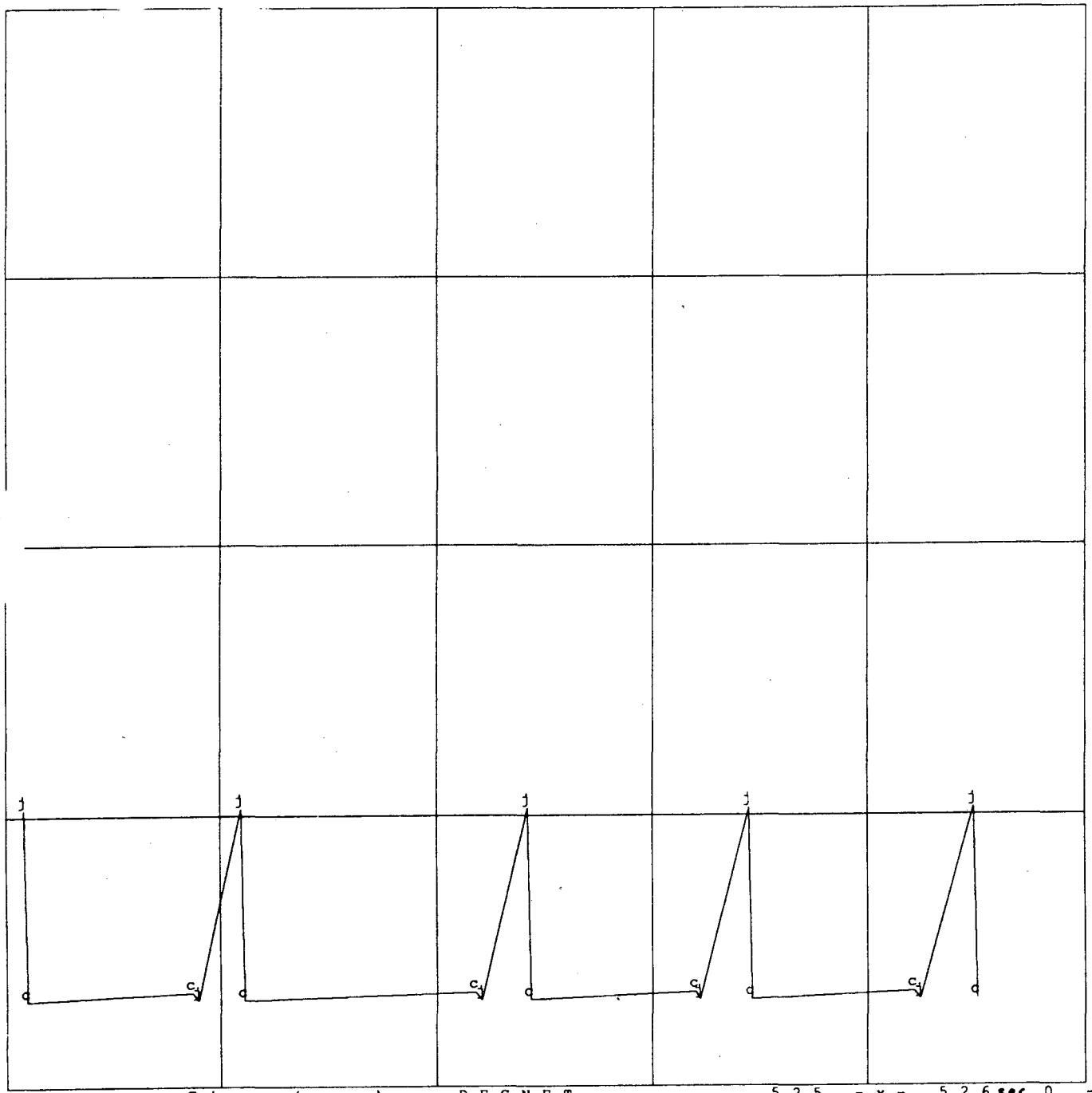
This investigation is still in progress. At this time the area of concentration is the response time. That is, the time interval between the time the packet was sent and its subsequent acknowledgement. It is evident that upon completion of this research some very interesting and worthwhile information on Ethernet performance will result.

APPENDIX

The following are some of the graphs produced from data collected over a period of one hour.

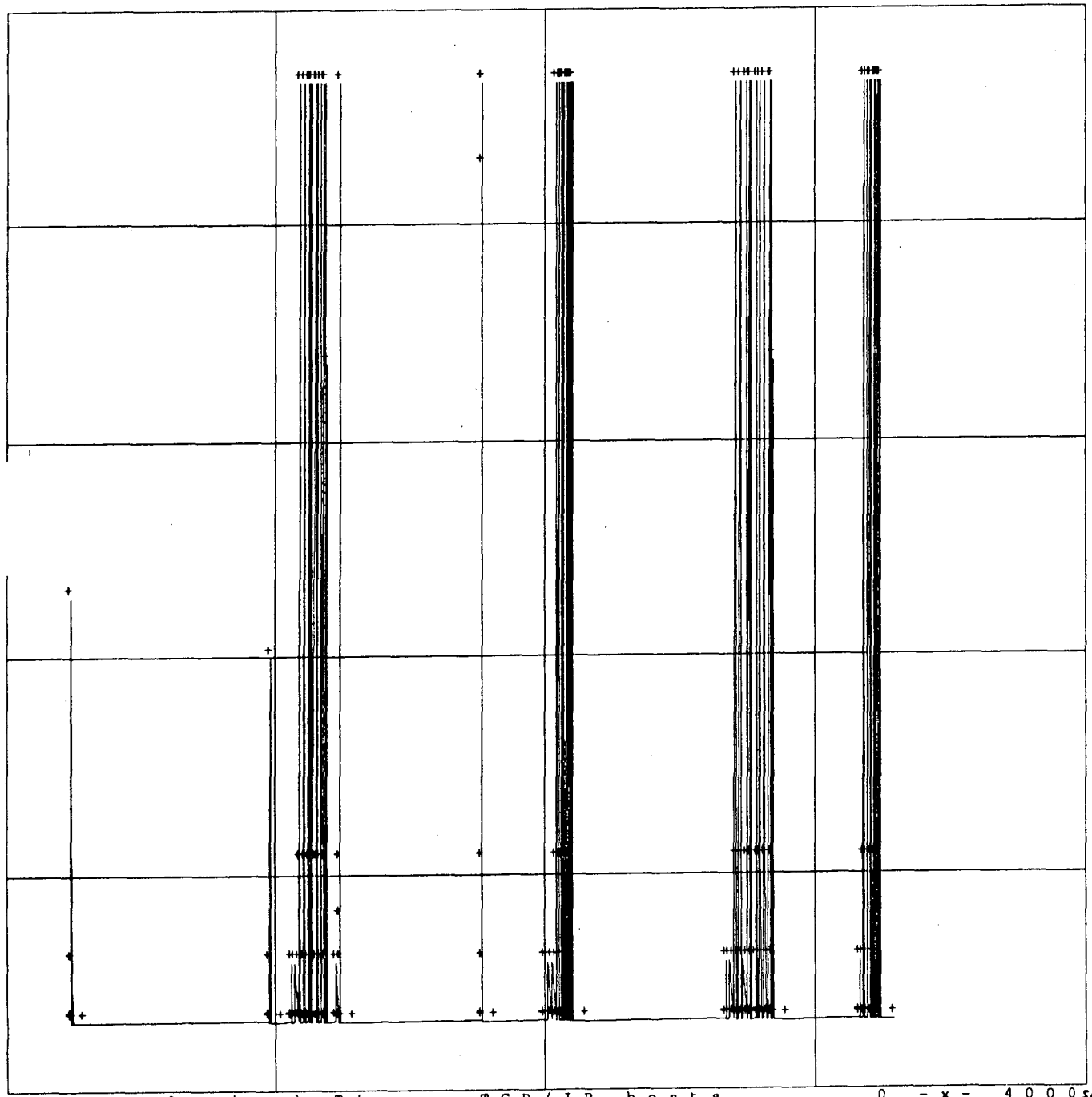


Size vs Arrival Time - DECNET hosts 0 -x- 4000 sec 0 -y- 800 bytes



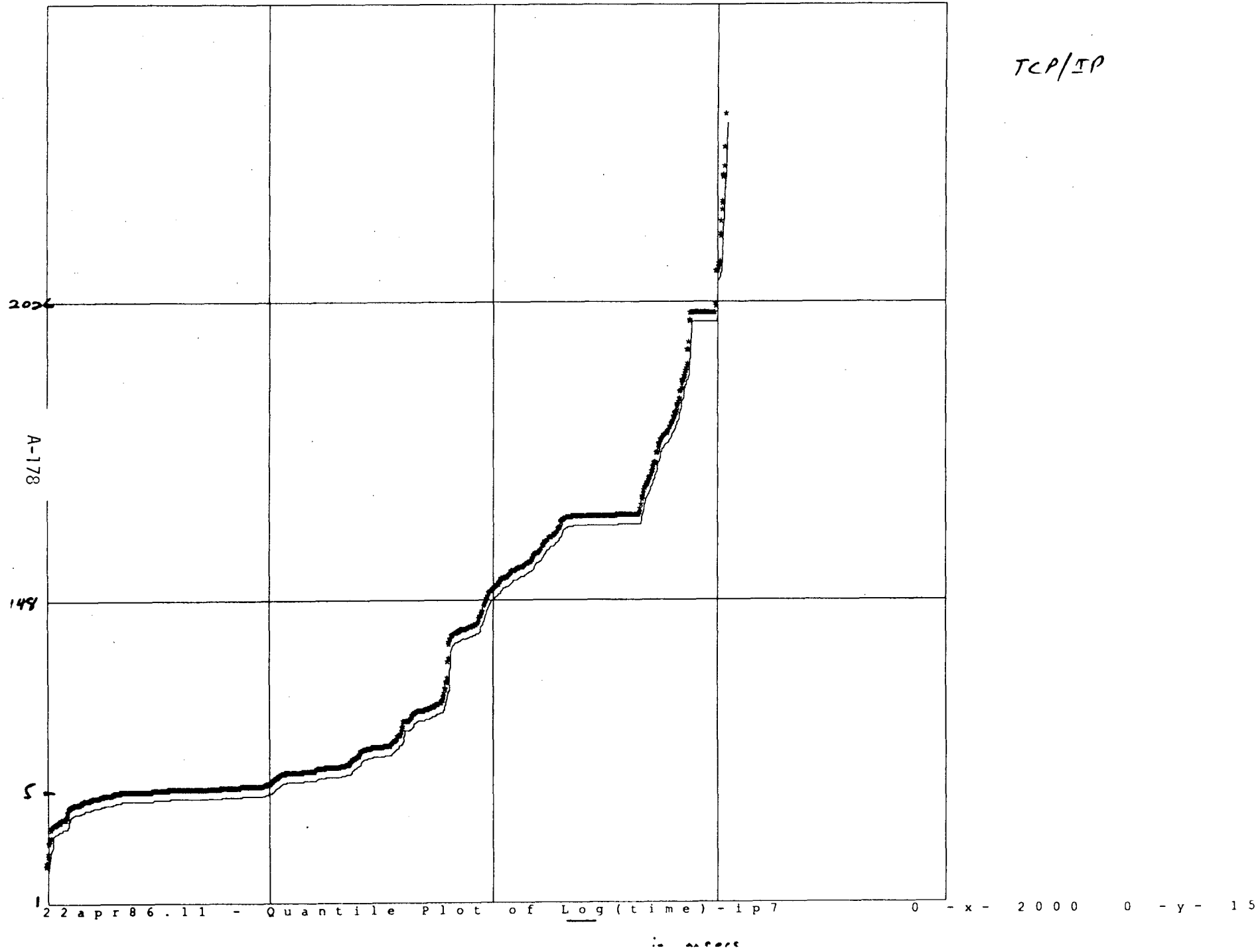
Size vs Time (sec) - DECNET 525 - x - 526sec 0 - y - 800 bytes

THIS IS AN EXPANDED PART OF THE PREVIOUS GRAPH.

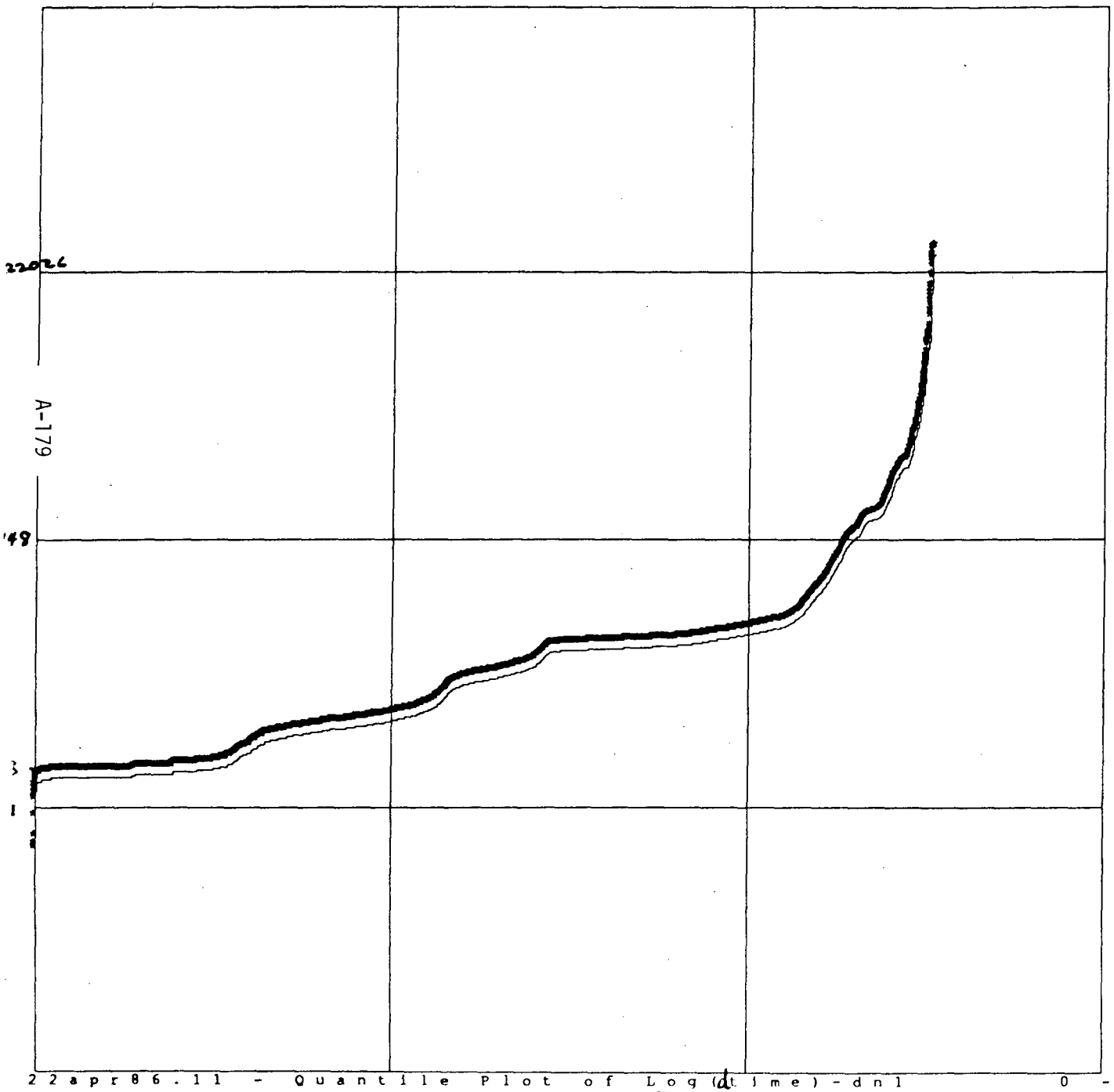


Size vs Arrival Time - TCP/IP hosts 0 -x- 4000 sec 0 -y- 1000 bytes

TCP/IP


2026
148
5
1
22apr86.11 - Quantile Plot of Log(time) - ip7
0 - x - 2000 0 - y - 15

DECNET



22apr86.11 - Quantile Plot of Log(dtime)-dnl 0 -x- 15000 -5 -y- 15

SOFTWARE MAINTENANCE AND PROGRAM CONVERSION

Robert Holt
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Research Sponsor: Dr. Ed Theil
Engineering Division
(Real Time Systems Group)

During the time I have been at LBL, I have been working under Ed Theil in the Real Time Systems Group. I have worked on two projects under his supervision in conjunction with the operators of the Cyclotron in building 88. I have translated a Pascal program to BASIC which enables the technicians in building 88 to read the trimcoils of the Cyclotron by computer instead of manually. I have also written two small programs to modify the data set of a large program which predicts trimcoils settings for different elements. The large program is called CYDE.

My first project involved program conversion. In that project I translated a Pascal program to BASIC and tested it on the computer upon which it was to be used. The purpose of the program was to enable a digital voltmeter to read the temperatures of seventeen trimcoils and print out this information so that it could be kept and saved for future reference. I did this program for a group in the 88" Cyclotron. A cyclotron is a device which shoots a high particle beam of different elements to obtain information about that element. The trimcoils are a part of the cyclotron and the temperature of those 17 trimcoils is vital information in determining information about the elements.

In converting the program from Pascal to BASIC, I encountered several problems which were eventually overcome. First and foremost, I had not taken Pascal before but had signed up to take a Pascal class on the UC-Berkeley campus so that problem

was alleviated. Second, the Pascal and BASIC which the system I was supposed to put the program on used was not standard which required me to read several manuals on Pascal and BASIC for that system to better understand the language for that particular system. Third, I had not been exposed to that type of computer before which required me to read some additional manuals on how to operate the system and I had to go to the building in which it was located and get some hands-on experience on how to operate it. Fourth and finally, the computer was in used the majority of the time so I had to schedule myself to go to work on the computer when it was not in use, which at first was at strange times of the day (and on one occasion night).

My other project dealt essentially with the same thing as the previous one (trimcoils) but the application was different. In my second project, I wrote two small programs to modify the data used in the CYDE program to allow the data set to be changed almost instantaneously instead of going about it tediously by hand. The CYDE program was designed to predict what to set the trimcoils to, given a certain amount of data. The CYDE program also gives some information about how the beam created should act. In explanation to that statement, it gives other information concerning the beam which is relevant to determining if the predictions are correct. In doing this project I used an old version of FORTRAN which is not used very much any more. I also had to learn a new operating system

because I had to write the program on the ModComp computer, which according to some scientists in RTSG is a somewhat difficult system to learn if you aren't already familiar with it.

In preparing myself to undertake this project, I had to review my FORTRAN. I also had to tutor myself on the workings of the ModComp so that I could understand how to compile and run my programs. After gaining some understanding of how to operate the ModComp I wrote the programs. In order to run the CYDE program, I had to learn to load disks and tapes so I had to learn a little about the hardware. To test the programs, I was given several test cases to run. After determining that the programs were correct I was given some real cases to run. I eventually ran over 100 cases for Dave Clark, the scientist that I was working with on this project.

Besides those two major projects, I also worked on bugs in the VAX system in building 46. These problems were brought up in meetings held by the group I was in (Central Support Group, a subgroup of RTSG) and each problem was assigned to someone in the group to work on and if possible, fix it. I was assigned several of these to work on during my time here. These helped me to understand how simple one word commands are created and that they are not as simple as they appear to be and made me realize that a great deal of work goes into making a computer system user friendly.

My job experience has taught me a number of things, both professionally and academically. Professionally, it has taught

me that in the LBL system, that every division is dependent upon each other in order for LBL to function efficiently as a whole. On a more personal level, it has taught me that each worker's job is dependent on his co-worker's and that closeness in a professional atmosphere gives the worker's a sense of pride about the company and makes working together go smoother and more efficient. Academically, I have been exposed to four computer environments, all having their similarities but each having it's distinct differences. I have been exposed Pascal and C programming languages and I have some knowledge of how to work with graphics as well as knowledge of how to debug system commands. All of these will be a definitely plus when I return to the academic atmosphere of Jackson State University. I have learned to relate better to my colleagues which will carry over to my return to Jackson State University and allow me to be able to better relate what I have learned to my fellow students.

The co-op relations with LBL are a starting point for something that should be developed with other companies to give more students the opportunity to be exposed to a professional atmosphere and perhaps gain some knowledge that they otherwise would not learned were they not given such an opportunity. This experience has enabled me to gain considerable knowledge in the operation of computers and also considerable knowledge in programming techniques.

In giving recommendations to future co-op students, I would suggest that students take more programming languages like FORTRAN, Pascal and Assembly to students in a similar work situation, it can not be stressed enough to ask questions. In asking questions, you are showing your employer that you want to learn learn. Another thing is that the student get to know people all over the company because they might be able to help you with some problem that you have workwise or other that the people in your department can not help you with. Course changes should emphasize more computer courses and a diverse group of other courses would help. Math and Physics are important but the emphasis is on computers so more computer courses should be taken.

This co-op experience at LBL has been a definite plus to my college career. It has given me some insight on how it will be when I graduate and what changes I need to make in my courses before then to better prepare myself. It will also make it easier for me to adjust to a professional atmosphere from the academic when the time comes. It has given me more knowledge that I would be glad to share with my fellow students upon my return to Jackson State University. It has allowed me to be exposed to a social atmosphere different from those I am used to thus making it easier for me to understand how someone from somewhere else feels when they come into the JSU atmosphere. It has also awakened my awareness to the fact that more minority

engineers and scientists are needed and that students in those fields should be encouraged to remain there and get their degrees and go as far as they can go in school in their respective majors because there are a lot of opportunities awaiting them upon graduation and they should be in a position to take advantage of them. Finally I would like to say that going on co-op is an opportunity that no student should pass up because it gives them the chance to experience firsthand what they will experience after graduation thus giving them the chance to make up for any skills they may be lacking after they graduate had they not went on co-op. Every student should try to co-op somewhere before they graduate. It is an experience they will never forget.

CELLULAR AND MOLECULAR RADIOBIOLOGY OF CHARGED PARTICLE RADIATION DAMAGE

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Research Sponsor: Eleanor Blakely
Biology & Medicine Division

I. BACKGROUND

Charged particles, otherwise known as heavy ions, are presently undergoing clinical trials to test their efficacy for treating human carcinomas. Cell killing by this method of radiation is more effective and productive than conventional radiations such as X-rays or γ-rays. The question of whether cells can repair potentially lethal damage (PLD - that component of radiation damage that can be influenced by postirradiation environmental conditions) from high linear-energy transfer (LET) radiations has not been clearly answered due to factors contributing to the complexity involved in identifying the potentially lethal lesion and in determining what modifies its repair.

Damage produced in cellular DNA by agents such as ionizing radiation disrupt the three-dimensional structure of chromatin; to survive such damage, cells must restore the correct topological structure to DNA after removing DNA lesions. It is known from studies that X-ray induced DNA strand breaks are repaired very rapidly by mammalian cells. Even after high doses most strand breaks are sealed. Base damage induced by ionizing radiation is also repaired. Since the lesions from high-LET radiations are known to be more severe than those from low LET radiation, it was of interest to study the effects of novobiocin on lesions produced by particle beams.

In the present study an attempt has been made to measure the effect of novobiocin on exponentially growing human cells. Secondly, measure the inhibition of potentially lethal damage (PLD) repair after X-rays in exponentially growing cells by novobiocin. Thirdly, measure the effect of novobiocin on PLD-repair after exposure to Bragg peak helium ions. These procedures were conducted using sterile tissue culture techniques essential for maintaining human cells in vitro.

The primary goals of my research work were:

1) to learn sterile tissue culture methods required to maintain human cells in vitro.
2) to measure the cytotoxicity of novobiocin on exponentially growing human cells.
3) to measure the inhibition of potentially lethal damage repair after X-rays in exponentially growing cells by novobiocin.
4) to measure the effect of novobiocin on potentially lethal damage repair after exposure to Bragg peak helium ions.

II. MATERIALS AND METHODS

Human T-1 cells were maintained at 37°C in a humidified atmosphere of 5 percent CO_2 in air in Eagle's minimum essential medium supplemented with 10 percent foetal bovine serum (Gibco) and L-glutamine (0.3 mg/ml).

Cells were initally grown at a low density in 35 mm diameter petri dishes to establish a growth curve. Afterwards, cells were inoculated at a given density in falcon flasks; they were then cultured to confluency, whereupon groups of cell were irradiated with X rays or helium irradiation. Immediately after irradiation at respective intervals, each group of cells were trypinsized, and the cells were subcultured at low density in fresh medium in the presence of novobiocin at various concentrations.

To examine the effect of novobiocin encytotoxicity, cells were replated in appropriate numbers in falcon flasks and cultured in the presence of various concentrations of novobiocin for various lengths of time, namely for 0, 1, 3, 4 hours respectively. After incubation for 11 days, the cultures were fixed and stained with filtered methylene blue, and the colonies containing more than 50 cells were scored as survivors.

Six experiments were completed during this project:

#1) an experiment to study normal cell growth

#2) and #5) experiments to study cytotoxicity of novobiocin

#3) and #6) helium experiments to measure PLDR with and without novobiocin

#4) X-ray experiment to measure PLDR with and without novobiocin

III. RESULTS AND SUMMARY

The effects of novobiocin (an inhibitor of DNA enzymes) on the repair of radiation damage from X rays or helium ions in exponentially growing human cells has been studied. Initial cytotoxicity experiments indicated that human T-1 cells were more sensitive than rodent cell lines to treatment with novobiocin. Experimental measurements indicated that exposure to novobiocin (100 μm) in a phosphate buffered saline (PBS) during a 2.5 hr. incubation after exposure to X rays inhibited the repair of potentially lethal damage observed in cultures held in PBS without the drug. These experiments may contribute to an understanding of the importance of higher-order DNA structure in the functional repair of radiation damage since the topoisomerases inhibited by novobiocin must be essential to cellular repair processes. Similar experiments to study the effects of novobiocin on repair of damage from helium ions are still in progress.

TUMOR RADIOBIOLOGY

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I. INTRODUCTION

My work at Lawrence Berkeley Laboratory dealt with the study of tumor radiobiology with heavy-charged-particle beams. These heavy-ion beams exist only at Lawrence Berkeley Laboratory (LBL). The heavy-ion beams are produced by a combination of two LBL accelerators, the Bevatron and the SuperHILAC, referred to as the Bevalac. Accelerators propel electrically charged particles called ions - either negatively charged electrons or positively charged protons and atoms stripped of some or all of their electrons, to speeds approaching that of light. This resulting beam is altered and shaped in such a way that its energy is released only to a predetermined area. The Bevalac Biomedical Facility combines the biological and physical sciences to develop a source of heavy ions for applications to radiation biology, radiation therapy, and diagnostic radiology.

The purpose of the research lab to which I was assigned is to evaluate the responses of rat rhabdomyosarcoma tumors to heavy-ion irradiation. In vivo and in vitro experiments are used to determine these responses. Some of the present areas of study include:

1) repair of potentially lethal damage,
2) tumor repopulation kinetics following fractionated doses of heavy ions, and
3) phase-specific cell survival following X irradiation.

Repair of potentially lethal damage is the repair that occurs during delayed plating after irradiation. This is an important factor for radiation therapy in designing treatment plans for cancer patients. Differences may exist in the kinetics of tumor cell repopulation following irradiation with heavy ions as compared to X rays. If differences are found, it is necessary to determine them so that correct doses of high- and low-LET radiation can be used on the patient. Phase-specific cell survival following irradiation is studied to

determine what types of cells are surviving irradiation so that other means of killing those cells can be found.

II. ABOUT THE TUMOR LINE AND THE RATS USED

Because of the nature of these experiments, a special tumor system has to be used. the tumor system is called rhabdomyosarcoma. This tumor system was originally developed at Rijswijk and brought to Lawrence Berkeley Laboratory from Holland. The major desirable characteristics of this tumor system are as follows:

a) It is adapted for growth both *in vitro* as a monolayer culture and *in vivo* as easily measurable spherical tumors in inbred WAG/Rij rats.

b) The tumors are homogeneous and free of large necrotic areas until they exceed 3 g.

c) Growth properties are reproducible, and there is no evidence of an immune response by the host animal.

d) Cell suspensions obtained by enzymatic dissociation of excised tumors can be grown *in vitro* with acceptable plating efficiencies, thus permitting cell survival curves to be obtained for tumors irradiated *in situ*.

Along with the tumor system, special rats were brought back, referred to as WAG/Rij rats. Needless to say, extreme care was given to these rats. A breeding system was developed that required records kept on every rat from the day it was born to the day it died. This is necessary so that if a bad or undesirable trait shows up, that particular line of rats can be removed from the colony. Following is a basic list for the upkeep of the breeding colony.

a) Weigh all currently breeding female rats, with the exception of nursing mothers; record all weights on the master sheet.

b) Isolate the pregnant females; determined by a sharp increase in weight, for instance $\geq$ 10 grams.

c) Separate and retire: bad mothers, sterile females, female breeders with more than 5 litters and their respective male partners.

d) Record the necessary information on the semi-weekly record sheets.

e) Record the necessary information on each rat's cage card.

f) Watch for any diseases which could occur within the breeding colony.

g) Watch for negative traits to the pedigree, i.e., Encephalitis, scruffy coats, hydrocephalic germ line, etc.

h) Watch for any problems that can upset breeders, i.e. temperature fluctuations, construction noise, ventilation and humidity, etc.

The rats are kept on schedules regulating feeding, cleaning, and mating times. To monitor these events, the rats are kept in special rooms. One room is desired for the breeding rats and one is for the rats that have been used in experiments. Because rats mate only at night, there is a special timer set on the lights in the breeding room. It is important to assimilate as closely as possible normal conditions for the rats. The breeding rats and the experimental rats are separated for several reasons. Some include: 1) each type needs a specific kind of care; 2) it is best not to cause undue stress on the breeders.

III. IN VITRO AND IN VIVO EXPERIMENTS

In Vitro

An *in vitro* experiment is one that uses living cells grown outside of the living body. These cells are kept alive by placing them in incubators set to the desired temperature, humidity, and CO_2 content to resemble living conditions. The cells are contained in special flasks with medium to provide them with the required nutrients. The medium consists of minimum essential medium supplemented with 10% fetal bovine serum, 10% newborn calf serum, 50 units/ml penicillin and 50 μg/ml streptomycin. The process used to continue to grow cells outside its natural habitat is called subculturing. The process is used to transfer cells from one flask to another containing fresh medium, therefore insuring healthy cells. Following is an outline of the technique.

The subculturing technique is performed in a special room free from bacteria and fungi by the use of ultraviolet light. There are incubators (set to the required specifications of: 37°C, 97.5% air - 25% CO_2 humidified gas mixture), a coulter counter (used to count the number of cells needed in each new flask), a refrigerator and freezer to hold the perishable items (medium, trypsin, etc.), and various other materials.

1) After closing the door to the germ free room, wash hands with ethanol and use a disposable mask.
2) Turn on the coulter counter and the pump. After the machine has warmed up, calibrate the lines of the window. (This is calibrated to make sure that all cells are counted.)
3) Take trypsin out of freezer and the medium out of the refrigerator and place in a 37°C water bath. Trypsin is an enzyme that detaches the membranes of the cells from the flask.
4) Take the culture flask out of the incubator.

5) Flush the coulter counter. This is to clean out the internal tubing and fill it with saline solution.

6) Set up vacuum pipet, and turn on the pipeter.

7) Suction out the used medium from the old flask. Then add trypsin and suction it out quickly. This is done to remove any excess medium. Then add the remaining portion of the trypsin. It is added to detach the membranes of the cells from the sides of the flask. Then you add medium to inactivate the trypsin before the cells are damaged.

8) Now place the solution formed in the flask to a test tube and centrifuge. This is done to separate the cells from the solution.

9) After centrifuging suction out the solution. Then add fresh medium. Mix it up so that the cells are not at the bottom.

10) To a vial of saline solution, add a small fraction of the new solution. This will be used in the coulter counter to determine the amount of cells in the solution.

11) Using different calculations, determine what fraction of solution will go into the new flasks.

12) Add the calculated amount to the new flasks already containing fresh medium and add to the incubator.

The technique described above can have different variations for different purposes. For example, if you were going to perform a radiation experiment on <u>in vitro</u> cells (cells plated in a flask), instead of adding a calculated amount of cell solution (steps 1-11 mentioned above) to large flasks, you would add them to smaller radiation flasks. This cell solution is also used to inject tumor cells into the rats.

In vivo

An in vivo experiment uses cells that are in a living body. The tumor cells used in this laboratory are grown in vivo through the transplanting of tumor cells from one rat to another or by using in vitro cells and injecting them into the rat. These techniques are as follows. When injecting the tumor cells into the rat, you would inject about 10^6 cells subcutaneously. They will grow to the size of about 1 cm^3 in the time period of 3 weeks. The tumors are now called "donor" tumors. The "donor" tumors are then excised, under sterile conditions, and cut into sections of about 1 mm^3. These sections are then surgically implanted subcutaneously into the rat. (All rats used for experiments are female. They are easier to manage, because of size and temper.) These tumor cells are allowed to grow for 3 weeks and again they reach the size of about 1 cm^3. They are now ready for use in an in vivo experiment. Usually the experiment would require the tumor to be irradiated with 225 kVp X-rays or with heavy ions. To study the survival of the tumor cells in vivo, they are excised under controlled conditions, minced with scissors, disassociated, planted, fixed and stained, and finally studied.

After irradiation of in vivo tumor cells, there is a period of time before you can actually see and study the results of irradiation. Because of this, it is very important to perform experiments of this nature both in vivo and in vitro. In vivo gives you the actual living conditions, while in vitro gives you the ability to observe and study every detail.

IV. LIST OF TECHNIQUES LEARNED

1. Mammalian Cell Culture
2. Breeding and the Upkeep of WAG/Rij Rat Colony
3. Injection of Tumor Cells in WAG/Rij Rat
4. Transplanting of Tumors in Rats
5. X-Irradiation of Tumors
6. Irradiation of Cells
7. Fixing, Staining, and Counting the Cell Colonies
8. Use of Various Equipment
    a. Coulter Counter
    b. Centrifuge
    c. Autoclave

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PROCOLLAGEN SECRETION AND SYNTHESIS

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The primary goal of our research is to possibly identify a procollagen receptor on the outside of the cell. Collagen has a unique structure and synthesizing process. We gather vital data by performing a series of experiments. These experiments and scientific techniques play a significant role obtaining results. The experiments are cell culture and extraction, a collagenase assay, and a electeophoresis.

All multicellular organism contain collagen, a family of fibrous proteins. It is the most abundant protein in mammals, constituting a quarter of the total. Collagen is the major fibrous element of skin, bone, tendon, cartilage, blood vessels, and teeth. Tropocollagen is the basic structural unit of collagen. Collagen has an unusual amino acid composition and sequence. The proportion of glycine residues in all collagen molecules is nearly one third, which is unusually high for proteins. Furthermore collagen contains two amino acids tha are present in very few other proteins, namely hydroxyproline and hydroxylysine. Glycine is critcal because it is small. The inside of the triple-stranded helical cable is very crowed. In fact, the only residue that can fit in an interior position is glycine.

The fundemental structural design of the collagen fiber is a quartered staggered array of tropocollagen molecules. The collagen polypeptide chains are synthesized on the membrane bound ribosomes and injected into the endoplasmic reticulum as larger precursors, called pro- alpha chain. there is indirect evidence that the hydroxyl groups of hydroxyproline residues form interchain hydrogen bonds that help to stabilize the triple helix. Condition that prevent hydroxylation

such as a deficiency of oxygen, of iron, or of ascorbic acid (vit. C) inhibits procollagen helix formation.

Procollagen is the biosynthetic precursor of collagen. The pro-alpha 1 (I) and pro-alpha 2 chains are synthesized by fibroblast cells and secreted into the extracellular spaces of connective tissues. The additional amino-terminal and carboxyl terminal peptide regions on these precursors chains are then cleaved by specific proteolytic enzymes called procollagen peptidases. Procollagen peptidases control fiber formation. Collagen fiber formation takes place in the extracellular fluid near the cell surface rather than inside the fibroblast because of the external peptide regions of theprecursor chains prevent the premature formation of fibers. They may also guide the transport of procollagen across the permeability barrier of the fibroblast. Collagen fibers are stabilized by the formation of covalent cross-links. Two kinds of cross- links are in the collagen fiber: intramolecular and intermolecular. The bonds formed appear to be unique to collagen and elastin, a related protein.

The most important and primary experiment we use to obtain data is a cell culture and extraction. We use primary avian tendon cells (PAT) cells . We incubte fertilized chicken eggs for sixteen days. On the sixteeth day the tendons are extracted and placed in a special enzyme solution to brake up the cell The cells are then placed in a collagenase enzyme for one hour. The cells are then separated into many different flasks. The cells are grown in vitro in a serum solution of medium and antibodies. They are preped for varies experiments.

A collagenase assay is an experiment that is done at least once a week. Its' primary purpose is to show the amount of pro-

collagen being synthesized and sercreted by the cells in different controled environments. ¼he main contol environment in the cell cultures is growing a group without ascorbic acid (vit. C) and a group with ascorbic acid. The set of +C/-C cells are then labeled with a radioactive proline solution each day. The cells and 2 ml of medium are removed from the flasks and placed in test tubes. A parallel set of tubes are set up and 500 lambda is placed in each parallel tube. A mixture of 1M HCl, QP buffer and 0.5M TES is added to each parallel tube and the original set is discarded. These samples will now be assayed in three different ways in duplicates. One set of samples will have nothing added and the other two sets will have 50 lambda of collagenase and pronase. These tubes will now be incubated for one hour. The reaction is stopped with a 10% TCA solution for five minutes. A vacuum filter is set up and four ml of 2% Tca is added to each tube and poured into a well and the tubes are rinsed with four ml of 2% TCA. The filter are dried under a heat lamp and placed in minivials with tridium. These tubes are placed in a Packard Scintillation Counter for the cells to be counted. The expected results are the cell procollagen secretion should increase with time grown in medium +C cells should secrete about 50% of its' procollagen and the -C cells should secrete 12-15% of its procollagen.

Another important experiment is done is called an electophoresis. The type of electrophoresis we use is a (SDS) Sodium dodecyl sulfate polyacrylamide gel electrophoresis which separate proteins based on their mass. This experiment is stsrted with a N&T gel prep. We label two flask of cells containing 2ml of medium with 100 lambda of radioactive

proline for three hours. The medium is centrifuged and the supernatant is discarded. To the cell that remain 50 lambda of lysodectoues is added and a 100 lambda of !00% TCA. The cells are centrifuged again and the supernatant is discarded. The remaining cells are plsced in a mixture of 100 lambda of lysis buffer and 5 lambda of PMSF and frozen at -70 degree overnight. The cells are scraped off the flask in 1ml of F_{12} serum. They are then centrifuged and the supernatant discarded. They are resuspended in 100 lambda of lysis buffer and 5 lambda of PMSF and frozen at -70 degree overnight. To finish the gel prep add 5 lambda of Tris and 5 lambda of Beta-mercaptoethanol and 2 lambda of bromophenol blue for five minutes and heat at 100 degrees. Now the SDS- polyacrylamide gel can be made . It consist of a resolving gel znd a stacking gel. The recipes consists of a mixture of 30% acrylamide, 1% cis-acrylamide, tris buffer, TEMED, and double distilled water which is degased for 15 minutes. After degasing a mixture of 10% SDS and 10% ammonium persulfate is added. A 50 ml syringe is used to pour the gel between two glass plates. Run a pipet of butonal across the gel to get ridid of excess water. You wait until the gel solidifyand start the stacking gel. It consist of the same material but in different volume one other exception a cone column is added to make wells for the gel prep material. The expected results should show separation of protein and different amounts of protein being secreted.

GENETIC RECOMBINATION DURING MITOSIS AND MEIOSIS OF SACCHAROMYCES CEREVISIAE

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Saccharomyces cerevisiae (baker's yeast) are unicellular Eukaryotes from the Ascomycete class of fungi that reproduce asexually by budding and sexually by forming ascospores (Figure 1). The formation of sexual spores in yeast, sporulation, is of great biological significance and enables yeast to undergo genetic recombination, mutation, hybridization and selection processes that lead to evolutionary changes. Both haploid and diploid cells can reproduce by budding. The nucleus of a diploid yeast cell arises through the fusion of nuclei of two haploid cells, ascospores, of opposite mating types and thus contains a 2N number of chromosomes. The diploid cell nucleus when depleted of its nitrogen source undergoes meiosis, spore formation and forms an ascus which contains 4 ascospores.

Saccharomyces cerevisiae contains 17 chromosomes and two mating types, MATa and MATα genes located on chromosome III. In the control experiment NLBL3 MATa was mated to NLBL1 MATα on YPD to make RX38 a MATa/MATα heteroallelic diploid (Table 1). The mating mixture was selected on leucineless-tryptophanless omission medium (LT) and incubated at 24^{0}C (Figure 2). Six, 50 ml liquid YPD cultures, each inoculated with a single 2.5 mm diameter LT grown colony were incubated at 24^{0}C and harvested during the exponential phase of cell increase (2-4 x 10^7 cells and buds/ml). Cultures were harvested individually, cells were washed in sterile deionized water and serially diluted and plated on synthetic complete medium, cycloheximide synthetic complete medium, leucineless-tryptophanless cycloheximide medium (LTC2), canavanine medium (CAN), methionine minus medium (MET) 24^{0}C/36^{0}C, lysine minus medium (LYS), tyrosine minus medium (TYR) and histidine minus medium (HIS). Red and white cycloheximide resistant segregants were recovered.

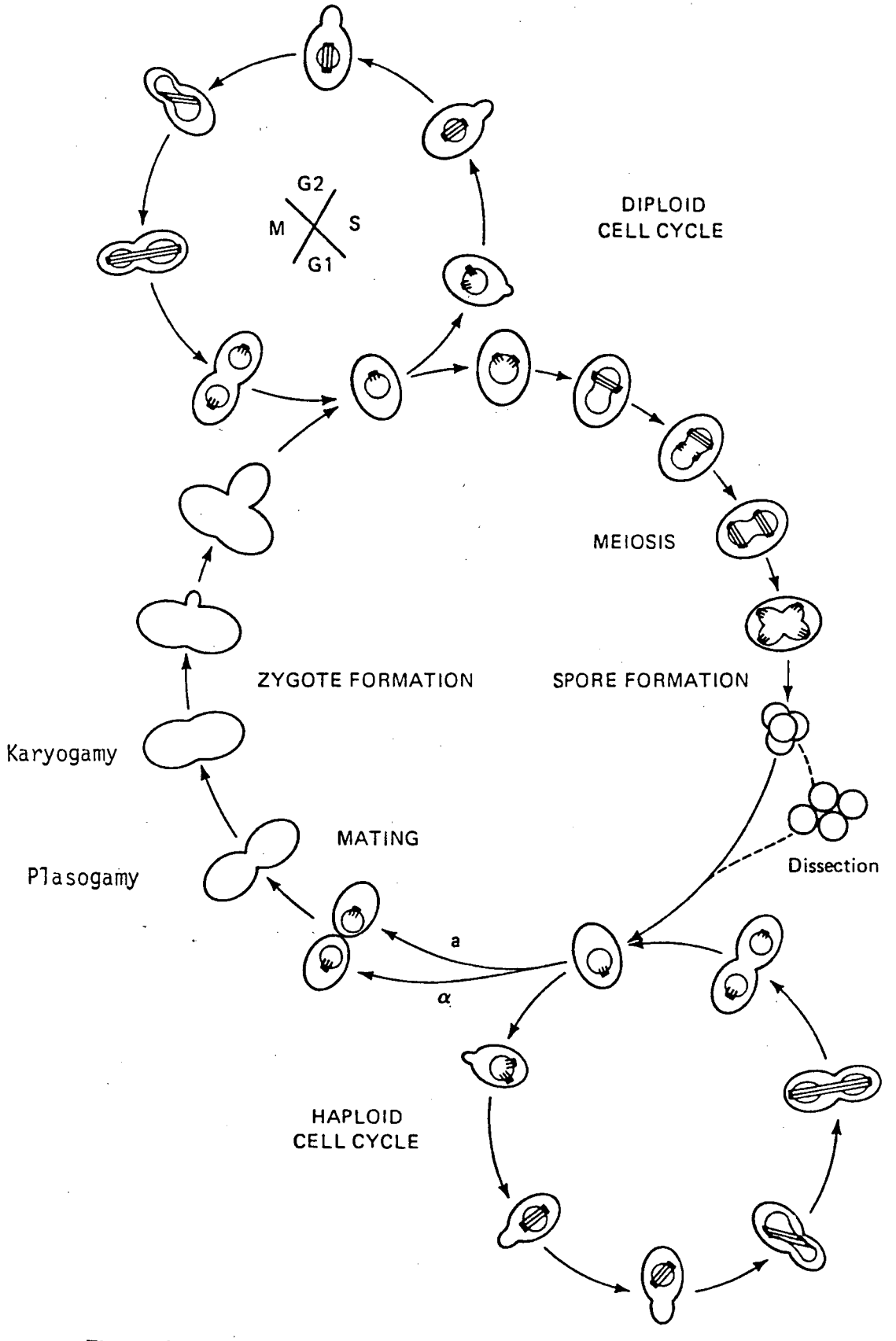


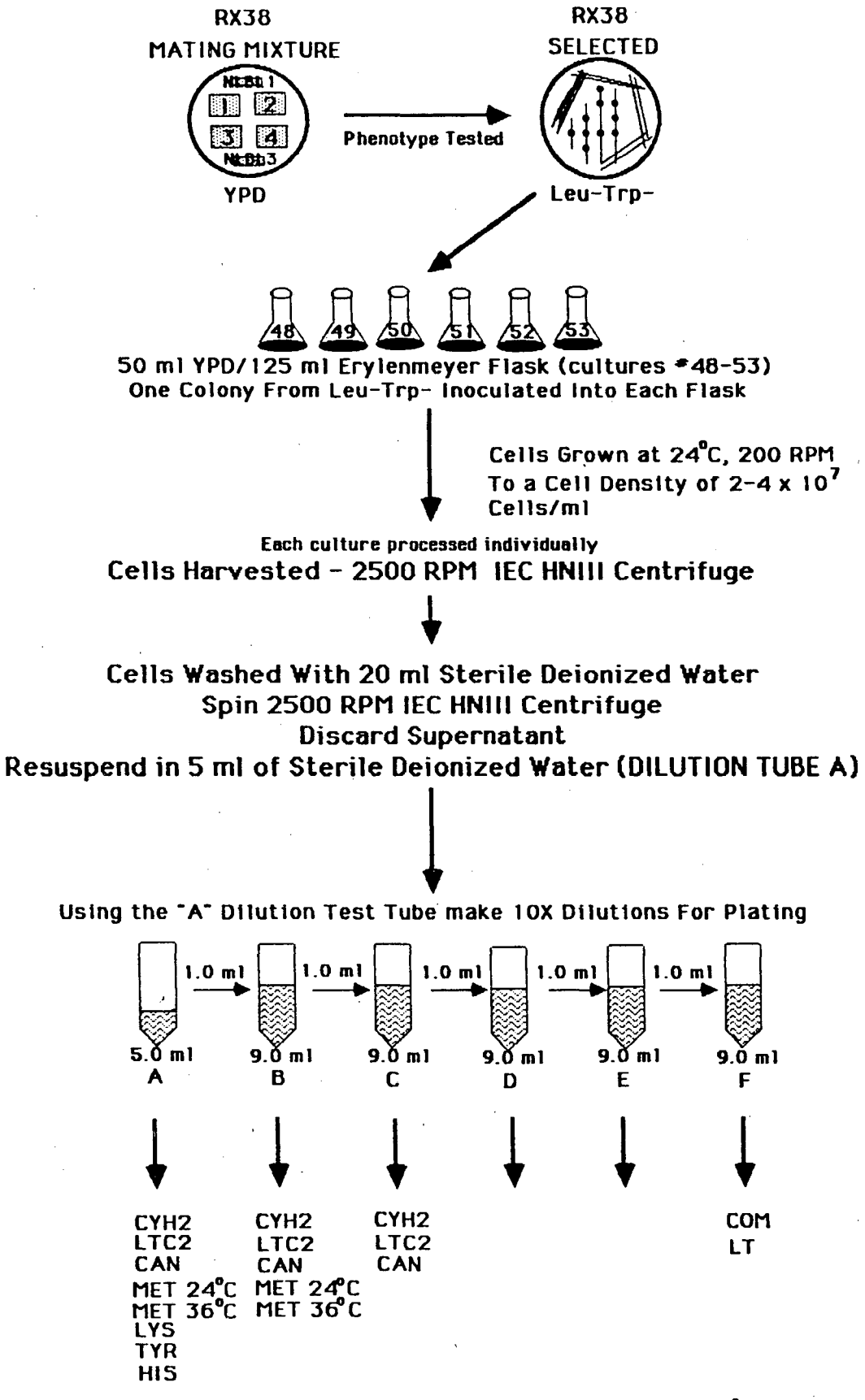
Figure 1. The life cycle of heterothallic strains of *Saccharomyces cerevisiae*. The nuclear configuration is indicated. G1, G2, S, and M refer to the phases of the cell division cycle.

Table 1. Principal Strains Employed.[a]

Strain	Genotype
LBL1	MATα ade5 met13-c cyh2^r trp5 LEU1 ade6 cly8
	ADE5 met13-d CYH2^s TRP5 leu1 ADE6 CLY8
	his7-1 tyr1-2 lys2-2 ade2-1 ura3-1 CAN1^s
NLBL1	MATα ade5 met13-c cyh2^r trp5 LEU1 ade6 cly8
	his7-1 tyr1-2 lys2-2 ade2-1 ura3-1 CAN1^s
NLBL3	MATa ADE5 met13-d CYH2^s TRP5 leu1 ADE6 CLY8
	his7-2 tyr1-1 lys2-1 ade2-1 ura3-1 can1^r
	III VII
RX38	MATa ADE5 met13-d CYH2^s TRP5 leu1 ADE6 CLY8
	MATα ade5 met13-c cyh2^r trp5 LEU1 ade6 cly8
	II XV V
	his7-2 tyr1-1 lys2-1 ade2-1 ura3-1 can1^r
	his7-1 tyr1-2 lys2-2 ade2-1 ura3-1 CAN1^s

[a]LBL1 is an n + 1 hyperhaploid strain disomic for chromosome VII that has been employed for isolation of rec mutations affecting spontaneous mitotic gene conversion and intergenic recombination. NLBL1 and NLBL3 are coisogenic Rec$^+$ haploid strains employed for genetic and biochemical studies. RX38 is a control Rec$^+$ REC46/REC46 hybrid constructed by mating NLBL1 to the MATα REC46 Rec$^+$ haploid strain NLBL3.

Figure 2. Plating Experiment RX38



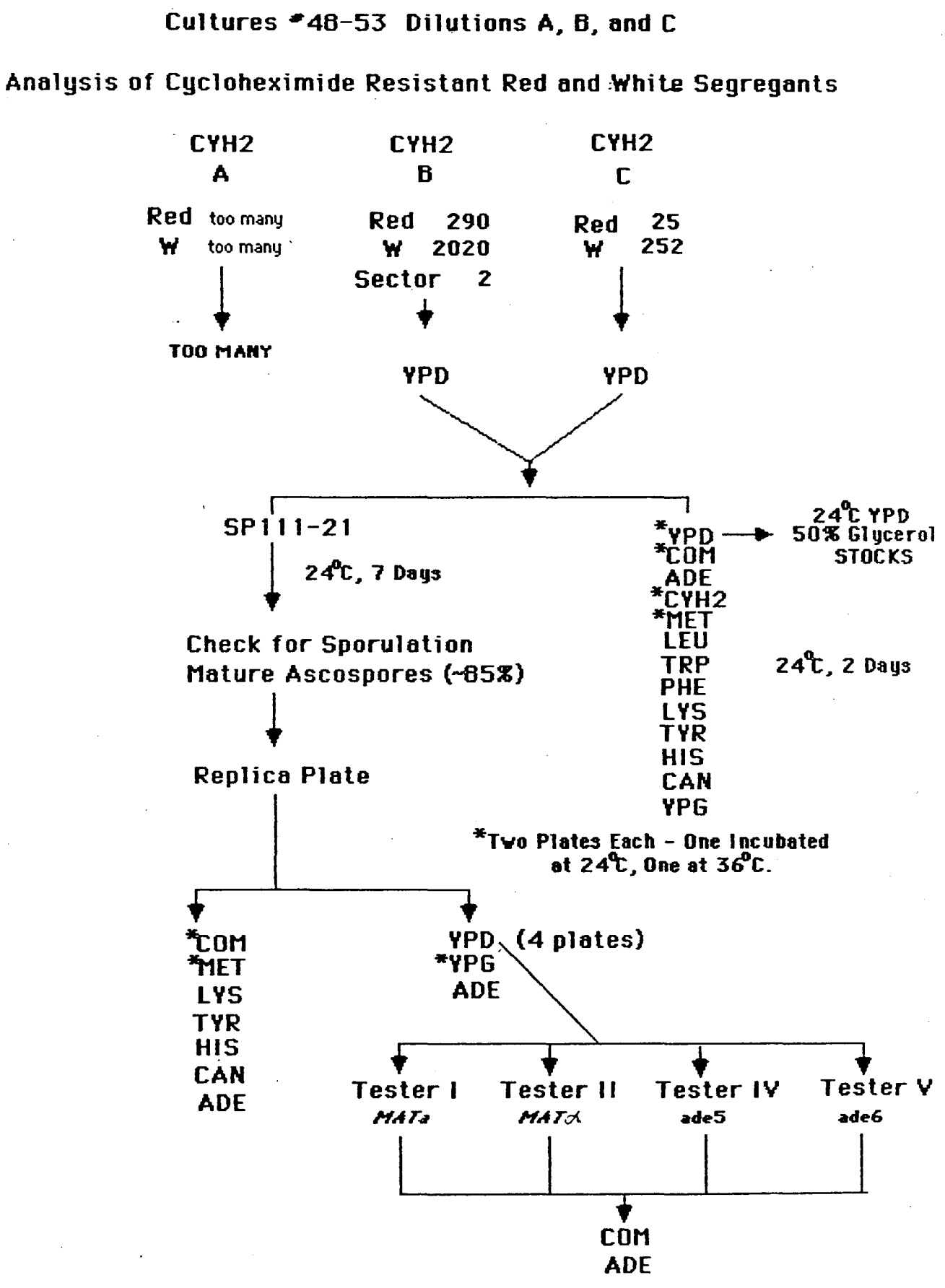
Except where indicated, plates were incubated at 24°C.
Each synthetic media was plated in triplicate
and received 0.1 ml cell suspension per plate.

The summer research project involved the analysis of the red and white cycloheximide resistant segregants from the control strain RX38 (Figure 3). Dilutions B and C were used to conduct the study. The A dilution contained too many cells and the cells were too close for isolation. YPD MASTERS numbered 1-18 were prepared from cycloheximide resistant red and white segregants on synthetic cyclohexide medium using sterile techniques and grown at 24^{0}C for two days. Yeast extract, Peptone, Dextrose nutrient medium (YPD) contains 20g/l dextrose, 20g/l peptone, 10g/l yeast extract and 15g/l agar.

Each YPD master was replica plated to synthetic diagnostic media and sporulation media. The synthetic media contains 20g/l glucose, 5g/l ammonium sulfate, 1.7g/l yeast nitrogen base and 15g/l Difco agar, .01g//l adenine, .05g/l arginine, .02/l histidine, .10g/l phenylalanine, .10g/l threonine, .05g/l tryptophan, .05g/l tyrosine and .02g/l uracil; pH5.8. The sporulation media contains 20g/l potassium acetate, 1g/l dextrose, 2..5g/l yeast extract and 15 g/l agar, 100mg/l L-threonine, and 75mg/l of each of the following nutrilites: adenine sulfate, L-arginine hydrochloride, L-histidine hydrochloride, L-leucine, L-lysine hydrochloride, L-methionine, L-phenylalanine, L-tryptophan, L-tyrosine, uracil; pH 7. The mitotic diagnostic media replicated consisted of COM 24^{0}C/36^{0}C, ADE 24^{0}C, CYH2 24^0/36^{0}C, MET 24^{0}C/36^{0}C, LEU, TRP, PHE, LYS, TYR, HIS, CAN 24^{0}C, YPD 24^{0}C/36^{0}C and YPG 24^{0}C and was incubated for two days. Sporulation media was incubated at 24^{0}C for seven days.

MET 24^{0}C/36^{0}C plates were used to detect growth and leaky alleles. met13-c the leaky allele is inhibited at 36^{0}C. COM 24^{0}C/36^{0}C examined cly8, temperature sensitive gene for cell lysis, and growth. A cell containing cly8 would be unable to grow at 36^{0}C and would be showing

Figure 3. Cycloheximide resistant segregants tested from RX38



confluent growth on COM media at 24^{0}C YPG tested for petites, the cells ability to utilize glycerol as a carbon source. ADE plates detected growth and revertants. CYH2 plates showed gene conversion and intergenic recombination and whether the genotype contained the CYH2^s or cyh2^r gene. LT tested allelic differences, i.e., gene conversion or crossing over at the TRP or LEU alleles in order to grow on LT. Aro mutation was detected by lack of growth on PHE.

After two days, mitotic diagnostic media was read according to color red, white, pink; and growth; confluent, heavy papillation, many papillae, few papillae and countable papillae. The results recorded the phenotype of the control strain RX38. The YPD cells were stocked in 50% glycerol, -70^{0}C, for future use. After seven days, SP111-21 plates were examined for mature ascospore formation (~85%) by light microscopy. The SP111-21 plates were replica plated to meiotic diagnostic media that consisted of COM 24^{0}C/36^{0}C, MET 24^{0}C / 36^{0}C, LYS, TYR, HIS, CAN, and ADE 24^{0}C, incubated for two days. A second SP111-21 plate was replicated to 4 YPD plates and mated to TESTERS I (MATa);II (MATα), IV(ade5), V(ade6). After two days at 24^{0}C The mating mixtures were replicated to COM, and ADE and incubated for two days at 24^{0}C. The results of mating type and ade alleles were usefull in characterizing genotypes.

The goal of the experiment was to check for gene conversion and recombination on Chromosome VII and intergenic recombination on Chromosome II between heteroalleles LYS, TYR and HIS. Results in the diploid RX38 showed 25% crossing over during mitosis at the TRP loci which is the same as Interval I of Rec46 (Tables 2, and 3). The mitotic and meiotic map distances, 25, Interval I and 35, Interval II, respectively for RX38 were the same. In another experiment, the heteroalleles LYS,TYR and

Table 2. RX38 Cycloheximide resistant segregants - preliminary results

RX38 CYCLOHEXIMIDE RESISTANT SEGREGANTS

	RED	WHITE	SECTOR	TOTAL	TRP⁻
48B	82	394		476	61/270W 24%
49B	48	491		539	117/208W 57%
50B	21	223		244	
51B	61	414	1 1/2R	475	
52B	78	498	1 1/2R	577	
	290	2,020	2	2,312	

	RED	WHITE	SECTOR	TOTAL	TRP⁻
48C	4	45		49	13 29%
49C	3	58		61	34 59%
50C	2	34		36	8 24%
51C	11	51		62	13 25%
52C	5	64		69	16 25%
	25	252		277	84 33%

	RED	WHITE	SECTOR	TOTAL	TRP⁻
48B&C	86	439		525	74/315 24%
49B&C	51	549		600	151/266 57%
50B&C	23	257		280	8/34 24%
51B&C	72	465		537	13/51 25%
52B&C	83	562		645	16/64 25%
	315	2,272	2	2,589	111/464 24% (omit 49)

Table 3. Sporulation deficiency of *rec46-1/rec46-1* hybrids

Hybrid	Genotype	Four and three-spored asci (%)	Two and one-spored asci (%)	Non-sporulated cells (%)	Total asci (%)
RX38	*REC46* / *REC46*	72.2	19.5	8.3	91.7
RX62	*REC46* / *rec46-1*	72.8	16.6	10.6	89.4
RX66	*REC46* / *rec46-1*	87.4	5.8	6.8	93.2
RX67	*REC46* / *rec46-1*	76.1	19.4	4.5	95.5
RX68	*REC46* / *rec46-1*	80.5	12.5	7.0	93.0
RX61	*rec46-1* / *rec46-1*	1.0	0.7	98.3	1.7
RX63	*rec46-1* / *rec46-1*	0.5	0.9	98.6	1.4
RX64	*rec46-1* / *rec46-1*	2.2	1.8	96.0	4.0
RX65	*rec46-1* / *rec46-1*	0.3	3.5	96.2	3.8

Table 4. Single strand analysis of meiotic segregants from *MATa/MATα REC46/REC46* and *MATa/MATα rec46-1/rec46-1* hybrids

Genotype: I	II	III	Category[a]	No. observed after sporulation	
LEU1	*TRP5*	*CYH2*	*MET13*	*REC46/REC46*	*rec46-1/rec46-1*
+	–	r	c	Parental 1	28	52
–	+	S	d	Parental 2	40	51
+	+	S	d	SCO I	8	11
–	–	r	c	SCO I	15	13
+	–	S	d	SCO II	22	34
–	+	r	c	SCO II	17	22
+	–	r	d	SCO III	7	14
–	+	S	c	SCO III	10	9
+	+	r	c	DCO I, II	9	13
–	–	S	d	DCO I, II	5	7
+	+	S	c	DCO I, III	2	1
–	–	r	d	DCO I, III	2	4
+	–	S	c	DCO II, III	2	8
–	+	r	d	DCO II, III	4	4
+	+	r	d	TCO I, II, III	1	0
–	–	S	c	TCO I, II, III	0	0
Totals				172	243
Recombination interval I (%)				25.0	19.8
Recombination interval II (%)				34.9	36.2
Recombination interval III (%)				12.2	16.5

[a] SCO single crossover; DCO double crossover; TCO triple crossover

HIS gave no crossing over results. The results obtained thus far are not complete, the research study is still being conducted.

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