PUERTO RICO NUCLEAR CENTER

ANNUAL REPORT 1967

OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT (40-1)-1833 FOR U. S. ATOMIC ENERGY COMMISSION
A great deal of PRNC's history is reflected in this photograph, taken during the 10th anniversary symposium. The two gentlemen on the left—Dr. Charles F. Bonilla and Dr. John C. Bigher—are former directors of PRNC. Next is Dr. Henry J. Gomberg, present director of PRNC. At far right is S. R. Sapirie, Manager of the US AEC's Oak Ridge Operations Office, who in 1957 signed the contract which established PRNC, to be operated under contract by the University of Puerto Rico. Today Dr. Bonilla is Head of the Chemical Engineering Department at New York's Columbia University; Dr. Bigher is on the USAEC's General Advisory Committee.

Dr. Gomberg receives a plaque commemorating PRNC's 10th anniversary from Mr. Sapirie.
INTRODUCTION

Though an annual report's key purpose is to describe activities during the previous year, the fact that 1967 marked the tenth anniversary of the Puerto Rico Nuclear Center merits a brief review of the past decade.

Looking backward ten years offers a valuable perspective of how PRNC has grown, both in size and scope. With its origin in President Dwight D. Eisenhower's "Atoms for Peace" proposal, PRNC has matured since its founding in 1957 as a training center operated by the University of Puerto Rico under contract with the US AEC to what is now a full-fledged teaching and research institution. PRNC's small staff nucleus in 1957 has since grown to over 300 scientists, technicians, and supporting personnel.

Much has been written about Puerto Rico's value as a "cultural bridge" between the North and South American continents. PRNC offers a small, but affirmative example of how this "bridge" can work. It is a bilingual institution, where scientists and students from the Spanish- and English-speaking nations of the hemisphere can work together without language barriers.

The scientific staff has a distinctly international flavor, with a strong contingent of Latin Americans. About one-third of its scientists are native-born Puerto Ricans. Another third are from South and Central America and other nations of the world, and the other third are North Americans.

PRNC has not neglected growth in the educational field, which is where it had its beginning. Fifty-nine students were trained during the first year of activity, nine of them non-U. S. citizens, with a major share of the U. S. citizens being native-born Puerto Ricans. In 1966, the student enrollment had grown to 173 (including 32 non-U. S. citizens), and in 1967 enrollment reached a record level with 236 students (including 37 non-U. S. citizens). In all, PRNC has trained 1,474 students, 251 of them from abroad, representing 18 different Latin American republics and other nations such as Japan, Germany, Spain, South Africa, India, the Philippines, Formosa, and Great Britain.

Perhaps the most dramatic change over the past decade, however, has been PRNC's expansion in scope; its research activities in the peaceful applications of nuclear energy are now as important as its efforts in education and training.
Today, biological and medical research programs include work in marine biology, terrestrial ecology, radiation chemistry, parasitology (investigation of *Schistosoma mansoni*, a tropical parasitic organism which causes Bilharzia) and entomology (tropical insect sterilization studies of the sugarcane borer). Physical research is being carried out in neutron diffraction, solid state physics and hot-atom chemistry. A preliminary study is in progress on the effects of mining with nuclear explosives and, in the field of isotopes development, PRNC researchers are examining the effects of radiation preservation of various tropical foodstuffs which are vital to the economies of Latin American nations.

The growth of research activity at PRNC is reflected in its publication output. Its staff produced 52 scientific publications last year, more than half the amount produced in the entire six-year period from 1957 through 1963.

A major expansion of facilities at the Puerto Rico Medical Center in Río Piedras is another manifestation of PRNC's growth. Construction of an entire new wing, costing over $1 million, is to begin in 1968, with completion scheduled for mid-1970.

The tenth year marked an important milestone for PRNC. It brought with it several gratifying instances of recognition. PRNC was selected to carry out the marine ecology studies in preparation for a proposed new sea-level Isthmus of Panama Canal. It became more active in helping the development of peaceful nuclear techniques in Latin America through its "sister laboratory" program with Colombia's Instituto de Asunto Nucleares, and by expanding its sponsorship of research in the US AEC Latin America Exhibits Program. Also, PRNC was designated by US AEC Chairman Seaborg as a regional "center of excellence" under the Punta del Este program of scientific cooperation with Latin America.

And finally, the 10th Anniversary Symposium in October 1967 brought together distinguished scientists and government officials from all sectors of the hemisphere. It was a stimulating event, a source of inspiration for even greater achievement in PRNC's second decade of life.

***

We are pleased to announce that during 1967 the eminent chemist Dr. W. O. Baker, Vice President for Research, Bell Telephone Laboratories, accepted PRNC's invitation to join its Advisory Committee. This now completes the eight-man committee (see Appendix for the full roster).
Dr. Jesse D. Perkinson, Chief of OAS Division of Science Development, speaks. At left Ben S. Stephansky, Deputy U. S. Representative to the OAS.

Above: US AEC Commissioner Ramey and IAEC Director General Eklund. Above right: Professor George Hodgson, University of Chile. Right: PRNC Assistant Director for Scientific Programs, Dr. Jorge Chiriboga, of Peru.
Teodoro Moscoso, Board Chairman of Commonwealth Oil Refining Company (and former Director of the U.S. Alliance for Progress), speaks on "The Development of Puerto Rico."

Dr. Juan J. Touya, technical director of the Nuclear Medicine Center, College of Medicine, Montevideo, Uruguay.

Dr. Paul B. Pearson, President of the Nutrition Foundation, New York City (and member of the PRNC Advisory Committee).

Dr. Antonio Bacigalupo, head of the Department of Zootechnics and Animal Nutrition, Agrarian University, Lima, Peru.

Dr. Fernando Lopez Della-mary of the Entomology Research Division of USDA's Agricultural Research Service in Mexico City.
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*F. LOWMAN (29)

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PUERTO RICO AREA OFFICE

APPROVED FOR CONTRACTOR

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PUERTO RICO NUCLEAR CENTER

TOTAL PERSONNEL : 303

CONTRACTOR : UNIVERSITY OF P. R.

LOCATION : PUERTO RICO

OPERATIONS : OAK RIDGE

CONTRACT NO. : AT-(R)-1-1833
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EDUCATIONAL AND TRAINING DIVISIONS

The educational and training divisions (also known as the 11 Program) offer training and research opportunities in the nuclear field for students at the graduate and postgraduate levels. The divisions either carry out individual phases of the training and research, or provide guidance and support. This area of PRNC activities, which represents about two-thirds of the overall program, is sponsored by the United States Atomic Energy Commission (US AEC) Division of Nuclear Education and Training.
Graduate students of Physics and Chemistry doing experimental work in ferroelectrics for their masters thesis, with Dr. J. A. Gonzalo. Below: Discussion of low temperature equipment used with X-ray sources in radiation effects studies. Left to right: Drs. P. Paraskevoudakis, B. Cruz, H. Gomberg, and F. Vazquez.
NUCLEAR SCIENCE

The Nuclear Science Division supports the M.S. degree programs in Chemistry and Physics of the University of Puerto Rico at Mayaguez by providing research opportunities for graduate students and faculty to teach specialized advanced courses. Research facilities are also made available to graduate students of Nuclear Engineering and Electrical Engineering as well as for pre- and post-doctoral students of other universities interested in working at PRNC.

A US AEC grant for research in "Hot-Atom Chemistry" and National Institute of Health grants for research in "Radiolysis of Peptides" and "Synthesis of Thiasteroids" have contributed to expand the chemistry program. Equipment has been acquired for gas shock-tube experiments, for work in mass spectrometry of volatile compounds, and for the study of electroreflectance in semiconductors. A special facility for handling radioactive iodine was built.

EDUCATIONAL ACTIVITIES

Graduate Courses

During 1967 the following five graduate courses were taught by PRNC personnel, with academic credit given by the UPR:

<table>
<thead>
<tr>
<th>Course</th>
<th>Professor</th>
<th>Enrollment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Kinetics</td>
<td>Dr. Owen H. Wheeler</td>
<td>3</td>
</tr>
<tr>
<td>Solid State Seminar</td>
<td>Dr. Julio Gonzalo</td>
<td>2</td>
</tr>
<tr>
<td>Radiochemistry</td>
<td>Dr. Rupert A. Lee</td>
<td>3</td>
</tr>
<tr>
<td>Solid State Physical Electronics</td>
<td>Dr. Florencio Vázquez</td>
<td>5</td>
</tr>
<tr>
<td>Advanced Electricity and Magnetism</td>
<td>Dr. Baltazar Cruz</td>
<td>4</td>
</tr>
</tbody>
</table>
**Thesis Research**

Sixteen graduate students from Puerto Rico, Colombia, and El Salvador are doing thesis research under Nuclear Science Division staff supervision:

<table>
<thead>
<tr>
<th>Thesis Title</th>
<th>Student</th>
<th>Advisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A physico chemical study of the Chapman rearrangement.</td>
<td>Fernanda Román</td>
<td>Dr. O. Rosado</td>
</tr>
<tr>
<td>Effect of gamma irradiation on essential oils.</td>
<td>Elba Díaz</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Synthesis of thiosteroids.</td>
<td>Wilfredo Rodríguez</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Radiolysis of peptides.</td>
<td>Dolores Julián</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Recoil labeling of aromatic compounds with halogen atoms.</td>
<td>Hilda López</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Reactivity of cyclic ketones.</td>
<td>Rosita Báez de Feliú</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Neutron activation of aromatic iodine compounds.</td>
<td>Carmen Lecumberbery</td>
<td>Dr. O.H. Wheeler</td>
</tr>
<tr>
<td>Ferroelectric properties of the alkaline selenites.</td>
<td>Luis Carlo Niño</td>
<td>Dr. J.A. Gonzalo</td>
</tr>
<tr>
<td>Radiation damage in KDP and ADP single crystals.</td>
<td>Iván Nazario</td>
<td>Dr. J.A. Gonzalo</td>
</tr>
<tr>
<td>High frequency behavior of antiferroelectric ADP.</td>
<td>Mario Beauchamp</td>
<td>Dr. J.A. Gonzalo</td>
</tr>
<tr>
<td>In-pile radiolysis of hydrogen chloride gas.</td>
<td>Luis Rivera Oyola</td>
<td>Dr. R.A. Lee</td>
</tr>
<tr>
<td>Leaching of copper ores.</td>
<td>Samuel Rodríguez</td>
<td>Mr. Fausto Muñoz</td>
</tr>
<tr>
<td>The effect of radiation on fluoroform.</td>
<td>José Mario Saca</td>
<td>Dr. R.A. Lee</td>
</tr>
</tbody>
</table>
Thesis Title
Color center energies of alkali halides. José Castro (Inst. of Modern Sciences)

The effect of incident photon energy on RbBr and KCl crystals.
Fernando Díaz

Electroreflectance on Ge, Julio Marrero Si, Mg2Si, Mg2Ge semiconductor crystals.

Advisor
Dr. I. Cantarell

Dr. B. Cruz

Dr. F. Vázquez

Oak Ridge Research Participation Program

Dr. Herbert S. Verter, Head of the Chemistry Department at Inter-American University, San Germán, Puerto Rico, spent the summer in the Division as an Oak Ridge Research Participant doing work on the synthesis of radiopharmaceuticals labeled with iodine-132. Dr. L.A. Felúi of the UPR Chemistry Department in Mayaguez was given an ad honorem appointment to collaborate in this investigation.

Doctoral Research

Two staff members successfully presented their doctoral dissertations. Dr. Baltazar A. Cruz received his Ph.D. in Physics from Harvard University (May 1967). His thesis research was on "F Center Formation at 78°C K in KBr During Exposure to Monochromatic X-ray Energies Around the Bromine K Edge." Dr. Rupert A. Lee obtained his Ph.D. in Chemistry at London University (June 1967). His thesis research was on "Radiolysis of Gaseous Hydrogen Halides."

Plowshare Training

Mr. Jaime Román, M.S., Assistant Professor in Analytical Chemistry, University of Puerto Rico at Mayaguez, and Mr. Carlos Ortíz, graduate student, spent the summer developing methods for the analysis of copper in minerals to initiate the Plowshare study of mining with nuclear explosives.

Research Completed

F-Centers Formation in KBr using Monochromatic X-rays - B. Cruz (Ph.D. Physics, May 1967). In KBr irradiated at 78°C K the rate of formation of F centers per unit energy retained in
the crystal was found to be the same whether 13.4 keV or 14.1 keV was the incident photon energy (the K edge of bromine is at 13.5 keV). At either incident photon energy the energy expenditure per F center formed was 1.21 x 10^3 eV ± 4.4%. This result indicates that K shell ionization in bromine does not affect the mechanism of formation of F centers in KBr. However, a large background of multiple ionized atoms is expected whether the incident photon energy is 13.4 keV or 14.1 keV. Each of these atoms becomes multiply ionized after losing an electron in the L shell. Thus, in a bromide the equality of the rate of formation of F centers above and below the K edge is not sufficient evidence to conclude that a Varley mechanism of F center formation is not operative. Each of the monochromatic beams was at the fluorescent output of RbCl filtered with a thin layer of NaBr or of Sr(NO3)2 filtered with RbCl. The output of a Machlett X-Ray Tube operated at 45 kv was used to excite the fluorescence. The current in the tube was regulated to achieve the same energy flux on the sample regardless of the incident photon energy. An air ionization chamber was calibrated with a calorimeter and was used to measure the flux. The half-width of each monochromatic beam was 330 eV. Measurements of the energy expenditure required to form one F center were: for KCl at 78°K, 6.9 x 10^2 eV ± 4.2%; for KBr irradiated at 300°K, 4.8 x 10^3 eV ± 5.7%; for RbBr at 78°K, 1.2 x 10^3 eV ± 17%; and for RbBr at 300°K, 1.3 x 10^3 eV ± 30%.

Radiolysis of Gaseous Hydrogen Halides - R.A. Lee (Ph.D. Chemistry, June 1967). Ion pair yields of 4.1 and 4.7 have been measured for the radiolysis of pure gaseous hydrogen chloride and hydrogen bromide, respectively, using 60Co gammas. These yields, together with the W values for HCl (24.8 eV) and HBr (24.4 eV) obtained, lead to calculated G-values of 8.3 and 9.6, respectively. The effect of the scavengers bromine and sulphur hexafluoride on the radiolysis of the two gases revealed that there are at least two hydrogen forming species present. One of these is a "thermal" hydrogen atom which may or may not have the electron as its precursor; the other is a "hot" hydrogen atom which cannot be scavenged. The results with chlorine as scavenger on the HCl radiolysis brought out the importance of back reactions taking place as the concentration of chlorine builds up during the irradiation of pure gaseous hydrogen chloride. A study of the effect of an applied electric field during the radiolysis of the two gases showed that reactions involving the recombination of ions were unimportant as a means of producing hydrogen. This study has shown that dissociative excitational processes
are as important as ionization processes in the decomposition of these two hydrogen halides. The HBr molecules appear to undergo dissociative excitational processes more readily than the HCl molecules.

Radiation damage at $T < T_c$ in TGS and Rochelle Salt - K. Okada (Nagoya Institute of Technology), J.A. Gonzalo, and J.M. Rivera. An experimental study of the effects of ionizing radiation on triglycine sulfate and Rochelle salt was carried out. The investigation was extended up to relatively high doses, in order to ascertain whether the reported drastic decreases and disappearance of the hysteresis loops and the well-known blurring of the peak in the dielectric constant vs. temperature were related to the destruction of ferroelectricity itself in the crystal, or, rather, to the progressive masking of ferroelectric characteristics by the presence of a large accumulation of radiation-induced defects in an essentially ferroelectric environment. Present experiments included measurements of the temperature dependence of hysteresis loops and dielectric constant at increasing values of the irradiation dose, under zero and variable d.c. fields. It was found that the ferroelectric characteristics remain well defined up to doses as high as 10 Mrad. for triglycine sulphate. Similar results were obtained for Rochelle salt. In this case, however, the ferroelectric characteristics of the crystal disappeared at about 2 Mrad. as a consequence of the collapse of both Curie points. The changes in domain structure of Rochelle salt were also investigated by using the polarizing microscope, and interpretation was given, which is consistent with the results of the dielectric observations.

Gamma Irradiation of Liquid Aldehydes - O.H. Wheeler and E. Díaz de Osborne (M.S. thesis, February 1968; on leave of absence from the Food Laboratory, University of Puerto Rico). The gamma radiolysis of decanal, citral, citronellal, and benzaldehyde was studied in the liquid phase. In the absence of air, the $G_M$ values were very low ($\sim 0.2$ for benzaldehyde and $\sim < 0.04$ for the other aldehydes). The only reaction detected was polymerization and the gaseous products were formed with $G < 10^{-5}$. In 0.8N acetic acid, citral and citronellal were destroyed with a $G$ value of 0.1, and peroxide formation was noted.
Radiation Protection of Glycine and Glycylglycine -

O.H. Wheeler, M. Santos Sánchez, R.A. Ribot, and M. Ramos (NIH project). The reduction in the G values for glycine and glycylglycine in aqueous solution was determined by measuring the unchanged substrate in the form of carbon-14 labeled compounds. The order of effectiveness for radiation protection for glycine was: cysteine > thiocurea > penicillamine > dimethylsulfoxide > methionine > phenylalanine. For glycylglycine the order was: thiocurea > cysteine > penicillamine > tyrosine > methionine > dimethylsulfoxide > phenylalanine.

Synthesis of Thiosteroids - O.H. Wheeler, E.E. Rodríguez and W. Rodríguez (M.S. thesis, March 1968) (NIH project). A steroid analog was synthesized from thiophene as ring A and successively adding on ring B in the α, β -positions of thiophene and then ring C with ring D as a six-membered ring. Attempts were made to link the thiophene rings in the 3, 3'-positions by first protecting the α, α1 - positions with iodide, carbomethoxy, methyl, or t-butyl groups. However, these groups either reduced the reactivity of the thiophene ring in Friedel-Craft reactions, or hindered the subsequent cyclization reactions.

RESEARCH IN PROGRESS

Piezoelectric Characteristics of LiH₃(SeO₃)₂ - J.M. Rivera and J.A. Gonzalo. A preliminary study of the piezoelectric and elastic properties of LiH₃(SeO₃)₂ at 93°C (close to the melting point) has been undertaken. Accurate measurements of the dielectric constant as a function of frequency showed resonance peaks in the 10⁵ - 10⁷ c/s region which gave reasonable estimates of the piezoelectric coefficient and Young's modulus along the ferroelectric axis, which is perpendicular to the (001) plane.

Dielectric Properties of Alkaline Selenites - L.C. Niño and J.A. Gonzalo. Single crystals of NaₓKₓ(1-x)H₃(SeO₃)₂ (with x = 0.95, 0.90, 0.80) have been grown in our laboratory, and measurements of dielectric constant and hysteresis loops are being undertaken with the object of determining the dielectric phase diagram of the solid solution. Some single crystals of the analogous systems NaₓLiₓ(1-x)H₃(SeO₃)₂ and NaₓCsₓ(1-x)H₃(SeO₃)₂ have been grown, and it is intended to complete both series for further dielectric characterization. The Curie temperature is expected to change gradually with composition.
Scope and camera used for hysteresis loops measurement in ferroelectrics at low temperatures.

Phase Transitions in NaH$_3$(SeO$_3$)$_2$ - L.C. Niño and J.A. Gonzalo

Dielectric constant and hysteresis loop measurements have been performed in single crystals of this material. The previously reported phase transition at -79°C was observed, and in addition to it a second phase change at around -165°C was detected, throughout the disappearance of hysteresis loops and a jump in the dielectric constant. Dielectric constant measurements as a function of temperature in the ferroelectric phase are not reproducible, unless a strong a.c. field is applied to the sample for a few minutes.

γ-irradiation of Simple Organic Fluorides: CH$_3$F and CHF$_3$

R.A. Lee. Work has begun on the γ-radiation of the simple organic fluorides CH$_3$F and CHF$_3$. In the chloride compounds, the hydrogen chloride formed is due to the abstraction of the halogen atom by a hydrogen atom. In the case of the fluorides, hydrogen would be expected in preference to HF. The products will be identified by mass spectrometry.
**Electroreflectance from Semiconductor Crystals** - F. Vázquez.

A study was made on the band properties of magnesium silicide, germanide and stannide. The technique used was introduced two years ago and is known as electroreflectance technique. An electric field was applied to the reflecting surface by introducing the sample in an electrolyte. From the reflectance data, information was obtained on the energy dependence of the dielectric constants and absorption coefficient. Finally, the transitions were studied in view of the available energy band calculations.

**Monochromatic X-ray Effects on the Formation of Color Centers in Alkali Halides** - E. Cruz and F. Díaz. A study of the effect of a halide K shell ionization in the coloration of KCl crystals is in progress. Similar work is being done on RbBr and on impurities in KI and KCl. Necessary equipment for variable temperature work, and for luminescence studies, is being acquired.

**Free Energy Expansion Coefficients from Double Hysteresis Loops** - J.A. Gonzalo. A direct method has been developed to determine the temperature dependence of the coefficients of the free energy expansion \( A = 1/2X^2 + 1/4 \xi P^4 + 1/6 \eta P^6 \ldots \) of a crystal spontaneously polarized in a temperature region where double hysteresis loops are observable (This implies that \( \xi < 0 \)). The features of the \( (P) \) vs. \( (E = \delta A/\delta P) \) relationship are fully displayed in the double hysteresis loop, thus allowing the simultaneous determination of \( \chi \), \( \xi \) and \( \zeta \). The coefficient \( \chi \) can be measured directly from the slope \( (\delta P/\delta E) \) of the straight line at the center of the double loop. We can define \( P_{c+} \) and \( P_{c-} \) as the polarizations before "switching" for decreasing and increasing field, respectively. This characterizes one of the single loops in the \( (P) vs (E) \) diagram, and one can easily compute \( \xi \) and \( \zeta \). This method has been applied to \( BaTiO_3 \). Oscillograms of the double hysteresis loops above \( T_0 = 111.5^\circ C \) were collected and the temperature dependence of \( \chi \), \( \xi \) and \( \zeta \) determined.

**Mechanism of Thermal Aromatic Rearrangements** - O.H. Wheeler, O. Rosado, and F. Roman (M.S. June 1968; on leave of absence from the Industrial Development Laboratory, Commonwealth of Puerto Rico). The mechanism of the Chapman rearrangement of phenyl benzimino ethers to benzycldiphenylamines is being studied by using carbon-14 labeled phenyl ethers. The allied rearrangement
of allyl benzimino ethers is also being investigated by using a tritium labeled allyl compound.

**Radiolysis of Peptides - O.H. Wheeler and D. Julián (M.S., March 1968)(NIH project).** Labeled glycyglycine, phenylalanyl-glycine, and glycylphenylalanine have been synthesized from carbon-14 labeled amino acids. Their radiolysis in aqueous solution is being studied using paper chromatography to separate and identify the products.

**STAFF**

Dr. Juan F. Facetti, a former member of our staff, who is now in charge of the Physics Department at the National University of Asunción, Paraguay, spent two weeks at PRNC during the 10th Anniversary celebration, and plans were arranged for cooperative research efforts in the future between his group and our staff.

Mr. Luis Rivera Oyola, research assistant and graduate student in nuclear engineering, was awarded an AEC traineeship in nuclear engineering in August.

Miss Milagros Santos, M.S., research associate on an NIH financed project concerning the "Radiolysis of Peptides," resigned in January to accept an appointment as chief chemist in a pharmaceutical laboratory.

Miss Carmen Lecumberry joined the staff of the Chemistry Department, University of Puerto Rico at Mayaguez in July.

Miss Dolores Julián, who was with the "Radiolysis of Peptides" project, joined the Chemistry Department, Inter American University in September. Both are completing their thesis studies in the Division.

Mrs. Myriam Ramos, also with the "Radiolysis of Peptides" project, joined the Department of Biology, Regional College of the University of Puerto Rico at Arecibo, in August.

Mr. Grafton Olivera, a former senior student who participated in research training, left PRNC to join the Research Laboratory of the ITT in San Juan, Puerto Rico.
Nuclear Engineering students working with the Division's Subcritical Assembly. Left to right: Antonio Rivera Cordero, Luis Rivera Oyola, and Raphael L. Ufret.
NUCLEAR ENGINEERING

The primary function of the Nuclear Engineering Division is to teach graduate courses for the students of nuclear engineering at the University of Puerto Rico at Mayagüez and to direct their research, and the thesis research of students from other universities in the United States and Latin America. This division also offers occasional short courses for scientists, engineers, and technicians, and for staff members engaged in individual research.

EDUCATIONAL ACTIVITIES

Master of Science Degree Program

UFR at Mayagüez, in close cooperation with PRNC's Nuclear Engineering Division, offers the Master of Science degree in Nuclear Engineering. The closeness of this relationship is illustrated by the fact that the faculty of the UFR Department of Nuclear Engineering is composed largely of staff members of the PRNC Nuclear Engineering Division, and the director of the UFR department is also the head of the PRNC division. In addition, the PRNC Nuclear Engineering Division provides the classrooms, offices, laboratories, equipment, and administrative personnel necessary for the education and training of the UFR nuclear engineering students. The requirements are 30 hours of graduate work, including the satisfactory completion of a thesis. Applicants for admission to this program must have a bachelor's degree in engineering or physics.

The basic pedagogical method is the presentation of lectures, strongly reinforced by laboratory work with various types of radiation counting equipment, the subcritical reactor, the I-77 low power reactor, and the PRNC one-megawatt reactor. Each student is also required to use both an analog and a digital computer and to present a seminar on his research to the PRNC staff. The students are encouraged to choose research topics related to their specific interests and those of their sponsoring countries or organizations. A description of the courses included in the nuclear engineering curriculum follows.

- Nuclear Reactor Technology Three lectures and one three-hour laboratory demonstration period per week. Steady-state and transient thermal conduction in fuel elements; thermal convection in heat-exchanger design; liquid metal systems; circulating fuel systems; time behavior of reactor systems; breeding and conversion;
an introduction to the economics of reactor operation; reactor engineering design problems.

- **Nuclear Measurements and Instrumentation** One lecture and two three-hour laboratories per week. Characteristics of operation and a thorough familiarization with the application of specialized techniques such as: coincidence and anticoincidence counting, pulse analysis, neutron spectrometry, gamma ray spectrometry, and so on.

- **Elements of Nuclear Engineering** Four lectures per week include characteristics of the atomic nucleus, radioactive decay, interaction of radiation and matter, and basic neutronics.

- **Graduate Seminar** Two hours per week include reports and discussions on special topics in nuclear science and engineering.

- **Reactor Theory** Three lectures per week. Consists of neutron balance equation, diffusion theory, and slowing down theory, bare homogeneous reactor, reflected reactor, heterogeneous reactor, time dependent reactor, perturbation theory and transport theory.

- **Advanced Reactor Theory** Three lectures per week. Advanced transport theory, reactor kinetics, and heterogeneous reactor theory.

- **Reactor Laboratory** One lecture and one three-hour design period per week. Laboratory problems involving the nuclear reactor.

- **Nuclear Engineering Application of Wavesmecanics I** Two lecture discussions per week. Physical behavior and properties of moderator nuclei. Fission properties of fuel nuclei. Collision theory. Quantum mechanics discussion of the Doppler Effect.

- **Nuclear Engineering Application of Wavesmecanics II** Two lecture discussions per week. A continuation of the same topics covered in the "Wave Mechanics I" course, including perturbation and control rod theory and the effect of anisotropic scattering.

- **Special Problems** One to three periods per week each semester related to the investigation of special problems in Nuclear Engineering. (Note: During the second semester of this
school year, Dr. Knud Pedersen conducted a three credit hour special problems course in Flowshare).

- **Research**  No credit. The student is awarded six credits for his thesis upon satisfactory completion and presentation of a thesis. One to twelve research periods per week. Research in the field of nuclear engineering.

- **Mathematics of Modern Science I**  Three lectures per week in determinants and matrices, finite differences, Fourier series and integrals, and Laplace transformation.

- **Mathematics of Modern Science II**  Three lectures per week. Partial differential equations, Bessel functions and Legendre polynomials, and complex variables.

**Supplementary Courses**

- **Nuclear Reactor Metallurgy**  Two lectures and one three-hour lab session each week. An introduction to elementary physical metallurgy of the principal reactor materials such as aluminum, zirconium, uranium, and high temperature alloys; mechanical properties; fabrication of nuclear fuels; radiation damage to reactor components.

- **Introduction to Nuclear Engineering**  For advanced undergraduate and non-nuclear engineering graduate students; three lectures each week in fission and chain reactions, elements of reactor design, utilization of nuclear energy for power, and radiation problems.

**Non-Degree Program**

In addition to the Master of Science degree program, the Division offered or participated in two special programs:

1) An intensive six-week seminar on Flowshare (peaceful uses of nuclear energy) was offered during the summer of 1967. The seminar was conducted by Dr. James A. Cheney of the Department of Civil Engineering and Dr. Wilson K. Talley of the Department of Applied Sciences of the University of California at Davis, and drew its participants from the staff of the FRNC and the faculty of the University of Puerto Rico.
Above: Control rods are adjusted before transfer function measurement on the L-77 reactor. Below: Transfer function measurement experiment on the L-77 reactor.
2) Dr. Donald S. Sasscer taught the physics part of an Office of Civil Defense, Nuclear Fallout Shelter Summer Institute. This institute was attended by professors representing 28 different universities from 20 different states.

**STAFF**

Dr. Knud B. Pedersen joined the division in June. For the past three years he has been teaching in the Departments of Nuclear Engineering and Engineering Science at Iowa State University. Before that Dr. Pedersen was a member of the staff of the Ames Laboratory of the AEC. He has been active in research in the areas of metal fatigue, nuclear cratering similitude, and reactor kinetics.

Dr. Donald Sasscer attended the International Conference on the Utilization of Research Reactors and Reactor Mathematics Computation in Mexico City, Mexico, May 2-4. During the second week of May, Dr. Sasscer participated in the Atoms in Action program in Panama by giving a series of lectures to the students of the University of Panama, and by consulting with them concerning research problems related to Nuclear Engineering.

Dr. Aviva E. Gileadi participated in the 13th Annual Conference of the American Nuclear Society held in San Diego, California, during July. Following this, Dr. Gileadi visited the Brookhaven National Laboratory for consultation concerning computer calculation related to neutron cross sections.

Dr. Knud Pedersen presented a paper entitled, "Measurement of Shutdown Margin," to the 14th Annual IEEE Nuclear Science Symposium held in Los Angeles, California, October 31 to November 5.

During August, Dr. Sasscer visited the Associated Midwestern Universities-Argonne National Laboratories, Summer Engineering Practice School at the Argonne National Laboratory for an evaluation of the research activities of Mr. Antonio Rivera Cordero, a graduate student in Nuclear Engineering at the UPR, who was attending the school. At the end of August, Dr. Sasscer attended the Nuclear Science and Engineering Fellowship Advisors meeting at Gatlinburg, Tennessee.
STUDENTS

During 1967, an Ecuadorian, a Mexican, a Cuban, a student from nationalist China, and seven students from Puerto Rico participated in the Masters Degree program. Three of the students were US AEC trainees and one an International Atomic Energy Agency fellow.

Three students presented papers to the Fifth Annual Student Conference of the American Nuclear Society held in Gatlinburg, Tennessee, in April:

1. Antonio Rivera - "Investigation of Puerto Rico Nuclear Center Beam Tube Explosion."

2. José E. Aguiar - "Design Criteria to Increase the Sensitivity of the Total Absorption Calorimeter for Soft X-Rays".

3. Francisco Jiménez - "G-Value Determination of the Fricke Dosimeter for Monochromatic X-Rays from 5 to 10 Kev".

Mr. Antonio Rivera attended the three-month Associated Midwestern Universities-Argonne National Laboratories, Summer Engineering Practice School held at Argonne National Laboratory during June, July and August.

Four students obtained the Master of Science degree in Nuclear Engineering from the Mayaguez Campus of the University of Puerto Rico under the research direction of the scientific staff of the PRNC (See Table 1).

The progress of the students active in the Nuclear Engineering program during 1967 is shown in Table 2.

Students enrolled in the Nuclear Engineering Master of Science degree program are given in Table 3.
# TABLE 1

Master of Science Degrees in Nuclear Engineering 1967

<table>
<thead>
<tr>
<th>Student</th>
<th>Thesis</th>
<th>Research Director</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aguiar Aramburu, José</td>
<td>&quot;Design Criteria to Increase the Sensitivity of a Total Absorption Calorimeter for Soft X-Rays&quot;</td>
<td>Dr. Peter Paraskevoudakis</td>
</tr>
<tr>
<td>Jiménez Rosado, Francisco</td>
<td>&quot;G-Value Determination of a Frick Dosimeter for Monochromatic X-Rays from 0 to 15 Kev&quot;</td>
<td>Dr. Peter Paraskevoudakis</td>
</tr>
<tr>
<td>Muñoz Ribadeneira, Fausto</td>
<td>&quot;Estudios de la Relación de Lixiviación de un Mineral de Uranio Tipo Carnotita con Acido Sulfúrico Empleando Métodos Comunes y Energía Ultrasónica&quot;</td>
<td>Dr. Rafael Muñoz Candelario</td>
</tr>
<tr>
<td>Reoyo Sánchez, Carlos</td>
<td>&quot;Fuel Element Maximum Temperature as a Result of a Loss of Coolant as a Function of Operating Power Before Shutdown&quot;</td>
<td>Prof. Kenneth Soderstrom</td>
</tr>
</tbody>
</table>

# TABLE 2

Progress of Nuclear Engineering Students in 1967

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Students who received the M.S. degree in Nuclear Engineering</td>
<td>4</td>
</tr>
<tr>
<td>Students who have completed all course work for degree (presently working on theses)</td>
<td>4</td>
</tr>
<tr>
<td>Students engaged in course work for M.S. degree</td>
<td>5</td>
</tr>
<tr>
<td>Name</td>
<td>Sponsor</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Antonio Castro Rosario, Puerto Rico</td>
<td>UPR</td>
</tr>
<tr>
<td>Cho-fu Lee, China</td>
<td>IAEA</td>
</tr>
<tr>
<td>Fernando López Carrasco, Mexico</td>
<td>CNEN</td>
</tr>
<tr>
<td>Gilberto Ramos Cuesta, Cuba</td>
<td>Self</td>
</tr>
<tr>
<td>Fernando Pérez Bracetti, Puerto Rico</td>
<td>UPR</td>
</tr>
<tr>
<td>Antonio Rivera Cordero, Puerto Rico</td>
<td>AEC</td>
</tr>
<tr>
<td>Luis Rivera Cyola, Puerto Rico</td>
<td>AEC</td>
</tr>
<tr>
<td>Francisco Rodríguez Perazza, Puerto Rico</td>
<td>UPR</td>
</tr>
<tr>
<td>Rafael L. Ufret Acevedo, Puerto Rico</td>
<td>AEC</td>
</tr>
<tr>
<td>Gilberto Vélez Delgado, Puerto Rico</td>
<td>PRWRA</td>
</tr>
</tbody>
</table>

(Puerto Rico Water Resources Authority)
RESEARCH PROJECTS

Design Criteria to Increase the Sensitivity of a Total Absorption Calorimeter for Soft X-Rays - Dr. Peter Paraskevoundakis and José E. Aguiar.

G-Value Determination of a Frick Dosimeter for Monochromatic X-Rays from 0 to 15 Kev - Dr. Peter Paraskevoundakis and Francisco Jiménez.


Estudios de la Relación de Lixiviación de un Mineral de Ura- nio Tipo Carnotita con Acido Sulfurico Empleando Métodos Comunes y Energia Ultrasónica - Dr. Rafael Muñoz Candelario and Fausto J. Muñoz-Ribadeneira.

Measurement of the Transfer Function of the L-77 Reactor - Dr. Aviva E. Gileadi and Fernando López.


Criticality Calculations - Dr. Aviva E. Gileadi and Manuel Rodríguez Perazza.

Investigation of Puerto Rico Nuclear Center Beam Tube Explosion - Dr. Donald S. Sasscer and Antonio Rivera.

Calculation of the Time and Space Dependent Neutron Densities Following an Underground Nuclear Explosion in Various Rocks (Plowshare Oriented Calculation) - Dr. Aviva E. Gileadi.

Stress Corrosion Cracking of Metal Alloys - Dr. Phillip Osborne and Erick Méndez.
Dr. Jose P.A. Castrillon of Argentina prepares apparatus for the chromatographic of the sulfoxide of thioxanthene.
PHYSICAL SCIENCES *

The long range objective of the Physical Sciences Division (formerly called the Radioisotope Applications Division) is to offer advanced training opportunities for Puerto Rican and Latin American trainees primarily through participation in research projects involving the use of high energy radiation and radioisotopes. Since this program is geared to regional needs, it includes an introductory training course in the use of isotopes, and requires a heavy participation of the scientific personnel in the academic activities of the natural sciences departments of the University of Puerto Rico, Río Piedras campus. The latter cooperative effort is encouraged through joint appointments.

EDUCATIONAL ACTIVITIES

Activities range from a four-week non-credit training course in the techniques of radioisotope applications to research training in the laboratories of the Center.

Radioisotopes Techniques Course

Offered four times during 1967. The distribution of the seventeen trainees (Table 1) by geographical origin shows seven participants from Puerto Rico, three from the Dominican Republic, two from Colombia, and one each from Uruguay, Perú, Chile, Bolivia, and Venezuela.

University Level Courses

- Radiochemistry A one-semester graduate course, taught by Dr. José P.A. Castrillón, Associate Scientist, with the participation of four students, and utilizing the PRNC laboratory facilities.

* As a reflection of the development in the division of research training opportunities in more general areas related to the nuclear field, the AEC approved the change in the name of the division from 'Radioisotope Applications' to 'Physical Sciences' on July 6, 1967.
<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Silvio Aristizabal, M.D.</td>
<td>Resident III in Radiotherapy (Had additional training in Clinical Applications, PRNC).</td>
</tr>
<tr>
<td>2.</td>
<td>Francisco Touya, M.D.</td>
<td>Member of the Faculty of Medicine-Nuclear Medicine Center, Montevideo, Uruguay. (Had special training in Clinical Applications and in Medical Sciences and Radiobiology, PRNC).</td>
</tr>
<tr>
<td>3.</td>
<td>Emperatriz Chavarro</td>
<td>Radioisotope Technologist, National Institute of Cancerology, Colombia (Had additional training in Clinical Applications, PRNC).</td>
</tr>
<tr>
<td>4.</td>
<td>M.J. Melo Batista, M.D.</td>
<td>Doctor in Medicine, Radioisotope Dept., Hospital Dr. S.B. Gautier, Sto. Domingo (Had additional training in Clinical Applications, PRNC).</td>
</tr>
<tr>
<td>5.</td>
<td>Alba L. Rico de Puente</td>
<td>Medical Technologist-Experimental Surgery, UPR, School of Medicine (Had additional training in Clinical Applications).</td>
</tr>
<tr>
<td>6.</td>
<td>Abel Rossy</td>
<td>Research Technician, Terrestrial Ecology Program, PRNC.</td>
</tr>
<tr>
<td>7.</td>
<td>Ilvia Millán</td>
<td>Medical Technologist-Pulmonary Function Studies, UPR School of Medicine (Had additional training in Clinical Applications).</td>
</tr>
<tr>
<td>8.</td>
<td>Lydia N. de Reyes, M.D.</td>
<td>Radiologist, University District Hospital, Medical Center, Río Piedras.</td>
</tr>
<tr>
<td>9.</td>
<td>Roger Ramos Aliaga</td>
<td>Biochemist, Faculty of Medicine, Cajamarca, Perú. (Had additional training in Clinical Applications, and is having special training in Medical Sciences and Radiobiology).</td>
</tr>
<tr>
<td>10.</td>
<td>Helen Rodríguez de Curet, M.D.</td>
<td>Clinical Research Center, UPR, School of Medicine.</td>
</tr>
<tr>
<td>11.</td>
<td>Sergio Silva</td>
<td>Graduate student in Biochemistry, UPR School of Medicine.</td>
</tr>
</tbody>
</table>
Dr. Manfred Eberhardt preparing equated samples for gamma-radiolysis studies.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TABLE 1</strong></td>
<td></td>
</tr>
<tr>
<td>PARTICIPANTS IN BASIC COURSE IN RADIOISOTOPE TECHNIQUES, 1967</td>
<td></td>
</tr>
<tr>
<td>(Cont.)</td>
<td></td>
</tr>
<tr>
<td>12. Claudio Zuñiga</td>
<td>Doctor in Medicine (Hematology), Clinical Hospital, University of Chile. (Had additional training in Clinical Applications). Is taking radioisotope training in Spain.</td>
</tr>
<tr>
<td>14. Mercedes Iñiguez de Nuño, M.D.</td>
<td>Doctor in Medicine, Hospital S.B. Gautier, Santo Domingo. (Had additional training in Clinical Applications, PRNC).</td>
</tr>
<tr>
<td>15. Jorge Mariaca, M.D.</td>
<td>Doctor in Medicine, Bolivian Commission of Nuclear Energy, Bolivia. Is having special training in Clinical Applications.</td>
</tr>
<tr>
<td>16. Zobeida Malpas</td>
<td>Radioisotope Technologist, University Hospital, Venezuela. (Had additional training in Clinical Applications).</td>
</tr>
<tr>
<td>17. Américo Rivera, Ph.D.</td>
<td>Research Biochemist, NIH, NINDB, Laboratory of Perinatal Physiology, San Juan</td>
</tr>
</tbody>
</table>
• Theory of Organic Chemistry A two-semester graduate course, taught by Dr. H. Harry Szram, Chief Scientist, with the participation of twelve students.

• Advanced Physical Chemistry A one-semester graduate course, taught by Dr. Alec Grimison, Chief Scientist, with the participation of twelve students.

• Chemical Kinetics A one-semester graduate course, taught during the summer session by Dr. George A. Simpson, Associate Scientist, with the participation of eight students.

• Instrumental Analysis and Advanced Inorganic Chemistry Two one-semester graduate courses, taught by Dr. Mariel Muir, Associate Scientist, with the participation of fifteen students in each course.

Graduate Research

The graduate students supervised by PRNC personnel, and their geographical origin, are shown in Table II.

During 1967 Miss Gladys Rodríguez completed her thesis work for the M.S. degree, and has joined the teaching staff of the Chemistry Department, Catholic University of Ponce, P.R.

Non-Credit Graduate Research Training

• During the summer of 1967 Dr. Martin Feldman, Assistant Professor at Howard University, Washington, D.C., worked on the gamma-radiolysis of dimethyl sulfoxide in freon and methyltetrahydrofuran matrices in collaboration with Drs. Simpson, Grimison, and Szram. Dr. Feldman's visit was under the auspices of the Oak Ridge Research Participation Program.

• Mr. Sergio Quadri, Assistant Professor, University of Concepción, Chile, received nine months' training which included work with Drs. G. Simpson and A. Grimison on gamma irradiation of heterocyclic molecules.

Undergraduate Research Training

The following senior science students took advantage of the research training opportunities of PRNC during 1967: Sonia Cruz Vega, Sandra Piñero Acosta, María de los Angeles Carrera, Humberto Reyes, and Angel Canales with Dr. M. Muir; Alex Bonilla, Carmen Meléndez, Donald Dexter and Félix Martínez with Drs. A. Grimison
### TABLE II
THESES RESEARCH SUPERVISED BY PRNC PERSONNEL DURING 1967

<table>
<thead>
<tr>
<th>Student</th>
<th>Country of Origin</th>
<th>Supervisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Birke, Arnoldo</td>
<td>Chile</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td>2. Cancio, Edith</td>
<td>Puerto Rico</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td>3. Castellanos, Jaime</td>
<td>Colombia</td>
<td>A. Cobas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z. Weisz</td>
</tr>
<tr>
<td>4. Colón, Jaime</td>
<td>Puerto Rico</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J.P.A. Castrillón</td>
</tr>
<tr>
<td>5. Costa, Agnes</td>
<td>Puerto Rico</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td>6. Fernández, Olga</td>
<td>Cuba</td>
<td>M. Muir</td>
</tr>
<tr>
<td>7. Gómez, Elsa</td>
<td>Venezuela</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td>9. Rechani, Pio</td>
<td>Puerto Rico</td>
<td>M. Muir</td>
</tr>
<tr>
<td>10. Riquelme, Ida</td>
<td>Cuba</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td>11. Rodríguez, Gladys</td>
<td>Puerto Rico</td>
<td>A. Grimison</td>
</tr>
<tr>
<td>12. Trujillo, Myrtha</td>
<td>Cuba</td>
<td>A. Grimison</td>
</tr>
<tr>
<td>13. Werr, Fayez</td>
<td>Lebanon</td>
<td>H.H. Szmant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. Eberhardt</td>
</tr>
<tr>
<td>15. Sprangle, Philip</td>
<td>U.S.A.</td>
<td>A. Grimison</td>
</tr>
</tbody>
</table>

Several of the graduate students are expected to complete their M.S. thesis requirements during 1968, and some plan to continue work toward the Ph.D. degree in Chemistry since this program is soon to be officially approved.
"Atoms in Action" Exhibit in Quito, Ecuador

In November, 1967, Dr. H. Harry Szmant participated in the AEC "Atoms in Action" Exhibit in Quito, Ecuador. Small research projects were initiated with two instructors of the Central University. Some results of previous participation by Dr. Szmant and Dr. Castrillón at the Costa Rica Exhibit presented at scientific meetings in Kingston, Jamaica, and Chicago, Illinois, are reported later.

STAFF

Dr. Manfred Eberhardt joined PRNC as Associate Scientist in September 1967. Dr. Eberhardt worked previously at the Radiochemistry Institute, Technical University of Munich. He will work on the gamma-radiolysis of dimethyl sulfoxide, and on steric effects in radiation chemistry.

During 1967 the following graduate students resigned their part-time PRNC research assistantships:

Mr. Jaime Colón, who continues his studies under an assistantship of the Department of Chemistry; Mr. Edgar Rodríguez and Mr. Jhaagdish Jhaveri, who discontinued graduate studies for personal reasons. New appointments were extended to Mrs. Agnes Costa, Miss Elsa Gómez, and Miss Myrtha Trujillo.

Participation in Scientific Meetings and Courses outside Puerto Rico.

Dr. H. Harry Szmant participated in the meeting of the Council of the American Chemical Society in Miami Beach, Florida, April 9-13, 1967. He formed part of the Organizing Committee for an "International Sulfur Chemistry Conference" to be held in Puerto Rico. On June 15, Dr. Szmant presented "Research in the Chemistry of Sulfoxides" as part of a cycle of lectures on "Contemporary Research in Organic Sulfur Chemistry" at Wayne State University, Detroit, Michigan.

In June and July 1967 at the Institute of Physical Chemistry,
Professor A. G. Maddock, Cambridge University (right), viewing the reactor facilities with PRNC staff.

University of Bologna, Italy, Dr. Alec Grimison participated in research on the theoretical spectra of heterocyclic radicals in collaboration with Professor Carlo Zauli, Director of the Institute.

On June 16, 1967 Dr. Szmant presented a seminar on "The Mechanism of the Wolff-Kishner Reaction" at the University of Detroit, Michigan.

On June 24-26, 1967 Dr. Szmant and Mr. Gerardo Molina received training in special techniques employed in hot-atom chemistry by Dr. Alfred Wolf at the Chemistry Department, Brookhaven National Laboratory.

On September 6-11, 1967 in an ACS Short Course at Chicago, Illinois, Dr. George A. Simpson received special training in Electron Spin Resonance Spectroscopy.

On September 11-15 at the American Chemical Society Meeting, Chicago, Illinois, Dr. Szmant also presided a scientific session of the Organic Division, and was elected a Fellow of the American Institute of Chemists.

On October 13, 1967 at the "Science in Research" sessions of the Southeastern Regional Conference of the National Science Teachers Association, San Juan, Dr. A. Cobas spoke on "Nuclear Experimentation in Puerto Rico," and Dr. H.H. Szmant on "Chemical Research at the University of Puerto Rico."

RESEARCH

The research programs of the division can be classified under the following headings: Radiation Effects, Radioisotopic Studies, and Supporting Research. The projects are described briefly below, with the senior investigators and graduate student trainees.

Radiation Effects

These projects study the effects of high-energy deposition in chemical systems. In some of the projects the emphasis is on the initial, or primary, products of radiation, while others emphasize the final products subsequent to secondary chemical reactions. However, the aim is always to trace the detailed mechanism by which radiation-induced changes occur.

**Gamma-Radiolysis of Dimethyl Sulfoxide** (H.H. Szmant, M. Eberhardt). The previous G values of several radiolysis products have now been determined with greater precision. In a typical experiment, G values of 2.24 for dimethyl sulfide formation, 0.37 for dimethyl-sulfone formation, 0.23 for formation of the methyl ester of methanethiosulfonic acid, and 0.5 for formation of a probable DMSO 'dimer' were obtained. The effect of dose and added water on these G values is currently under study. Graduate Student trainees: Agnes Costa, Edgar Rodriguez.

**Stereochemical Effects in the Radiolysis of Cis and Trans-1-2 dimethylcyclohexane** (M. Eberhardt). The effects of stereochemistry on the radiolysis of cis and trans-1-2 dimethylcyclohexane are being studied, in particular the steric effects on the formation of molecular hydrogen. This is a project initiated by Dr. Eberhardt at the Technische Hochschule, Munich, Germany, which is now to be continued at PRNC.
Dr. Manfred Eberhardt and Mr. Alfonso Mata, a student from Costa Rica, operating a vapor phase chromatograph.

**Radiation-induced Addition of Thiols to Olefinic Bonds** (HH Szmant, M. Eberhardt). The stereochemistry of radiation-induced homolytic addition of thiols to olefinic bonds is being studied, and the results compared with those in analogous chemical reactions. Graduate student trainee: Irma Zea Ponce.

**Radiation Chemistry and Photochemistry of Heterocyclic Molecules** (A. Grimison, G.A. Simpson). This project receives support from the AEC Division of Biology and Medicine, and studies the nature of primary species formed by gamma-irradiation of heterocyclic molecules. The work is described fully elsewhere in this Annual Report under Biological and Medical Research Programs. Graduate student trainee: Myrtha Trujillo Sánchez.

**Radiation Damage in Organic Crystals** (A. Cobas, S.Z. Weisz). This project receives support from the AEC Physical Sciences Division. Radiation damage in well-defined crystalline organic materials is studied by conductivity measurements. The work is described fully elsewhere in this Annual Report under the Physical Research Program. Graduate student trainee: Jaime Castellanos.

**Tritium Distribution in the Recoil Labeling of Organic Lithium Salts** (J.P.A. Castrillón). This recently initiated project investigates the radiation damage, label yield, and, principally, the tritium distribution in the parent molecule following neutron irradiation of organic lithium salts. Preliminary
results for phenylacetic acid indicate that irradiation of the lithium salt rather than the free acid results in a completely different tritium distribution, with much higher label yields. Thus 20% of the total activity is located on the α carbon atom, as against less than 2% with the standard technique. Graduate student trainee: Pio Rechani (part-time participation).

**Photochemistry of Transition Metal Complexes (M. Muir).** This new project studies the ultraviolet irradiation of a series of complexes of Pt(II) with ligands, such as pyridine, bipyridine, phenanthroline, and ethylenediamine. The major reaction suggested by the preliminary work is photosolvation, proceeding to a different extent with the various ligands.

**Radioisotope Studies**

These projects mainly involve the use of incorporated radioactive tracer atoms, as a diagnostic aid to the study of reaction mechanisms.

**The Chromic Acid Oxidation of 1,1-di(p-iodophenyl) ethane-2-C-14 (H.H. Szmant).** The mechanism of the chromic acid oxidation of 1,1-di (p-iodophenyl) ethane-2-C-14 continues to be studied. Graduate student trainee: Jaime L. Colón.

**Preparation of C-14-labeled Dimethyl Sulfoxides (H.H. Szmant, G. Molina).** C-14-labeled dimethyl sulfoxide has been prepared by the reaction of dimethylsulfoxide with C-14 labeled methyl iodide, and the subsequent decomposition of the oxosulfonium salt with pyridine. This material will be used in other research projects.


**Preparation of Tritiated Benzophenone-hydrazone (H.H. Szmant, G. Molina).** This preparation is now completed. The material will be used in the study of the Mechanism of the Wolff-Kishner reaction.

**Supporting Research**

The projects described under this heading do not directly involve the use of radiation of radioisotopes. However, they
exist in order to provide support for the previous projects by producing essential information on the systems of interest.

**Decomposition of Benzhydryldiazine (H.H. Szmant).** This project studies the preparation and base-catalyzed decomposition of benzhydryldiazine. This decomposition is directly related to the study of the Wolff-Kishner Mechanism.

**Studies of the Anions of Diaryl Ketone Hydrazones (H.H. Szmant).** The kinetics of the generation and Wolff-Kishner reaction of the anions of diaryl ketone hydrazones are being studied. Graduate student trainee: Arnoldo Birke.


**The Stereochemistry of the Thiol-Olefin Addition (H.H. Szmant, J.P.A. Castrillón).** The stereochemistry of the homolytic addition reactions of thiols to olefinic bonds is being studied, as support for the research on the radiation-induced addition. Graduate student trainees: Edith Cancio and Janice Petrovich.

**Molecular Orbital Calculations on Heterocyclic Molecules (A. Grimison, W. Adam).** A series of all-valence electron calculations on heterocyclic molecules and radicals is being carried out, using the Extended Hückel Theory. This work aims at the elucidation of the electronic structures of these systems, and derived properties. Work during 1967 was mainly concentrated on the calculation of nuclear magnetic resonance parameters from all-valence electron wavefunctions. Treatments of aryne intermediates, and of hydrogen bonding between pyridine and water, and pyridine and methanol were also completed. Graduate student trainees: Gladys Rodríguez, Phillip Sprangale.
The Division of Clinical Applications of Radioisotopes is primarily oriented to Latin American and Puerto Rican physicians who wish to acquire special skills in the human applications of radioisotopes for diagnostic and therapeutic purposes. The main training activity, the basic Clinical Applications Course, is offered to physicians wishing to qualify for obtaining a license to practice nuclear medicine or use radionuclides in humans in other connections. The Division also offers extended participation to trainees in special fields of interest, such as: extended practice in a field of the trainee's choice, a special training project, or a formal course of nuclear applications in a medical specialty. In addition, the Division offers informal teaching to the Medical Faculty, Medical Students, Nursing Staff at clinics, wards and courses through medical consultations, rounds and lectures. A semester course for medical residents (orientation clinical uses of radioisotopes) has been discontinued. Long term training for students desiring at least one year's experience is also offered.

The Division is aware of the needs of preparation of personnel with more advanced skills. As the clinical uses of radioisotopes become more generalized the need for stratification is more apparent. Special clinical radioisotopes procedures are not available to the majority of radioisotopes facilities everywhere. Hence, the need for training in this area. The Division anticipates future efforts to meet these needs.

EDUCATIONAL ACTIVITIES

Training program in the Clinical Uses of Radioisotopes. The courses offered are:

• Basic Clinical Radioisotope Applications Course

This formal eight week Course offers the trainee an opportunity to learn by direct personal experience the human uses of radioisotopes in diagnosis and therapy of diseases; provides sufficient exposure to clinical material to facilitate the correlations between laboratory and clinical findings; permits the trainee to acquire sufficient technical proficiency in diagnostic and therapeutic radioisotope procedures to enable him to qualify for license requirements. This is accomplished by a formal program of lectures which include diagnostic procedures that are currently in use in the following areas of medical diagnosis:
1. Thyroid disorders
2. Cardiovascular system
3. Renal tract
4. The liver
5. Gastrointestinal absorption
6. Hematological applications
7. Fluid compartments and electrolytes
8. Tumor localization and organ visualization
9. Radioisotope therapy

The lectures are followed by clinical radioisotope laboratory exercises: clinical discussions of incoming patients for diagnostic work up; observation of laboratory procedure; demonstrations, and personal participation or direct performance of work by the trainee under close guidance by clinical and laboratory staff.

By the end of the course trainees usually complete an average of over 100 diagnostic procedures, the minimum performance being 80 adequately performed procedures for the entire period (see Table 1).

- **Clinical Applications of Radioisotopes in Pediatrics**

This course was intended to attract pediatricians to study this phase of nuclear medicine in their specialty. It was organized with help from the Department of Pediatrics and a staff member of the Division who is a pediatrician doing radioisotope work jointly with the Department of Pediatrics of the University Hospital, School of Medicine. Since nuclear applications in Pediatrics are moving slowly in Puerto Rico, enrollees for this course have not yet appeared. But this Division has trained three pediatricians on the University Hospital medical staff who are now leaders in their respective subspecialties: one is engaged in pediatrics neurology, another in pediatric hematology, and a third is doing pediatric endocrinology.

- **Informal Courses**

These are extended periods of practical training in one of the major areas of nuclear medicine under the guidance of the clinical and laboratory staff. The areas of greater interest for trainees have been radioisotope localization studies, hematology and renal diseases.
Participants in the training program during FY 1967 are listed in Table II. A numerical tabulation of the professional background and countries of origin of the participants in the training program is presented in Table III.

Table IV presents procedures used in the teaching program by and for the students.

Table V illustrates the diagnostic and therapeutic procedures carried out at the Division.

Table VI shows the relation of the research program to the teaching units of the clinical radioisotope course.

** TABLE - I**

Two Month Training Program on Clinical Applications of Radioisotopes

<table>
<thead>
<tr>
<th>UNITS OF STUDY</th>
<th>AVERAGE NUMBER OF PROCEDURES PER TRAINEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid Function</td>
<td>35</td>
</tr>
<tr>
<td>Routine uptake measurements</td>
<td>(15)</td>
</tr>
<tr>
<td>Assay of radioactive thyroid hormone levels in blood</td>
<td>(15)</td>
</tr>
<tr>
<td>Modified tests of thyroid function</td>
<td>(5)</td>
</tr>
<tr>
<td>Dynamic functions of the hepatic, renal, and vascular systems</td>
<td>20</td>
</tr>
<tr>
<td>Hematologic applications of radioisotopes</td>
<td>5</td>
</tr>
<tr>
<td>Tumor Localization studies</td>
<td>10</td>
</tr>
<tr>
<td>Gastrointestinal absorption</td>
<td>5</td>
</tr>
<tr>
<td>Electrolyte and fluid balance</td>
<td>2</td>
</tr>
<tr>
<td>Therapeutic procedures</td>
<td>3</td>
</tr>
</tbody>
</table>

**TOTAL** 80
TABLE - 2

Basic Course in Clinical Applications of Radioisotopes

Dec. 19, 1966 to February 10, 1967
1. Hugo Ramírez Madrid
   Chile
2. Yocasta Saverví Bonilla
   Dominican Republic

February 14 to March 17, 1967
3. Marcelino Rispoli
   Argentina

April 3 to May 26, 1967
4. Emperatriz Chavarrro Rojas
   Colombia
5. Eduardo Touya
   Uruguay
6. Luis F. Barragán
   Bolivia
7. Silvio Aristizabal
   Colombia

June 12 to August 4, 1967
8. Alba L. Rico de Puente
   Puerto Rico
9. Mercedes Melo Bautista
   Dominican Republic

August 7 to September 29, 1967
10. Ilvia L. Millán Zaccheus
    Puerto Rico
11. Lidia D. Nieves de Reyes
    Puerto Rico
12. Claudio Zúñiga García
    Chile

TABLE - 3

Number of Trainees

<table>
<thead>
<tr>
<th>TYPE OF STUDENT</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.D.</td>
<td>8</td>
</tr>
<tr>
<td>Medical Technicians</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puerto Rico</td>
<td>3</td>
</tr>
<tr>
<td>Latin America</td>
<td>9</td>
</tr>
<tr>
<td>Chile</td>
<td>(2)</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>(2)</td>
</tr>
<tr>
<td>Colombia</td>
<td>(2)</td>
</tr>
<tr>
<td>Argentina</td>
<td>(1)</td>
</tr>
<tr>
<td>Bolivia</td>
<td>(1)</td>
</tr>
<tr>
<td>Uruguay</td>
<td>(1)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>12</td>
</tr>
</tbody>
</table>
### TABLE 4

Procedures completed, observed or discussed by students

<table>
<thead>
<tr>
<th>TRAINEE</th>
<th>COURSE</th>
<th>Observed</th>
<th>Done</th>
<th>Discussed at Clin. Sessions</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hugo Ramírez Madrid, M.D.</td>
<td>Clin. Applic. Rad.</td>
<td>71</td>
<td>96</td>
<td>255</td>
<td>422</td>
</tr>
<tr>
<td>Yocasta Suberví Bonilla</td>
<td>&quot;</td>
<td>9</td>
<td>82</td>
<td>279</td>
<td>370</td>
</tr>
<tr>
<td>Marcelino Rispoli, M.D.</td>
<td>Nephrology</td>
<td>-</td>
<td>78</td>
<td>-</td>
<td>78</td>
</tr>
<tr>
<td>Emperatriz Chavarro Rojas</td>
<td>Clin. Applic. Rad.</td>
<td>88</td>
<td>104</td>
<td>256</td>
<td>448</td>
</tr>
<tr>
<td>Eduardo Touyá, M.D.</td>
<td>&quot;</td>
<td>82</td>
<td>113</td>
<td>256</td>
<td>451</td>
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<tr>
<td>Luis F. Barragán, M.D.</td>
<td>&quot;</td>
<td>87</td>
<td>110</td>
<td>256</td>
<td>453</td>
</tr>
<tr>
<td>Silvio Aristizábal, M.D.</td>
<td>&quot;</td>
<td>79</td>
<td>115</td>
<td>249</td>
<td>443</td>
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<tr>
<td>Alba L. Rico de Puente</td>
<td>&quot;</td>
<td>89</td>
<td>110</td>
<td>244</td>
<td>443</td>
</tr>
<tr>
<td>Mercedes Melo Bautista, M.D.</td>
<td>&quot;</td>
<td>91</td>
<td>104</td>
<td>244</td>
<td>439</td>
</tr>
<tr>
<td>Ilvia L. Millán</td>
<td>&quot;</td>
<td>141</td>
<td>111</td>
<td>228</td>
<td>480</td>
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<tr>
<td>Lidia D. Nieves de Reyes, M.D.</td>
<td>&quot;</td>
<td>144</td>
<td>116</td>
<td>218</td>
<td>478</td>
</tr>
<tr>
<td>Claudio Zúñiga García, M.D.</td>
<td>&quot;</td>
<td>144</td>
<td>116</td>
<td>218</td>
<td>478</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td></td>
<td>1025</td>
<td>1255</td>
<td>2703</td>
<td>4983</td>
</tr>
</tbody>
</table>
### TABLE - 5

**Diagnostic and therapeutic procedures Carried Out During 1967**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid Studies</td>
<td>2764</td>
</tr>
<tr>
<td>Liver Studies</td>
<td>135</td>
</tr>
<tr>
<td>Heart Studies</td>
<td>63</td>
</tr>
<tr>
<td>Kidney Studies</td>
<td>349</td>
</tr>
<tr>
<td>Gastro-Intestinal Studies</td>
<td>16</td>
</tr>
<tr>
<td>Blood Studies</td>
<td>7</td>
</tr>
<tr>
<td>Tumor Localization</td>
<td>49</td>
</tr>
<tr>
<td>Training Procedures</td>
<td>2174</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>5557</td>
</tr>
</tbody>
</table>

### TABLE - 6

<table>
<thead>
<tr>
<th>Clinical Applications Study Unit</th>
<th>Research Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tests of thyroid function</td>
<td>Clinical and laboratory evaluation of thyroid disorders. Effect of radiation of Thyroid Tissue in Patients.</td>
</tr>
<tr>
<td>3. Liver function</td>
<td>I-131 rose bengal localization and dynamic studies: scintigraphy, hepatogram. Experimental pathology of liver with Thioacetamide.</td>
</tr>
<tr>
<td>4. Hematology</td>
<td>The absorption of B-12 in patients with Tropical Sprue.</td>
</tr>
<tr>
<td>5. Tumor Localization</td>
<td>Clinical-pathologic correlation of scanning studies.</td>
</tr>
</tbody>
</table>
Student Research

Students attempt to solve specific problems of their own choice or which have been assigned them. Last year we informed on work done by a student on the labeling of human platelets in normal and abnormal states with 51 Cr with which he studied survival of these blood corpuscles. During the present year we are reporting on the work done by three of our trainees.

- Consideraciones acerca de un programa para un curso de Aplicaciones Clínicas de Radiomucleidos, (Considerations about a Program on the Clinical Applications of Radiomuclides). Dr. Claudio Zúñiga, trainee from the University of Chile, analyzed relevant factors in the preparation of an adequate training program as seen from the standpoint of a Latin American practicing physician. Dr. Zúñiga notes the fundamental needs of the Latin American physician as a whole, and then his particular needs as an individual. For the first part he offers an outline of subjects for a basic program. For the second part, he suggests specialized training, preferably in the form of research work, guided by the trainee's special interest. Such training, supported in part by the Center, could be best carried out in association with one of the teaching hospitals of the Medical Center, preferably the University Hospital. The points raised by Dr. Zúñiga are worth considering. Some of his suggestions have been in operation for some time.

- Summary of work done by trainee during one month following the basic clinical course. This is a summary of activities of Dr. Luis F. Barragán, trainee from Bolivia, who spent one additional month of training. During this month Dr. Barragán worked on pulmonary scanning in the diagnosis of pulmonary embolism; he learned the technique of preparation of albumin I-131 macroaggregates, the agent employed to label pulmonary vascularity to diagnose pulmonary embolism; he studied the chromatographic method used to detect thyroid gland aminoacid with the aid of labeled thyroxine and triiodothyronine.

The trainee reviewed the pertinent bibliography on pulmonary scanning and studied the work previously done at this Division, which was largely based on clinical material referred from the University Hospital with signs and symptoms of pulmonary embolism. During his stay at the Division, he performed 6 lung scanings on 6 patients in whom the radioisotope technique demonstrated abnormal patterns of significant diagnostic value. Five patients had signs and symptoms of pulmonary embolism, and one of acute bronchitis.
The preparation of albumin macroaggregates was performed in three steps:

1. Following the technique of James L. Quinn, macroaggregates of human albumin (nonradioactive) were prepared without any aseptic controls. The colloid was checked by microscopic examination for size range by comparison with red blood cells. Size range found varied between 20 and 150 micra.

2. Step 1 above plus aseptic precautions and bacteriologic control of samples prepared. These were checked for bacterial sterility in sabouraud dextrose agar, trypticase soy agar and Thyoglycollate culture media. Preparations remained sterile for 15 days; thereafter positivity appeared in the Thyoglycollate medium.

3. I-131 macroaggregates were then prepared following steps 1 and 2 above using commercially available radioalbumin. This was successfully accomplished and tried in a dog in which an adequate image of the lung was obtained. Via subsequent re-scanning the material was seen to leave the lungs in 2 1/2 hours.

Radiochromatography of thyroid hormones: Blood obtained from hyperthyroid patients treated with I-131 was treated chromatographically according to the technique of Raurog: Detection of labeled thyroid hormones T₄ and T₃ was successfully accomplished by autoradiography of the chromatographic paper strips.

- Measurements of Effective Renal Plasma Flow and Glomerular Filtration Rate by Isotope Techniques

This report is based on work submitted by Dr. Hugo Ramírez Madrid. He studied 42 subjects employing clearance methods of renal function described by their authors: The effective renal plasma flow (ERF) according to R.D. Wagoner et al and the Glomerular filtration rate (GFR) according to C.D. Farmer et al.

A brief description of the ERPF, also applicable to the GFR, follows: after intravenous administration of the labeled substances orthoiodochippurate I-131 for ERPF or Diatrizoate I-131 for GFR the plasma clearance of these substances is followed up at 5 minute intervals. Plotting the activity on semi-logarithmic paper gives a biexponential curve: one with rapid decay, gives a line with slope denominated α₁ and intercept at 0 denominated β ; the second exponential curve gives a straight line with slope α₂ and intercept at 0 denominated A. T 1/2 of A
and B is determined for each function by the expression:

\[ T_{1/2} = \frac{0.693}{\alpha} \]

and the clearance for the labeled substance is obtained from the application of Sapirstein double exponential formula; thus

\[ \text{Clearance} = \frac{I \alpha_1 \alpha_2}{A T_{1/2} + B T_{1/2}} \]

I = injected activity  
\( \alpha \) = slopes lines B + A  
B = intercept of B line at 0  
A = intercept of A line at 0  
\( T_{1/2} \) = halflife of lines A and B, respectively

Clearance of diatrizoate I-131 represents glomerular filtration rate (GFR) and should be compared with that of inulin, the standard test of GFR; while clearance of hippurate I-131 represents effective renal plasma flow (ERPF: clearance of para-aminobenzonic acid, PAH). The following observations will be presented under 3 items: a) technical difficulties, b) results, c) conclusions.

1. There were 3 major technical difficulties:

   a. Vein clotting precluded the obtaining of samples occasionally at the pre-scheduled time. Difficulty best handled by appropriate use of heparin as an anticoagulant.

   b. Criticality of injected dose. Loss of a tiny amount of volume of injection adversely affected the estimated activity injected (I) by giving it a higher value; giving thus a higher value for clearance.

   c. No correlation of counted activity 8-10 minutes after injection and injected dose. According to authors, activity to be expected at 8-10 minutes was to be much greater than what was observed. A lower cpm of samples lowers values for A and B and hence increases the values for clearance.

2. Results: Healthy subjects. A group of 10 healthy subjects composed of 7 males and 3 females with average age of 35
(range 15-58) were studied. The GFR determined averaged $132.8 \pm 5.8$ ml/min., which is somewhat higher to the standard inulin test: $112 \pm 15$ ml/min.

The ERPF was determined in 7 healthy subjects. The results averaged $641.1 \pm 29.3$ ml/min. This result compares favorably with the standard test of PAH clearance for ERPF which is $612 \pm 68$ ml/min.

Patients: Eleven patients with renal disease and hypertension were examined. Results of the GFR correlated with the clinical picture yielded values of impaired function and in two instances in which renal function did not seem to be impaired (normal blood chemistry), the GFR detected a minor degree of impaired function.

The ERPF was investigated in 14 patients with renal disease and hypertension. Again the results of impaired renal function correlated well with the clinical picture. This test seems to reflect better the state of normal renal function than the GFR.

3. Conclusion: The methods employed for determination of ERPF and GFR appear to be reliable means to determine renal function in healthy individuals and patients with renal diseases. Its application to the study of patients with renal disease complicated with hemodynamic and extracellular fluid space changes may prove equally helpful, provided the physical assumptions upon which the test is based would hold under these conditions.

The participants in the courses offered during 1967 are listed in Table 2. A numerical tabulation of the professional backgrounds and countries of origin of the participants in the training program is presented in Table 3. A large portion of the laboratory activity is made available to student participation. A tabulation of the number of completed procedures by each trainee is given in Table 4.

The ongoing diagnostic radioisotopes services insure an adequate variety of clinical material for the training program. Table 5 presents a numerical analysis of the diagnostic and therapeutic services rendered during 1967. The clinical research program generates new ideas which are incorporated in the curriculum, adding stimuli and a fresh outlook to the teaching effort. Research activities related to training are shown in Table 6.
Dose Levels in the treatment of Hyperthyroidism with I-131 in Puerto Rico. A total of 120 patients with hyperthyroidism have been treated over the past 7 years at PRNC. Analysis of dose and therapeutic effects observed indicate that response rate to full therapeutic dose was of the order of 90% of the treated patients. This includes response to one or more doses (as necessary) of the I-131; the average dose needed to achieve this result was of the order of 6.0 mCi (6.6mCi), not very far from responders to a single dose which averaged 4.5 mCi ± 1.4 mCi. Radiation dose in rads estimation at tissue level was approximately 5500 rad. While the dose level employed in this group of patients is generally lower than in other places, the post treatment sequelae of hypothyroidism is within the limits observed everywhere (10-30%). The incidence of hypothyroidism observed in this group was 19.4%.
Use of Iodine-131 Rose Bengal excretion test in the differential diagnosis of obstructive jaundice in infants. Biliary obstruction in infants in the first few months of life is a perplexing problem because conventional liver function tests are unable to differentiate intrahepatic jaundice (usually hepatitis) from extrahepatic jaundice (usually biliary atresia). The first is a medical condition in which delay in diagnosis is not of paramount importance; in the second type, the best chances of adequate treatment and cure are insured only by a correct and early diagnosis.

Rose Bengal I-131 is a dye taken up by liver cells and excreted via the biliary system into the intestine. Patients with blocked biliary tree and undamaged liver tissue may show good uptake but poor release of the dye into the intestine, whereas those with injury of hepatic cells may fail to show a good liver uptake, and yet be able to excrete whatever is taken up in a short time, because the biliary tree does not suffer a complete or permanent degree of blockade.

This work was performed in 10 infants from the University Hospital who were suffering from neonatal jaundice. There were 8 male babies and 2 females, with age range of 3 weeks to 3 1/2 months, and body weights varying from 3 1/2 to 11 pounds. Jaundice lasted 1 week to 3 months. Liver function tests showed marked alteration of hepatocellular function. Final diagnosis was: 8 patients had hepatitis and 2 biliary atresia. Six of the 8 patients with hepatitis were unequivocally identified by entrance of tracer material into the intestine. Four patients who failed to show entrance of tracer into the intestine turned out to be: 2 with hepatitis and 2 with biliary atresia.

The diagnostic significance of the I-131 Rose Bengal excretion test is that it could correctly detect the patients with biliary atresia, and was able to clearly identify 3/4 of patients not having this condition. It reduces the differentiation to 1/4 of the population of infants with hepatitis in which this test cannot differentiate one way or another.

Perfil Funcional Hepático durante la Experimentación con la Tioacetamida (Liver Profile during the Experimental Administration of Thioacetamide to Dogs). The administration of thioacetamide to dogs induces hepatic toxicity of such degree that it may result in two types of liver cirrhosis, depending on dosage and exposure. Acute hepatic necrosis with profound alteration
of liver biochemistry and inability of the liver to image on a scan record after administration of I-131 Rose Bengal occurs within a period of 3-4 weeks. Animals surviving 3 to 6 months develop a hypertrophic cirrhosis with fine granularity, while those surviving longer (over 6 months) develop atrophic Laennec-like nodular cirrhosis. All these changes are accompanied by biochemical alterations, characterized chiefly by low levels of albuminemia, and high levels of Globulinemia. Poor uptake of I-131 Rose Bengal indicates persistent hepatic injury but recovery of at least part of the reticuloendothelial system is apparent by improved liver uptake of colloidal gold-198, which in the initial phase of acute hepatic necrosis had been somewhat affected.

Plasma B-12 Co 57 Levels at 8 and 12 hours as indices of B-12 absorption. B-12 absorption levels in plasma at 8 and 12 hours were examined in 20 patients and 6 nonpatients (controls). Since the values of 8 and 12 hours did not differ markedly, only values of 8 hours were considered. Fifteen patients with malabsorption syndrome (sprue or pernicious anemia) showed a level of 0.37% dose per liter of plasma with a range of 0.07 to 0.81% per liter. Five patients with no absorption difficulties gave a value of 1.81% dose per liter of plasma with range 0.19 to 3.78%/L. A control group of persons gave a similar value with 1.23%/L and range 0.52 to 2.16%/L.

The variability of the instrument, the low level of counting, and the marked degree of overlapping because of the group of individuals analyzed (patients with absorption defects, patients with no absorption defects, and nonpatients) made us consider that the test was of no clinical benefit for the diagnosis of intestinal malabsorption of vitamin B-12.

RESEARCH IN PROGRESS

- The use of radiiodine studies to assess response of hyperthyroid patients to antithyroid medication. Radiiodine is used to predict pharmacologic response of the thyroid gland of hyperthyroid patients to antithyroid treatment with drugs currently used in clinical management: Tapazole and Propylthiouracil. These drugs inhibit binding of free iodide trapped by the thyroid gland and thus inhibit synthesis of thyroid hormones. There are two basic ways in which this pharmacologic effect may come under observation with the use of radiiodine studies:

1. Thyroid uptake at an early time, say 3 hours vs. 24 hours.
2. Thyroid uptake at 3 hours followed by observation of release of trapped free iodide upon the administration of a thiocyanate salt.

In both instances one wishes to measure how much iodide gets trapped and then released, without actually having entered the usual metabolic cycle of hormone formation. In the first instance, iodide levels obtained at 3 hours may or may not be present at 24 hours; in the second instance iodide trapped at 3 hours may or not be released by the gland. We measure the amount released by the amount retained in the gland, which is compared to a baseline or pre-medication value.

When the gland is pre-medicated with tapazole or propylthiouracil, a certain degree of blockade of thyroid hormone occurs in all instances. In the patients studied, a retention of more than 25% of the baseline value at 24 hours indicates a substantial amount of iodide going through the blockade to thyroid hormone formation. Thus, we arbitrarily set a 25% residual thyroid uptake at 24 hours or 1 1/2 hours after administration of thiocyanate salt as indicative of a nonresponse level.

Four groups of patients have been studied:

18 patients with Tapazole
18 patients with Propylthiouracil
70 patients with Tapazole, iodide discharge with KCNS
11 patients with Propylthiouracil, iodide discharge with KCNS

Of 18 patients with tapazole, 14 responded and 4 did not; in the group of propylthiouracil, 2 responded well and 16 did not. Again, this sharp difference between responses to tapazole and propylthiouracil is observed in the following two groups:

1) In 70 patients with tapazole and iodide discharged from the thyroid gland measured after the administration of KCNS, 56 responded well and 14 did not.

2) The same procedure in 11 patients treated with propylthiouracil showed 2 good responders, and 9 poor responders.

Since antithyroid drug therapy is a long term proposition for the patient and a treatment with a significant amount of risks,
which drug allergies, insensitivity, and bone marrow toxicity are
the more troublesome, it is important to know in advance or to
predict the probable response of the patient since it may take a
year before that judgment can be made by clinical means.

- Thyroid I-131 uptake in Puerto Rican Children. 24-Hour Thy-
roid uptake studies were performed on 115 children from the Fol-
low Through Project of the Head Start Program to which we are
offering collaboration. The group consisted of 60 boys and 55
girls with ages ranging from 6 to 10 years.

The mean thyroid uptake was $17.4 \pm 4.3\%$ with range 7.3 to
32.8\%. At the 95\% confidence level 4 subjects were excluded:
one below with value of 7.3\%, and three above with values 26.2,
30.9 and 32.8\%. It appears in this group that while average
thyroid uptake is close to that of adult values obtained at this
Division, the upper range of normal, and even the highest value
obtained in all the group, is much lower than in adults, which
usually varies from 8 to 45\%, and in some instances may extend
well above 45\% in clinically euthyroid individuals.

Thyroid uptake was also determined in a heterogeneous group
of 22; six were said to be "normal" and the remaining 16 were
suffering from nonthyroid diseases. Some of these patients were
on medication, which at the time of examination was presumed to
exert no influence on thyroid function. The group consisted of
14 females and 8 males with age range from 2 weeks to 11 years.

The mean uptake was 18.3 and the range was 3.4 to 35.2\%. Two
patients showed low uptakes (3.4 and 6.2\%).

This group is small and shows wide scattering of values.
While no conclusions can be drawn, the scattering suggests some
effect of dissimilarity of the composition of the group as com-
pared to the one above, and perhaps some influence of the non-
thyroid disease process or its treatment on thyroid function.

- Radiation of the Neck Region and Its Effect on Thyroid
Function. Twenty-seven patients with head and neck cancer were
irradiated to the neck; the thyroid region underwent irradiation
concurrent with the treatment. Radiiodine studies consisting
of 24 hour uptake, Protein Bound Conversion Ratios of I-131, Pro-
tein Bound Plasma Levels of I-131 and scintiscanning of the thy-
roid gland were performed immediately before, during and imme-
diately after the conclusion of the radiotherapy. Observations
of the functional tests performed demonstrated a marked decline of thyroid uptake during the radiotherapy period, with a rebound to recovery one month after completion of radiation. (See Table I and Graph I).

In spite of heavy radiation to the thyroid tissue estimated in the neighborhood of 7000 r in 7 weeks, and the apparent depression of thyroid uptake of I-131, the scintiscanning studies failed to reveal any areas of sharp localization of defective iodine I-131 distribution. It may be that radiation affected the tissue in a general and diffuse manner, so that a depression of function was not detectable by mapping because there might not have been any areas of isolated major tissue injury.

Table I

Radiiodine Studies of Thyroid Gland and Radiation

<table>
<thead>
<tr>
<th>RAI Test</th>
<th>Radiotherapy (RT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>24 Hour I-131 Uptake</td>
<td>18.5%</td>
</tr>
<tr>
<td>Protein Bound Conversion Ratio I-131</td>
<td>9.4%</td>
</tr>
<tr>
<td>Protein Bound Plasma Level I-131</td>
<td>0.042%</td>
</tr>
</tbody>
</table>

- Treatment of Hyperthyroidism with I-131. A total of 120 patients with hyperthyroidism treated with I-131 were evaluated in follow-up post therapy. Three new cases of post treatment hypothyroidism were detected in a group of 53 patients within one year of previous evaluation. Average thyroid uptake observed last year and this year are within normal limits (22.3 and 19.1% respectively). Contrary to expectations from reported experience elsewhere the incidence of post treatment hypothyroidism in this group does not follow a regular pattern, nor is there a tendency to increase during the period of observation (7 years).


- Lung Scanning in Pulmonary Embolism. This project is carried out in collaboration with the Pulmonary Laboratory of the UPR School of Medicine under the direction of Dr. Adelaida Elías. It is proposed to correlate data of clinical value with other laboratory diagnostic procedures including pulmonary function tests, enzyme studies, electrocardiograms, angiograms and pulmonary scanning.

The diagnosis of pulmonary embolism and its differentiation from other acute or chronic pulmonary or cardiovascular problems is of utmost importance, particularly when massive emboli obstruct major pulmonary arteries, in which case prompt surgical intervention to relieve the obstruction is a life saving procedure. Equally important is the detection of other degrees of embolization since adequate management of the patient depends on this.

During the present year, 55 lung scans have been performed on 46 patients, the majority of which were referred to this Division to rule out the diagnosis of pulmonary embolism. A group of 10 patients in which the final diagnosis of pulmonary embolism was unequivocally established by clinical laboratory tests (which included WBC, EKG, enzyme studies, and chest x-rays), angiography, and post mortem examination had abnormal lung perfusion patterns; (poor circulation) by scanning. The lung scan was also abnormal in 10 disease categories involving the lungs, besides embolic phenomena. These included a heterogeneous group of diseases such as lung tumors (primary or metastatic), acute exacerbation in chronic Bronchitis; chronic Bronchial Asthma, Pneumonia, Pleural Effusion, cardiac disease, Bronchiectasis, etc. In another group of patients with strong suspicion of pulmonary embolism there was a marked discrepancy in the findings detected by pulmonary scan and by angiography of the pulmonary arteries. The angiogram was positive in only one instance, while the scan was definitely abnormal in all of them.

Lung scanning is very useful in the detection of pulmonary emboli of all kinds: large, medium, or small. It is also sensitive to other pathologic or vascular abnormalities of the lung parenchyma. Its capability to detect emboli when no other diagnostic procedures can is probably its greatest merit. Further experience with the method and its correlation with other diagnostic procedures and clinical information is being sought to increase the chances of accurate diagnosis and appropriate management.
STAFF

Dr. J.O. Morales, Specialist in Internal Medicine, was appointed jointly between the Nuclear Center and the School of Medicine for one year to work part time at the Department of Medicine and Radioisotope Laboratory of the University Hospital.

Miss Myrna Rivera resigned her position as Research Technician to accept a position as a chemist at the Laboratory of Toxicology of the Medical Center.

Miss Norma Gándara resigned her position as Research Technician to accept a position as chemist at the Public Health Laboratory of the Department of Health.

TRAVEL

During the past year Dr. Aldo E. Ianaro traveled to Uruguay and Argentina to attend two scientific meetings there and to present two works (listed in the Appendix).

At these meetings he made new contacts with professional people interested in our courses. He also revisited earlier contacts at teaching institutions and laboratories to promote interest in prospective candidates for our courses.

Measurement of a fluid sample in a well counter.
Mrs. M. M. Palacios de Lozano, Research Associate, and Mr. Michael Gileadi, Research Assistant, working on the Li F dosimetry project, a feasibility study of the use of lithium fluoride microrods for the solution of radiotherapeutic problems.
RADIOThERAPY AND CANCER

The Radiotherapy and Cancer Division program is designed to provide education, research and service in the application of radiation therapy to the treatment of cancer.

The Division functions as the Radiotherapy Department of the Dr. I. González Martínez Oncologic Hospital, which is adjacent to the PRNC Bio-Medical Building in the Puerto Rico Medical Center. The Hospital provides some of the paramedical personnel, beds, operating rooms, clinical laboratories, outpatient facilities, equipment, space, and other medical services essential for the care of cancer patients.

The Oncologic Hospital renders service to over two-thirds of the indigent cancer patients in Puerto Rico. It has also provided all the radiotherapy services offered to patients in the Puerto Rico Medical Center since May 1966.

At the academic level, the Division operates as the Radiotherapy Section of the UPR School of Medicine. It also works in close collaboration with the Cancer Control Program of the Puerto Rico Department of Health.

Partial support for the Division program is received through a National Cancer Institute Training Grant to the UPR School of Medicine.

EDUCATION

Description of Courses

A brief description of the principal formal educational activities follows.

Radiotherapy Residency Program. This program is designed to prepare qualified radiation therapists and meets the requirements of the American Board of Radiology. The trainees are physicians with a year of internship or equivalent clinical experience. The training period is three years, but trainees are required to take an additional fourth year of supervised practice (preceptorship) before admission to the specialty examinations. Diagnosis of cancer, determination of the extent and radiosensitivity of tumors, selection of appropriate treatment, and the planning and conducting of radiological therapy are included in the curriculum. Background in clinical oncology is imparted to residents through su-
supervised work with new, follow-up, and hospitalized cancer patients. Radiation therapy experience is acquired by working with roentgentherapy machines of various voltages, cobalt and cesium teletherapy units, and the internal application of radioactive material such as radium, strontium, cobalt, iridium, and cesium in solid (needles, tubes, wire) sources.

Special Short-Term Radiotherapy Course. This course is prepared according to the needs of the individual requesting the training; the person must have had previous radiotherapy experience. Participants may engage in research and may participate in all training activities of the Division; however, they are not extended the privilege of patient responsibility. A minimum of one month of training is required.

In-Service Cancer Course for Medical Students. This course is designed for future physicians with clinical problems and current research in cancer and radiation therapy. The minimum length for this course is one month.

In-Service Training for Radiological Physics Personnel. This course is offered periodically as requested.

In-Service Training for Radiotherapy Technicians. This course is offered as requested.

Radiotherapy of Cancer Lecture Course. This course for third year medical students is offered yearly as part of the UPR School of Medicine curriculum. Twelve lecture hours are offered, highlighting: epidemiology of cancer, radiological physics, radiobiology, clinical radiotherapy, and radioisotopes in therapy.

Educational Activities

The educational activities offered to physicians included lectures, seminars, demonstrations, and patient care under supervision with rotation through the various sections of the Division: PRNC treatment area, Oncologic Hospital treatment area, Curie-therapy and Hospital inpatient area, follow-up, and radiological physics. The Residents rotated through the Pathology Department of the Oncologic Hospital and also took the PRNC Radioisotope Training courses. (Table 1 presents a summary of the regular activities of the Radiotherapy Division; Table 2 provides information on the trainees in the Division courses; Table 3 presents
**TABLE 1**

**REGULAR ACTIVITIES OF THE RADIOTHERAPY DIVISION**

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>8:00 - 12:00 a.m.</td>
<td>Curietherapy patients evaluation and planning clinic</td>
</tr>
<tr>
<td></td>
<td>8:00 - 12:00 a.m.</td>
<td>Head and neck cases follow-up clinic</td>
</tr>
<tr>
<td></td>
<td>12:00 - 1:00 p.m.</td>
<td>Oncologic Hospital new patients conference</td>
</tr>
<tr>
<td></td>
<td>2:00 - 5:00 p.m.</td>
<td>Examination of new patients and consultation</td>
</tr>
<tr>
<td>Tuesday</td>
<td>7:30 - 8:30 a.m.</td>
<td>Journal Club</td>
</tr>
<tr>
<td></td>
<td>8:00 - 12:00 a.m.</td>
<td>Application of radioactive material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pelvic cases follow-up clinic</td>
</tr>
<tr>
<td></td>
<td>12:00 - 2:00 p.m.</td>
<td>Oncologic Hospital new patients conference</td>
</tr>
<tr>
<td></td>
<td>1:00 - 2:00 p.m.</td>
<td>University Hospital tumor conference</td>
</tr>
<tr>
<td></td>
<td>1:30 - 3:00 p.m.</td>
<td>Municipal Hospital tumor conference</td>
</tr>
<tr>
<td></td>
<td>1:00 - 5:00 p.m.</td>
<td>Examination of new patients and consultation</td>
</tr>
<tr>
<td>Wednesday</td>
<td>8:00 - 10:00 a.m.</td>
<td>Radiotherapy Grand Rounds</td>
</tr>
<tr>
<td></td>
<td>10:00 - 12:00 a.m.</td>
<td>Radiotherapy Staff and consultation conference</td>
</tr>
<tr>
<td></td>
<td>12:00 - 1:00 p.m.</td>
<td>Oncologic Hospital new patients conference</td>
</tr>
<tr>
<td></td>
<td>1:00 - 5:00 p.m.</td>
<td>Examination of new patients and consultation</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>Curietherapy cases evaluation</td>
</tr>
<tr>
<td>Thursday</td>
<td>8:00 - 10:00 a.m.</td>
<td>Esophagus cases follow-up clinic</td>
</tr>
<tr>
<td></td>
<td>11:30 - 12:00 a.m.</td>
<td>Oncologic Hospital morbidity conference</td>
</tr>
<tr>
<td></td>
<td>12:00 - 1:00 p.m.</td>
<td>Oncologic Hospital new patients conference</td>
</tr>
<tr>
<td></td>
<td>1:00 - 5:00 p.m.</td>
<td>Examination of new patients and consultation</td>
</tr>
<tr>
<td>Friday</td>
<td>8:00 - 12:00 a.m.</td>
<td>General follow-up clinic</td>
</tr>
<tr>
<td></td>
<td>12:00 - 1:30 p.m.</td>
<td>FRNC Seminar</td>
</tr>
<tr>
<td></td>
<td>2:30 - 3:30 p.m.</td>
<td>Radiotherapy Weekly Conference</td>
</tr>
</tbody>
</table>

**NOTE:** Teletherapy applications are scheduled for the FRNC and Oncologic treatment areas from 8:00 to 12:00 a.m. and 1:00 to 5:00 p.m.
TABLE 2

RADIONTHERAPY AND CANCER DIVISION TRAINEES, 1967

RADIONTHERAPY RESIDENCY PROGRAM


SPECIAL SHORT TERM RADIONTHERAPY TRAINING

1. Dr. Herman Mussbaum - U.S.A. Experienced radiologist receiving a year of advanced training at the San Francisco Tumor Institute; spent the month of March 1967 training at the Radiontherapy Division. His training was supported by the U.S. Public Health Service.

2. Dr. Jacobo Ramos - New York - November 1967. Dr. H. Treitel - New York - December 1967. One month training for residents in Radionology at the Veterans Administration Hospital in Bronx, New York, by special arrangement with Dr. B. Roswit, Chief of Radionation Therapy Service at that Institution.

CANCER COURSE FOR MEDICAL STUDENTS

One month training for third and fourth year medical students at the U.P.R. School of Medicine under the auspices of the Cancer Control Division of the P.R. Department of Health and the National Cancer Institute Radiation Therapy Training Grant.

<table>
<thead>
<tr>
<th>1967</th>
<th>U.S.A. - 4th Year</th>
<th>1967</th>
<th>U.S.A. - 4th Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>José A. Lozada</td>
<td>October</td>
<td>Carlos M. Rivas</td>
</tr>
<tr>
<td></td>
<td>Frank Kolodziej</td>
<td>December</td>
<td>Carlos M. Ramírez</td>
</tr>
<tr>
<td>March</td>
<td>Juan M. Aranda</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jorge A. Blanco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>Robert M. Friedman</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>Juan A. Torres</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luz M. López</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>Nitza A. Vanga</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luis A. Vázquez</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>Manuel Náter</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luis A. Oliver</td>
<td>June</td>
<td>Luis A. Torres</td>
</tr>
<tr>
<td></td>
<td>José V. Figueroa</td>
<td>July</td>
<td>Antonio R. Villamil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Juan M. Rosa</td>
</tr>
</tbody>
</table>
TABLE 3

CLINICAL RADIATION THERAPY ACTIVITIES

A. Distribution of New Cases Treated in the Radiotherapy and Cancer Division

<table>
<thead>
<tr>
<th>BY SITE</th>
<th>65</th>
<th>FEMALE GENITAL ORGANS</th>
<th>269</th>
<th>MALE GENITAL ORGANS</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAL CAVITY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior 2/3 tongue</td>
<td>19</td>
<td>Cervix Uteri</td>
<td>217</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>25</td>
<td>Endometrium</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>21</td>
<td>Ovary</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OROPHARYNX</td>
<td>105</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base of tongue</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYPOPHARYNX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86</td>
</tr>
<tr>
<td>NASOPHARYNX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RESPIRATORY SYSTEM</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paranasal sinuses</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIGESTIVE SYSTEM</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BREAST</td>
<td>103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Teletherapy Applications
   (Co60, X-ray, and Cesium)  26,169

C. Curietherapy
   (Co60, Radium, Iridium)  259

D. Follow-up  5,393

E. Consultations  315

**TOTAL**  974
TABLE 4

FORMER PRNC TRAINEES NOW CONTRIBUTING TO RADIOThERAPY TRAINING PROGRAMS

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Mario Vuksanovic</td>
<td>University of Miami</td>
</tr>
<tr>
<td>Dr. Efraín Navarro</td>
<td>National Cancer Institute, Mexico City</td>
</tr>
<tr>
<td>Dr. Modesto Rivero</td>
<td>National University, Caracas</td>
</tr>
<tr>
<td>Dr. Juan Reusche</td>
<td>Radiology Institute Cayetano Heredia, Lima</td>
</tr>
<tr>
<td>Dr. Oriel Alva</td>
<td>Roffo Cancer Institute, Buenos Aires</td>
</tr>
<tr>
<td>Dr. Ernesto Amadey</td>
<td>Universidad Nacional del Nordeste, Corrientes</td>
</tr>
<tr>
<td>Dr. Lucas Di Rienzo</td>
<td>Clínica Regional Dr. Di Rienzo, Cordoba</td>
</tr>
<tr>
<td>Dr. Arturo Valencia</td>
<td>Hospital San Jorge, Pereira</td>
</tr>
<tr>
<td>Dr. Napoleón Matos</td>
<td>Instituto Nacional de Enfermedades Neoplásicas, Lima</td>
</tr>
</tbody>
</table>

Miss C. Ramirez examining mould work used in radiotherapy.
information on the distribution of cases treated in the Radio-
therapy and Cancer Division; and Table 4 provides information on
former trainees."

The training program was enhanced during 1967 by the partici-
pation of the following consultants and prominent specialists from
other institutions: Dr. Alfred Gellhorn, Chief, Institute of Can-
cer Research, Columbia University and Head, Department of Medicine,
Francis Delafield Hospital, New York City; Dr. Jerome Vaeth, Di-
rector and Chief of Radiotherapy, San Francisco Tumor Institute,
San Francisco; Dr. Carl Hansen, Director, Radiation Research Pro-
gram, National Cancer Institute, Bethesda; Dr. Bernard Roswit,
Chief Radiation Therapy Service, Veterans Administration Hospital,
Bronx, New York City; Dr. Herman Suit, Associate Radiotherapist,
Radiotherapy Department, M.D. Anderson Hospital and Tumor Insti-
tute, Houston; Dr. William Caldwell, Associate Professor of Ra-
diology and Chief, Radiotherapy Division, School of Medicine,
Vanderbilt University, Nashville; and Dr. A.H.W. Mias, Principal
Research Officer, Patterson Laboratories, Christie Hospital and
Holt Radium Institute, Manchester, England.

The Division staff also participated in the teaching of other
PRNC training courses, the weekly Tumor Conference of the UPR
School of Medicine, the weekly Tumor Conference of the San Juan
City Hospital, and the daily New Patients Conference of the Onco-
logic Hospital. Dr. Antonio Bosch continued supervising the Ra-
diotherapy Technicians Course for the Oncologic Hospital and other
members of the Division staff participated in the teaching program
until the course terminated in May.

**RESEARCH IN PROGRESS**

The following research projects were active at the end of the
year:

Dose-time relationships in the external irradiation of carcino-
ma of the uterine cervix: comparison of 4,500 R vs. 5,000 R.

Fractionation in radiation therapy of carcinoma of the uter-
ine cervix: 3 vs. 5 fractions per week.

Fractionation in radiation therapy of inoperable breast can-
cer: 1 vs. 5 fractions per week.
In the photos, clockwise: Dr. Ghelman loading radioactive sources for curie-therapy; Mrs. Lozano engaged in dosimetry work with the Telecesium unit; Dr. Arenas and technician plan radiation therapy of oral cancer in a patient.
Fractionation in radiation therapy of head and neck cancer: 3 vs. 5 fractions per week.

Split-course technique in radiation therapy of cancer.

Study of results of treatment techniques in adenocarcinoma of the endometrium.

Surgical adjuvant breast project (participating in a National study).

Carcinoma of the cervix in sterilized women.

Study of prophylactic irradiation of adjoining anatomic lymphatic regions in Hodgkin's Disease (participation in a National project).

Radiotherapy for carcinoma of the prostate - Stage C (participation in a National study).


RESEARCH COMPLETED

Study of the optimal tumor dose in radiation therapy of cancer of the esophagus: 5000 R in four weeks vs. 6000 R in 6 weeks. This project was ended when a total of 200 cases were accumulated. The data will be published when a minimal two-year follow-up is achieved for all cases. It appears that the results to date confirm the preliminary impressions reported in the PRNC 1966 Annual Report, page 64.

The significance of persistent tumor cells in the vaginal smear after radiation therapy of carcinoma of the uterine cervix: prognostic significance. This project was ended and its results presented at the annual meeting of the American Radium Society in Toronto in May 1967. The paper has been accepted for publication in the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine.
Preliminary analysis of the study: Fractionation in radiation therapy of carcinoma of the uterine cervix: Two year results of prospective study of 3 vs. 5 fractions per week. Two hundred cases were analyzed. The preliminary results of this study suggest a more favorable situation regarding completeness of treatment and survival, when the stated weekly dose is delivered in three fractions versus the conventional fractionation; this is more notable in the advanced cases.

Study of modified "split-course" technique in the treatment of cancer of the esophagus (see PRNC 1966 Annual Report, page 66). A total of 14 cases were included in the study. It was found that this technique is well tolerated by patients. Due to time limitations caused by continual use of the therapy machines, the project was discontinued, to be resumed at a later date.

A pilot study of a "split-course" technique in the treatment of advanced cancer of the urinary bladder and of the uterine cervix. Details of this study appear on page 66 of the PRNC 1966 Annual Report. It has been found that two courses of 2500 R administered in 10 daily fractions of 250 R each in a two week period, separated by a rest period of 2-3 weeks prior to curietherapy, is well tolerated by patients with advanced cancer of the uterine cervix. Advanced bladder cancer cases tolerated well two and, at times, three courses of irradiation with daily fractions of 250 R for 10 fractions, separated by rest periods of two to three weeks.

The influence of the intracavitary curietherapy applicator in the prognosis of cases with carcinoma of the uterine cervix. This project was reported in the PRNC 1966 Annual Report on page 66. The study has been completed and is being prepared for publication.

Evaluation of treatment results in carcinoma of the bladder. From 1950 to 1965, a total of 179 cases with bladder cancer were seen at the Oncologic Hospital. Surgery was the preferred treatment, with radiation therapy used for inoperable cases or post-surgical recurrence. The overall five-year survival achieved was 28%. A complete course of radiation therapy for surgical recurrence or persistence produced a five-year survival of 30%.
Radiation therapy in tumors of the central nervous system. A revision of the experience with tumors of the central nervous system at the Oncologic Hospital from 1941 to 1966 disclosed that 59 cases were seen in that period (including 10 pituitary cases). A total of 51 cases were submitted to radiation therapy because of inoperability or post-surgical persistence; the five-year survival achieved in the group with non-pituitary lesions (49 cases) was 42.4%.

STAFF

Dr. Guillermo Gómez Cárdenas, Radiotherapist at the Instituto Nacional de Cancerología in Bogotá, Colombia, joined the Division staff on May 15, 1967, to serve as a Visiting Radiotherapist. While Dr. Gómez is carrying out educational and clinical research activities, he is receiving advanced training in radiation therapy.

At the end of 1967 the Division staff included 4 radiotherapists, 1 visiting radiotherapist, 1 physicist, 1 assistant physicist, and 1 biostatistician. Additional paramedical services were provided by 1 research medical records librarian, 3 graduate nurses, 3 office employees, and a photographer-electronic technician. The Division staff is still not large enough to handle the present needs, especially at the radiotherapist level. The present load should be handled by 6 radiotherapists.

SCIENTIFIC MEETINGS

During 1967 various staff members presented scientific papers at professional meetings and these are listed in the Appendix. In addition, Dr. Víctor A. Marcial attended the First Gray Memorial Conference at the Mount Vernon Hospital in London, England on September 20-22, 1967. Dr. Jeannie Ubiñas and Mrs. María Palacios de Lozano attended a meeting on Computer Dosimetry sponsored by Washington University in St. Louis, Missouri on November 11-12, 1967. Dr. José M. Tomé attended the Annual Meeting of the Radiological Society of North America in Chicago during the period of November 26 - December 1, 1967.
Dr. Ferrer Monge studies the cytogenetic effect of ionizing radiation, using irradiated root tips of *Vicia faba*. Below: Drs. Koo and Kamath at the control console of the x-ray spectrometer used to produce monoenergetic x-rays.
AGRICULTURAL BIO-SCIENCES

The purpose of the Agricultural Bio-Sciences Division remains a two-fold one: first, the training of students in agricultural research in its broadest sense, emphasizing nuclear techniques; and second, the continuation of basic research programs which are concerned with problems in tropical agriculture that can uniquely be studied by nuclear techniques.

EDUCATIONAL ACTIVITIES

In 1967, the Division continued to further the application of nuclear technology in the agricultural and biological sciences. This was done in part by training students in agricultural and biological research, with emphasis upon nuclear techniques. Training was frequently related with the Division's basic research activities, which are outlined in a later section.

During 1967, the emphasis upon training continued to be directed toward the graduate and post-graduate level, since graduate programs in science at both the Río Piedras and Mayaguez campuses of the University of Puerto Rico are now well established. As honorary members of the various science departments of the University, Division staff offered the following courses during the year:

Instrumental Techniques in Biological Research - Dr. S.H. Kamath. This course was offered through the UPR School of Medicine and was attended by six graduate students, two of whom were faculty members. It was the first time a course in instrumental analysis with biological orientation was offered at the University.

Radiobiology - Dr. R.A. Luse, Dr. J.A. Ferrer-Monge, Mr. J. Cuevas. Five graduate students at the University in Mayaguez attended this new course, which made extensive use of the PRNC gamma facility for laboratory experiments--an uncommon feature in such courses.

Cytogenetics - Dr. J. Ferrer-Monge. This course serves as core for one of the three areas of specialization chosen for emphasis in the graduate program by the Biology Department in Mayaguez.

Biology: Research and Thesis. - Dr. F.K.S. Koo. This course was taken by students doing M.S. thesis work in biology.
Agronomy: Research and Thesis - Dr. R.A. Luse. This course was taken by students doing M.S. theses in agriculture.

The staff also contributed lectures and laboratory sessions to the PRNC basic course in radiisotope techniques, the University's course in radiochemistry, and the PRNC course in tissue culture.

Graduate Research

The number of graduate students engaged in research for M.S. degrees in biology or agriculture continues at the 1966 level, which was double that previously. Their research topics reflect the broad interests of the Division:

Effects of ionizing radiation on enzyme kinetics - Adriana Báez (Puerto Rico). This project is designed to determine quantitatively the relative radiosensitivities of the two enzymatic activities of a bifunctional system. The peptidase and esterase activities of carboxypeptidase A were chosen for study. This work was discontinued due to lack of funds.

Effect of temperature on the mitotic cycle of Vicia faba - Carmen E. Cintron (Puerto Rico). The effect of temperature (in the range 0 - 40°C) on the mitotic frequency, synthesis of DNA, and frequency of chromosome aberrations in the broad bean is being studied. Completion is expected late in 1968.

Radiation inactivation of the enzyme polyphenol oxidase - Aida Garcia de Fournier (Puerto Rico). Estimation of the molecular weight of this enzyme, which may be a monomer or tetramer, has been made from the radiation sensitive volume and found to agree well with the tetrameric weight. This thesis will be completed by March, 1968.

Effect of ionizing radiation on levels of plant nucleic acids - Tsu Hui Lin (Taiwan). New chromatographic methods using MAK columns effect separation of several fractions of plant nucleic acids and permit their quantitative analysis following various irradiation treatments. This work was discontinued after seven months when student transferred to Texas A&M University.

Fixation of phosphates in tropical soils - José Narvaez (Nicaragua). The fixation of phosphorus by four Puerto Rican soils was determined by addition of 32P-labeled phosphate salts to soil columns, their elution with water or acid, and subsequent measurement of eluted ("non-fixed") phosphorus by radioactivity and chemical techniques. Thesis was completed in August and student has returned to Nicaragua to conduct soils research.
Above: Dr. Koo and Mr. Colon place guava seedlings in position for gamma irradiation in the Bio-Medical Building's irradiation room. Left: The kinetics of enzymes after radiation inactivation are measured by graduate student A. Fournier, while Dr. Kamath looks on.
Radiation-induced delay in ripening of bananas - Joaquín Oliver (Puerto Rico). The extent of delay in ripening of Johnson and Montecristo variety bananas given low doses of gamma irradiation has been determined. The interaction of radiation delay with subsequent artificial ripening by chemicals also has been studied. This thesis will be completed in the spring of 1968.

Host plant preference of Diatraea saccharalis (Fab.) - Victoriano Quintana (Puerto Rico). Seasonal surveys of the infestation by the sugar cane borer in 120 species of grasses have been made, using the University's world grass collection. The eight grass species with greatest borer infestivity are now under study to determine the oviposition rate, survival rate, and development time of the borer in each host. Thesis should be completed by October, 1968.

Combined effects of chemicals and gamma rays on the production of chromosomal aberrations in Tradescantia paludosa - Edith Robles de Irizarry (Puerto Rico). Three chemicals (8-ethoxycaffeine, 5-aminouracil, kinetin) and gamma radiation have been used separately and in combination to produce chromosome aberrations in Tradescantia paludosa root-tips. When the chemical and radiation were applied in combination, synergistic effect was observed in the production of chromosome breakages and interchanges at different recovery times. The latter result is of special interest because it indicates an interaction between chromosome breakages produced by two agents. Thesis will be completed by May, 1968.

Influence of ionizing radiation on methionine utilization by E. coli - Frederick Rushford (Puerto Rico). Rapidly multiplying populations of E. coli exposed to various doses of gamma irradiation are being studied as to their ability to incorporate $^{35}$S-labeled methionine into cell constituents. Thesis is expected to be completed in early 1969.

Degradation by gamma radiation of pectinic acid and related polymers - Gregory Telek (Puerto Rico). Tonically-bonded gels of citrus pectinic acid were studied after their gamma irradiation to determine the extent of depolymerization produced by the radiation and the radioprotection offered by this type of bonding. Experimental work has been discontinued by student.
Right: Atomic absorption spectrophotometry permits measurements of trace metals in plant material at the parts per million level or less. Here, Technician A. Colon runs plant digests. Below right: Dr. Kamath collects fractions following a chromatographic run to separate components of irradiated biochemicals on the basis of molecular weight. Below: Technician M. Pagan prepares a sample of E. coli cells for irradiation with monoenergetic x-rays in a special micro-chamber.
Cytogenetic effects of chronic gamma irradiation on microsporogenesis in Palicourea riparia - Robert Venator (Puerto Rico). Palicourea riparia was studied cytologically following a short term chronic gamma-irradiation in the rainforest at El Verde. In general, the results show that the meiotic abnormalities in microsporogenesis increased with dose and these aberrations appeared to decay with time. Pollen abortion was found to be relatively low, presumably due to the small chromosomes and polyploidy nature of this species. Also uncovered in this species were several inherent cytological anomalies such as cytomixis, binucleate sporocyte formation, and polyplory. This thesis will be completed in the summer of 1968.

Advanced Training

At a more advanced level of training, the Division provided a nine-month period of training to an IAEA Fellow, Sergio Quadri C., Assistant Professor at the University of Concepción, Chile. This was a further example of the individualized training which has been offered by the staff to four other trainees (sponsored by ORINS, IAEA, the Peace Corps, and OAS) since 1965. Mr. Quadri's research consisted of the examination--by optical absorption, photobleaching behavior, electron spin resonance, and thin layer chromatography--of the radiation damage produced in thymidine, ascorbic acid, and vitamin B_12. Working in cooperation with members of the Physical Sciences Division, Mr. Quadri was able to irradiate these compounds as solids in an organic glass matrix at 77°K to stabilize reactive intermediates. Upon completion of these studies in December, 1967, Mr. Quadri returned to Chile where he has become a member of a group studying radiation preservation of Chilean food products.

"Atoms in Action" Exhibit

The Division staff continued to serve as Scientific Advisors at the USAEC "Atómicos en Acción" Exhibit in Latin America. In 1967 their activities were as follows:

<table>
<thead>
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This contribution of time and effort towards encouraging scientific research in Latin America has attained positive results; research groups in El Salvador and Guatemala have requested and obtained USAID or local national government support for their research in the past two years. Projects for insect control by radiation sterilization are in active progress in Guatemala, El Salvador, and Ecuador. Numerous theses have been supervised, so that both students and faculty members have been brought into contact with nuclear techniques. It is felt that such cooperation with Latin American scientists will continue to foster application of nuclear techniques in Central and South American agriculture.

**RESEARCH ACTIVITIES**

The Division continued active in basic research, in problems in tropical agriculture that can uniquely be studied by nuclear techniques. Division research in this area may be discussed within three main categories:

1. **Radiobotany of sugarcane.**

   The induction of plant mutants having high sucrose content offers a solution to the declining yields returned by sugarcane, still the most important crop in Puerto Rico's economy. Initial experiments to determine the radiosensitivity of seeds and buds to the mixed gamma-neutron radiation produced in the PRNC megawatt reactor were completed in 1964. Subsequently, thousands of seeds and vegetative buds have been irradiated, germinated and planted in the field. Second and third generation material, likely to contain a higher frequency of mutants, has been mass screened during the past year using automated analytical techniques. Small tissue samples are taken from each stalk; those individuals showing high sugar content are vegetatively propagated and grown for later evaluation in the Sugarcane Improvement Program of the University Agricultural Station. This year over 5,000 assays were run in the chemical screening program, with about 40 superior mutants found and propagated. Visible mutations such as wider, stiffer leaves indicate that other favorable characteristics may be induced.

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Another project directed toward improved sugarcane yields was that of the radiation induction of resistance to the mosaic virus disease. Seven strains of sugarcane showing desirable breeding
characteristics but susceptible to the virus were irradiated with mixed gamma-neutron radiation from the megawatt reactor. Over 2,000 seed pieces were irradiated at 2-3 kilorad doses and grown in the field. At present, some 1500 cuttings from the first generation plants (grown from the irradiated seed) have been grown to the second generation crop and tested for their virus resistance through a sandblast infection technique. Seedlings showing resistance will be incorporated into the University's breeding program.

2. **Radiation Sterilization of the Sugarcane Borer.**

Division activity in this research area is carried out by Dr. D.W. Walker, with primary financial support provided by the USAEC Division of Biology and Medicine, and is reported elsewhere in this Annual Report.

3. **Radiation Preservation of Tropical Fruits.**

Within the Division, considerable effort is now being directed to the underlying processes associated with radiation preservation of tropical fruits such as mango and banana. This research supplements the feasibility studies which are supported by the USAEC Division of Isotope Development.

To study the relationship between degradation of structural polysaccharides in the fruit tissue and fruit softening, pectic constituents were extracted from irradiated and non-irradiated mangos. Here fruits of the Mayaguezana and Sandaresha varieties were irradiated to doses of 500 to 1,500 Krad and their pectic constituents from the alcohol-insoluble residue were fractionated on the basis of solubility and extent of methylation by successive extraction with cold water, 0.5% EDTA solution, and 0.05N hydrochloric acid. Each fraction has been characterized by determination of total uronide content, degree of methylation, and molecular weight (both weight and number average). It was found that there was a nearly linear decrease in number and weight average molecular weights over the dose range studied. Water soluble pectinic acid exhibited considerable change in number average weight at doses above 1000 Krad and may be considered more radiation sensitive than the other pectin fractions. The rather small changes in extent of pectin depolymerization (5-15 percent for 500 Krad doses) result in appreciable loss of firmness in the irradiated mango. Methoxyl content of the fractions decreased to ap-
approximately two-thirds of control values when fruits were irradiated to 1000-1500 Krads. There was no appreciable change in the total uronide content of the various pectin fractions with increasing radiation dose.

Preliminary studies were carried out on the volatile components so important for flavor in the mango. Macerated fruit was swept with nitrogen and the volatiles trapped at liquid nitrogen temperature. Gas chromatographic analysis indicated a predominance of carbonyl compounds. New chromatography equipment is now being calibrated and a new low temperature extraction system is being built to permit quantitative studies of flavor in both irradiated and non-irradiated fruit.

Other Research

In addition to the Agricultural Bio-Sciences Division's basic research in agriculture, two projects of fundamental radiobiological importance are incorporated within the Division. These may be summarized as follows:

1. Resonance in Radiation Effects.

It has been shown that there exists a resonance in radiation effect in onion root tips containing BUDR, i.e., there occurs a maximum of chromosome damage for a given dose of monochromatic x-rays at a photon energy at or near the K-absorption edge of bromine. These studies have been extended to Tradescantia paludosa root tip cells treated with BUDR and monochromatic x-radiation. Chromosome aberration frequency passed through a maximum at 13.5 Kev as x-ray photon energies were varied from 12.5 to 15.5 Kev, with 68 breaks per 100 cells found at 13.5 Kev (Br K-edge) compared with approximately 23 at 12.5 and 15.5 Kev. No such peak was observed in similarly x-irradiated cells containing no BUDR. These results seem to provide further support for the existence of the resonance in radiation phenomenon.

To test if this resonance effect is also expressed in mutation induction, experiments have been carried out using Escherichia coli and Corynebacterium equi. In the present studies the same target atom, bromine, was incorporated into the bacterial DNA by treating the culture at log phase for five hours with 100 µg/ml concentration of BUDR. The samples were exposed for 18-20 hours to monochromatic X-rays of various energies. The irradiated as well as
controls were plated on agar media for mutation and survival assay. Presented in the following Table are the results for all the series treated with both BUdR and X-rays (these results are not corrected for the slight changes in beam intensities and mass absorption coefficients at various photon energies).

<table>
<thead>
<tr>
<th>Photon energy (Kev.)</th>
<th>Reversion rate in E. coli (x 10^-6)</th>
<th>Color mutation rate in C. equi (x 10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>0.21</td>
<td>0.36</td>
</tr>
<tr>
<td>13.5</td>
<td>1.02</td>
<td>0.85</td>
</tr>
<tr>
<td>14.1</td>
<td>0.39</td>
<td>--</td>
</tr>
<tr>
<td>15.5</td>
<td>0.11</td>
<td>0.40</td>
</tr>
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</table>

Apparently in both organisms the mutation rates at the K-edge energy (13.48 kev) were significantly higher than those below and above the K-edge energy. The reversion rates (from tyrosine-dependent to independent) for E. coli obtained with X-ray treatment alone at these same energy levels were very low, ranging from as low as the spontaneous reversion rate up to 0.007 x 10^-6. The average reversion rate for BUdR treatment alone was about 0.15 x 10^-6. For color mutations (from the original orange color to red, yellow, white, etc.) in C. equi the mutation rates for X-ray treatment alone were again very low at all energies studied, ranging from the spontaneous rate to 0.05 x 10^-3. The average mutation rate for BUdR treatment alone was about 0.09 x 10^-3. These results once again unequivocally confirm that the resonance phenomenon can be produced in genetic systems by low energy monochromatic X-rays.

Previous work with metalloenzymes likewise has indicated that there is an energy dependence of damage (inactivation) related to the constituent metal. Catalase shows a ten-fold increase in inactivation at 7.1 Kev, the K-edge of iron, compared with damage a few kev above or below this energy (constant dose). To determine more precisely the nature of the damage produced by x-irradiation at the iron K-edge, irradiated catalase was studied by some of the latest techniques of protein chemistry (molecular gel sieving, electrophoresis, fluorescent tagging), to determine the structural changes in the enzyme induced by such radiation. No cleavage of the protein portion of the molecule was indicated at totally inactivating doses. Study of the release of iron from the enzyme's porphyrin structure is now in progress using very highly sensitive methods (fluorescence spectrophotometry, atomic absorption spectrophotometry, electron spin resonance spectrometry).
2. Radiobiological effects of neutrons in plant systems.

In order to further a world-wide program being established by the International Atomic Energy Agency, the Division has started experiments on the effects of fast and thermal neutrons in plant systems. The first of these, most directly related to the Agency's program, is to utilize barley seeds as a biological dosimeter of neutron irradiators (such as reactors). By measurement of seedling growth following germination of irradiated seeds, a useful index of the neutron flux and relative energy distribution may be deduced without reliance on elaborate physical characterization. A second series of experiments now underway by Dr. Ferrer involves the effect of temperature on frequency and type of chromosome aberration in neutron irradiated plant tissue (seeds, root tips). A third series of experiments initiated to determine biochemically the changes in plant nucleic acid following sub-lethal neutron doses was abrogated due to loss of personnel.

STAFF

Dr. Luse served in 1967 as President of the Sociedad Americana de Ciencias Agrícolas ("American Society of Agricultural Sciences), and Mr. Cuevas as Secretary-Treasurer. Dr. Koo, in conjunction with members of the University of Puerto Rico faculty, organized a seminar group called the "Geneticscope Club" to promote exchange of new findings and ideas in the field of genetics and its allied disciplines.

Dr. Luse was an invited participant in the FAO/IAEA Study Group Conference on "Coordination of Research on the Use of Neutrons in Seed Irradiation" held in Vienna, December 11-18.

The year 1967 saw the Division in new quarters in Mayaguez. The new Plant Sciences Building adjacent to the Greenhouse provides space for the two walk-in plant growth chambers, as well as two laboratories and a low temperature room. A new cytogenetics laboratory and a new biochemistry laboratory were constructed in space in the Reactor Building made available by moving the Shops. The Division continues to carry out field work in many sites on the island: a new plot south of Mayaguez (Cornelia Hill), the two acre banana plantation near Mayaguez, and sugarcane plantings in Rio Piedras and Gurabo.
Right: Dr. Ramiro Martinez Silva using lamina flow hood in tissue culture. Below: Mr. Roger Ramos Aliaga with Dr. Jorge Chiriboga viewing the special automatic spectrophotometer. Mr. Ramos Aliaga, from the School of Pharmacy and Bio-Chemistry, University of San Marcos, Lima, Peru, is here studying enzymatic kinetics for his doctoral thesis.
MEDICAL SCIENCE AND RADIObIOLOGY

The Medical Sciences and Radiobiology Division offers training and research opportunities in fundamental aspects of radiation biology, biochemistry, parasitology, virology, and medicine. Research is directed in large part toward biological problems encountered in tropical areas such as Puerto Rico. Facilities include a tissue culture unit, an animal house containing a mouse colony and a snail colony, a biochemistry laboratory, and other modern equipment and laboratory facilities.

EDUCATIONAL ACTIVITIES

The following courses were offered during 1967:

1. Tissue Culture and Radioisotope Techniques at Cellular and Subcellular Levels. This course was offered in November, 1967 under the direction of Dr. Martínez Silva, in collaboration with the UPR School of Medicine's Department of Microbiology. Participants were:

<table>
<thead>
<tr>
<th>NAME</th>
<th>COUNTRY</th>
<th>LECTURES</th>
<th>LABORATORY</th>
</tr>
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<tbody>
<tr>
<td>Antonio Rodríguez, M.D.</td>
<td>Colombia</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Humberto Rendón, M.D.</td>
<td>Perú</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Berta Morales Lira, M.D.</td>
<td>Perú</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Antonio Pedraza Ruiz</td>
<td>Spain</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ivonne Lastra, Ph. D.</td>
<td>Puerto Rico</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Carmen Romaguerra, M.S.</td>
<td>Puerto Rico</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Lydia Esther París</td>
<td>Puerto Rico</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Angel H. Torres Rivera</td>
<td>Puerto Rico</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Carmen M. Walsh de Rivera</td>
<td>Puerto Rico</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Aida Mercedes Díaz</td>
<td>Puerto Rico</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Carmen Minerva Nieves</td>
<td>Puerto Rico</td>
<td>X</td>
<td></td>
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2. Graduate Course in Virology. Sponsored by the UPR School of Medicine's Department of Microbiology, this course is offered at PRNC under the direction of Dr. Julio I. Colón, Associate Scientist (ad honorem). Participants, all from Puerto Rico, were: Dr. Ricardo Rosa, Nilda Alicia Méndez Nieves, Guadalberto L. Borrero Aldahondo, and Muri R. Rodríguez del Valle.
3. **Special Training.** The following persons were given training in the topics listed:

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<td>Nair Olsen</td>
<td>Uruguay</td>
<td>Medical student</td>
<td>Radiobiological techniques applied to Parasitology.</td>
</tr>
<tr>
<td>Boje de Touya</td>
<td>Puerto Rico</td>
<td>Medical technician, Municipal Hospital</td>
<td>Chromatographic separation of aminoacids.</td>
</tr>
<tr>
<td>Rosaura Rivera</td>
<td>Puerto Rico</td>
<td>Public Health Service</td>
<td>Labeling of snail and cercariae of Schistosoma mansoni.</td>
</tr>
<tr>
<td>Wilda B. Knight</td>
<td>Puerto Rico</td>
<td></td>
<td>Radiocisotopic techniques in immunology.</td>
</tr>
<tr>
<td>George V. Hyllier</td>
<td>Puerto Rico</td>
<td>Veterans Administration</td>
<td>Labeling of antibodies for the diagnosis of tumors.</td>
</tr>
<tr>
<td>Eduardo Touya, M.D.</td>
<td>Uruguay</td>
<td>7 months</td>
<td>Chromosomal techniques.</td>
</tr>
<tr>
<td>Berta Morales</td>
<td>Perú</td>
<td>2 months</td>
<td>Chromatographic techniques for separation of labeling compounds.</td>
</tr>
<tr>
<td>Lira, M.D.</td>
<td></td>
<td></td>
<td>Chromatographic purification of labeled compounds.</td>
</tr>
<tr>
<td>Luis Barragán, M.D.</td>
<td>Bolivia</td>
<td>1 month</td>
<td></td>
</tr>
<tr>
<td>Hugo Ramírez, M.D.</td>
<td>Chile</td>
<td>1 month</td>
<td></td>
</tr>
</tbody>
</table>

4. **Radiobiology and Isotope Techniques.** A special 3-day course at PRNC was offered to the following students as a contribution to a graduate course in Embryology at UPR, Río Piedras:

- Melania Rodríguez, Dominican Republic; Zaidi Bagar, Pakistan, and Carmelo Camacho, Olga Vázquez, María Bobonis, Rafael Goytía, Miguel Bisbal and Ivonne Lastra, Ph. D., all from Puerto Rico.

5. **Orientation on PRNC Program in Tropical Nuclear Biology and Medicine.** A special orientation was given on July 26, 1987 to the following postgraduate fellows attending a Tropical Medicine Course at Louisiana State University: Allan James Cooper, M.D.;
Robert S. Goldsmith, M.D.; Harry W. Huizinga, M.D.; Edwin M. Ingersoll, M.D.; Christian Mathot, Ph.D.

6. **Thesis Research.** The following students initiated their thesis research:

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Degree</th>
<th>Source of Sponsorship</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmen Rivera</td>
<td>P.R.</td>
<td>Master, Microbiology</td>
<td>NIH</td>
<td>Effect of irradiation in interferon levels</td>
</tr>
<tr>
<td>Ricardo Rosa, M.D.</td>
<td>P.R.</td>
<td>Master, Microbiology</td>
<td>NIH</td>
<td>Effect of irradiation of the antigenicity of Echo viruses</td>
</tr>
<tr>
<td>Roger Ramos Aliaga</td>
<td>Perú</td>
<td>Ph.D. in Biochemistry</td>
<td>PAHO</td>
<td>Labeling of cocaine and metabolic studies</td>
</tr>
<tr>
<td>Orlando Rendón</td>
<td>P.R.</td>
<td>Ph.D. in Physiology of Medicine</td>
<td>School of Medicine</td>
<td>Effect of irradiation on chromosome function on human diploid cells</td>
</tr>
</tbody>
</table>

**COOPERATIVE RESEARCH AND TRAINING**

Cooperative research and training programs are maintained with the following institutions and agencies:

A. **School of Medicine, UPR**

1. Department of Microbiology - Dr. Julio I. Colón, virologist, has been assigned to work at PRNC as an "ad honorem" member of the staff.

2. Department of Parasitology - The interchange of information and biological material with this department has been continued.

3. Department of Biochemistry and Nutrition - Dr. E. Toro Goyco, Associate Professor of Biochemistry and Nutrition, has begun a study of the biochemistry of *Schistosoma mansoni* eggs; he is receiving help from the Division.
4. Dr. Jorge Chiriboga, Professor of Biochemistry (ad honorem) at the UFR School of Medicine, has lectured at the Department of Biochemistry.

B. U.S. Public Health Service

Cooperation on Schistosomiasis research with the group headed by Dr. Frederick Ferguson has continued. Biomphalaria glabrata snails are labeled with $^{89}$Sr and Schistosoma cercariae with $^{75}$Se to study the biological characteristics. It is hoped that these studies will eventually contribute to the control of this disease. Mrs. Wilda B. Knight of the USPHS is assigned to PRNC on a part-time basis.

C. Veterans Administration Hospital

A project on the resistance of mice to Schistosoma mansoni introduced into the peritoneal cavity in Millipore diffusion chambers is being conducted by Dr. Menéndez Corrada and Mr. George Hyllier of the Veterans Administration Hospital in collaboration with PRNC. A note with the results is ready to be sent for publication.

Dr. P. Crosby of the V.A. Hospital is conducting research on xanthene-oxidase levels in mice infected with normal and irradiated cercariae provided by PRNC.

D. Schistosomiasis Laboratory

Dr. José Pellegrino, Director of the Universidad Federal of Minas Gerais, Belo Horizonte, Brazil, spent one month at PRNC with the Schistosomiasis group.

Dr. Celio Murilo de Carvalho Valle spent three months at PRNC working on the rhythm of shedding of the Schistosoma cercariae by Biomphalaria glabrata snails, studying the effects of radiation on host-parasite relationships of snails infected with Schistosoma mansoni, and learning radiobiological and isotopic techniques in parasitology.

E. Walter Reed Army Institute of Research

Dr. Lawrence S. Ritchie of the Walter Reed Army Institute of Research in Washington, D.C., has been temporarily assigned to
PRNC to collaborate with the Schistosomiasis group as an ad honorem staff member.

RESEARCH ACTIVITIES

Research is associated with radiobiological training activities offered at animal, cell and subcellular levels. Special emphasis has been given to the study of tumors and the effects of radiation on the host-parasite relationship in various biological systems using tissue culture, arthropods, snails, and other animals. Schistosomiasis research is described separately in this Report. An account of current research, organized by sections, follows:

A. Mammalian Metabolism Section

1. Studies with Scandium-46 to Test its Utility in Population Dynamics Research. The study of the biological behavior of $^{46}$Sc in the vectors of various diseases was continued. When injected into the mouse either intraperitoneally or intravenously, $^{46}$Sc is permanently retained. When injected in the stomach it is eliminated within the first 24 hours. If $^{46}$Sc comes in contact with the skin it is not absorbed.

When the isotope was injected intravenously, a blood clearance of 4.6 hours was found. Lizards, frogs, and different snails such as Marisa cornuarietii and Biomphalaria glabrata were successfully labeled with $^{46}$Sc.

The liver is the organ that picks up most of the activity when $^{46}$Sc is injected intravenously. After cell fractionation of the liver, the nuclei and cell debris have the largest activity.

Following intraperitoneal injection, most of the activity was present in the intestine.

Results thus far indicate that $^{46}$Sc is satisfactory for population dynamics studies.

The feasibility of adding two or more radioactive labels that could be detected by whole body counting with gamma spectroscopy to the predator of the snail, Biomphalaria glabrata, was
explored. This snail is one of the hosts of Schistosomiasis, a parasite which affects man.

2. **N-demethylation of Cocaine and Nutritional Status** - Ten million natives of South America's Andean regions chew coca leaves, which contain cocaine. This addiction is associated with the low nutritional levels prevalent among the population. In rats it was demonstrated that cocaine reduces to normal levels the high lipid content in the liver of the animals being fed a low protein diet. One of the goals is to label cocaine with $^{14}$C in the methyl group of tertiary amine in order to learn about the mechanism of methyl donation. The enzymatic N-demethylation of cocaine has been studied as a possible important pathway in the metabolism of rats and mice. The Km of the enzymatic reaction for cocaine is $1.09 \times 10^{-6}$ M (See Figure 1).

The enzymatic reaction is catalyzed by a microsomal system of the liver. The enzymatic system uses NADPH$_2$ and Mg$^{++}$ as cofactors. The system needs a continuous generator to produce a reduced coenzyme. The animal's nutritional state changes the rate of the reaction. Animals with low protein diet have a 70% reduction compared with the controls.

Chromatographic methods and chemical synthesis of the metabolites has been accomplished.

3. **Correlation between body burden and Schistosoma pigment in the liver of mice experimentally infected with Schistosoma mansoni.** *

4. **The study of the effectiveness of Schistosomidal drugs with the aid of $^{59}$Fe.** *

B. **Mammalian Radiobiology Section**

1. **Radiation effect ($^{60}$Co) on the host-parasite relationship in mice infected with Schistosoma mansoni.** *

2. **Susceptibility of mice to Trypanosoma cruzi.**

   a. **Effect of irradiation.** Because of numerous

* See Schistosoma report elsewhere in this Annual Report.
direct or indirect physiological effects of radiation on animals (anemia, leucopenia, plasma, electrolyte and protein disturbance, ulceration of the gastrointestinal tract, etc.), it is expected that the host parasite relationship in mice infected with Trypanosoma cruzi will be affected by radiation. In one experiment a group of newborn mice was irradiated with 400 Rads and another group was kept as control. Both groups were inoculated by the intracerebral route with .025 ml of a five-day culture of Trypanosoma cruzi; thirteen days later the animals were bled from the tail and tested for parasites. When the mice died, the tissues were examined in search of parasites. The results are summarized in the following table:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of Positives</th>
<th>No. Examined</th>
<th>No. of Positives</th>
<th>No. Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

As can be observed, irradiated mice showed 100% parasitemia when the controls showed only 14%. The other organs showed a still more striking difference.

(1) As can be seen in Figure 2, a radiation dose of 400 Rads induces death of the infected animals at an earlier stage than in non-irradiated animals. Under these circumstances, the LD50 occurs in the irradiated group on the 17th day after inoculation: in the non-irradiated group, the same effect takes place 26 days after inoculation.

b. Time of radiation relative to infection. It has been reported that the moment selected for radiation during the course of an infection is of great importance. An experiment was designed in which mice were exposed to 400 Rads and infected at different time intervals. The infective doses of Trypanosoma cruzi
Figure 1

Dependence of initial velocity of \( \text{N-demethylation} \) reaction on cocaine concentration. Lineweaver-Burk plot.

\[ \text{km} = 1.09 \times 10^{-4} \text{ M} \]

\[ \frac{1}{\text{km}} \]

\[ \frac{1}{\text{k} / \text{M}} \]

\[ \frac{1}{\text{S}} \]

Figure 2

T. Cruzi in Normal and Irradiated Mice - Cumulative Lethality by Intracerebral Inoculation
were administered by the intracerebral route 7, 2, and 1 days before irradiation, immediately after irradiation, and 1, 2, and 7 days after irradiation. The results, summarized in Table 1, showed an increased effect on mortality by radiation, more evident when given one day after infection.

c. Resistance in mice to *Trypanosoma cruzi* infections induced by irradiated culture. In order to know if irradiated *Trypanosoma cruzi* could multiply in the mouse host without producing disease but inducing resistance, one experiment was designed in which the strain Corpus Christi was subjected to different radiation doses. A non-irradiated group was used as a control. The six mice groups treated did not show parasitemia within the first 24 days. Five of these groups were challenged with the Tulamien strain and the results are summarized in Table 2.

3. Radiation activation of latent viruses in wild arthropods and vertebrates.

a. Effect of Gamma irradiation on the infection of mice and rats with Coxsackie virus. Adult white mice and adult wild rats (*Rattus rattus*), normally resistant to coxsackie infection, were exposed to whole body radiation with gamma rays from a cobalt source of 1510 curies. Immediately afterward, they were inoculated with coxsackie virus type A10. Nonirradiated controls were inoculated with the virus at the same time. Nonirradiated un inoculated controls were also included in the experiment. These groups of animals were bled daily and the virus content of the blood was determined in suckling mice.

The mortality rates in irradiated mice inoculated with coxsackie virus type A10 are shown in Table 3. Virus inoculations in unirradiated mice and in the group of mice irradiated with less than 400 Rads caused no deaths over a period of 21 days. The deaths shown in Table 1, that appeared in the groups of mice irradiated with 400 or more Rads and inoculated with virus, were doubtless due to the irradiation and not to the virus. The pattern of deaths was very irregular when the inoculated and uninoculated sub groups were compared, but the death rate increases and the average day of death decreases with the increase of radiation, regardless of the presence of virus (Table 4). These results indicated that there was not a synergistic effect of virus and gamma radiation when only death of the animal was taken as the criterion. However, when infectivity was taken as the
criterion, a consistently higher concentration of virus was demonstrated in the blood and tissues of the irradiated ones (Table 5). The viremia lasted for several days, depending on the radiation doses. Groups of mice irradiated with 100, 200, 300, and 400 Rads had a viremia that lasted up to 3 days. In the groups of mice receiving 500, 600, 700, and 800 Rads, the viremia lasted from 4 to 8 days, depending on the radiation dose. At present, we do not know if the virus is indeed replicating in the mice tissues or if it is just the inoculum that persists longer in the irradiated animals. However, the high titer of virus recovered does indicate that some multiplication is taking place in some of the irradiated groups.

The results obtained with the rats are very similar to those obtained previously with adult mice. Among the rats irradiated with 400 Rads and inoculated with virus a significantly high concentration of virus was found in the blood from the first to the 7th day after irradiation and inoculation with the virus. The rats irradiated with 800 Rads and inoculated with virus showed viremia on the 1st day which lasted for more than 7 days (Table 6). Although not at a very high titer, virus seems to persist longer in animals irradiated with 800 Rads. Rats that were not irradiated and inoculated with virus showed a very low titer viremia for only one day.

b. Activation of coxsackie virus by radiation in adult mice and adult wild rats. After an observation 22 days following the original treatment, surviving mice were again tested for the presence of virus in the blood, feces and from some selected organs of animals that were sacrificed. No viral activity was found in the blood of any of these animals nor in the organs of those sacrificed. A second dose of irradiation (400 Rads) was then given to these mice. After 48 hours they were bled, sacrificed, and the following organs were selected and harvested individually: brain, lungs, heart, liver, pancreas, and spleen. Pools of similar organs from animals of the same group were made and tested for the presence of virus in suckling mice. Viral activity was found in the spleen, brain, heart, liver, and pancreas (Table 7). Only the original virus, coxsackie A10, was found in these organs. The important point here is that after the second irradiation treatment virus was isolated from groups of mice that just previously had shown no virus in the blood, nor in any of the organs tested. Either the active virus had been present in very low concentration, and was not detectable by our methods or the virus was in a latent state and was activated by radiation.
The wild rats that survived 25 days after the original treatment showed no viral activity from the 16th day after treatment. These animals were further irradiated with 800 Rads. Fifteen days later they were bled, sacrificed and the following organs tested for viral activity: lungs, heart, liver, pancreas, and spleen. Coxsackie A10 was again isolated from the pancreas of rats that were previously irradiated and inoculated with coxsackie virus. As shown earlier in the mice, here we also have an indication of activation of a latent infection by radiation.

4. An attempt to induce immunity in mice to Schistosoma mansoni using milipore diffusion technique.*

C. Invertebrate Metabolism Section

1. Labeling of Biomphalaria glabrata with Radioseelenium*

2. Labeling of Marissa cornuarietis with Radioseelenium and Strontium 89.*

3. Periodicity of cercarial emergency.*

4. Labeling of cercariae of Schistosoma mansoni with radioisotopes.*

5. The antigens of Biomphalaria glabrata haemolymp. Characterization and identification of bands by Immunoelectrophoresis.*

6. Labeling the Trypanosoma cruzi with 75Se. Trypanosoma cruzi in the exponential growth phase was given 30 µC of 75Se. After 7 days the culture contained 1.72 x 107 trypanosomes and 60,400 cpm. After centrifugation at 5,000 rpm and resuspension in a new selenium-free medium, 3 groups of mice were inoculated by the muscular, peritoneal, cerebral, and venous routes. In order to determine the fate of the inoculated parasites, mice were sacrificed at different intervals and total body counts, as well as of organs were made. Results are summarized in Table 8.

D. Invertebrate Radiobiology Section

1. Effect of irradiation on the reproductive potential of Biomphalaria glabrata.*

* See Schistosoma report elsewhere in this Annual Report.
2. Effect of irradiation (\textsuperscript{60}Cobalt) on the host-parasite relationships in snails infected with \textit{Schistosoma mansoni}.*

E. Cellular Radiobiology Section

1. Cellular lines derived from a murine chondrosarcoma. It was reported previously that at PRNC three cellular lines had been isolated and serially propagated in vitro from a murine chondrosarcoma. Work is being carried out in order to determine characteristics related to the chromosomal complement and immunology of the cells.

The methods used to obtain the cellular lines as well as the chromosome study were reported in the 1966 Annual Report, page 85.

It was reported in the PRNC 1966 Annual Report that the DC II-2 cells showed a bimodal distribution of chromosomes with peaks of 74 and 128. After cloning the cells it was possible to obtain a clone with a distribution of 69, while one of the clones showed 14\% of the cells with 75 chromosomes and 86\% of the cells with 136. The studies with DC-II-4 have showed a clone with a modal distribution of 70 chromosomes.

2. Effect of irradiation on \textit{Trypanosoma cruzi}. Further studies have been conducted in order to determine the mechanism of action by radiation on \textit{Trypanosoma cruzi}.

Besides the Corpus Christi and Bartoldo strains reported on in PRNC 1966 Annual Report, the Tulahuen strain of \textit{Trypanosoma} (kindly provided by Dr. Irving Kagan, from the National Communicable Disease Center, Atlanta, Ga.) was used.

Results concerned with modifications by the different doses of radiation under Morphology of \textit{Trypanosoma cruzi} have been reported previously.

Reproduction: After exposure to doses ranging from 100 to 100,000 Rads the lag phase is increased in relation to the dose. With 1,000,000 Rads, viability is entirely abolished.

In order to determine the ability of irradiation exposed \textit{trypanosoma} to incorporate thymadine into DNA, one experiment was designed and the counts per minute pt \textsuperscript{3}H\textsubscript{3} labeled thymidine determined per 3 days every 24 hours. The results, summarized in Figure 3 show a close relationship with the growth in vitro.
Infectivity in mice: The ability to infect suckling mice by the i.c. follows a pattern very similar to that shown for infectivity in cells (Figure 4).

3. Effect of Irradiation in human diploid cells. - Human lung fibroblasts of fetal origin (WI-38) can be cultivated in vitro for about 50 passages. When the cells have reached this state they are no longer able to divide and gradually die. This process has been compared with aging in the superior animals and might be used as a tool for studying the effect of radiation on the aging process at cellular level.

During the 20th passage, WI-38 cells seeded in Leighton tubes (100,000 cells per ml) incubated for 4 hours at 37°C. While attached to the glass they were subjected to the following doses from a 60Co source: 25, 50, 100, 200, and 400 Rads. After radiation the medium (Eagle’s plus 10% calf serum) was changed and the cells incubated again at 37°C. The cells were observed daily. When they reached a confluent sheet in the tubes that had received the higher doses of radiation, they were trypsinized, counted and split 1 to 2 in new Leighton tubes. After 24 hours of incubation, one tube in each series was removed and the chromosomes were counted. The other tube was further incubated and observed daily until sheets were present. They were trypsinized, split 1 to 2 and the same process repeated. The number of splits of the control was considered the reference and compared with the irradiated cells.

Six days after irradiation the cells that received 25 and 50 Rads reached approximately the same population as the controls. However, the cells treated with 100 Rads reached only 50% of the control population, and those exposed to 400 Rads showed a greater delay in division, reaching only 20% of the control group after 6 days. Even though the cells exposed to 400 Rads were the most damaged, and always showed a smaller population, this effect is not observed in the total number of cell divisions. They reach the same level of multiplication and the cumulative number of cells is not significantly smaller.

Chromosome alterations: When designing this experiment it was hypothesized that chromosome aberrations induced by radiation might lead to heteroploidy and, therefore, to the appearance of a new cell line. In the experiments conducted so far, it has not been possible to obtain a new line of cells even with the highest
radiation dose (400 Rads). However, qualitative alterations of the chromosomes were obtained from split 1 to split 22 when the cells had reached the limit of their reproductive capacity.

New experiments are being planned in order to study the metabolism of DNA and RNA that could serve as a clue to understanding the observed alterations.

4. Effect of irradiation on the host-parasite relationship of Trypanosoma cruzi at cellular level. As previously reported, cell monolayers irradiated with doses from 100 to 50,000 Rads were able to support the cellular multiplication of Trypanosoma cruzi. It was observed that the number of parasites per cell was greater with increasing radiation doses. In order to study the radiation effect on cells infected with Trypanosoma cruzi, one group of cell monolayers in Leighton tubes was irradiated with 6,400 Rads. The medium was replaced and the cells inoculated with 1,000,000 culture forms of Trypanosoma cruzi. The control group was treated in a similar way, but it was not irradiated. Every 24 hours one tube of each series was removed, fixed, and stained.

The results (Table 9) showed a greater number of parasites in the irradiated cells at an earlier stage. The mechanism accounting for this phenomenon could be an alteration of the cellular membrane by radiation that would permit the earlier entrance of the trypanosomes. Once inside the cells, the parasites multiply by binary fission with a generation time of approximately 12 hours. When the multiplication of the Leishman-Donovan bodies reach a certain number inside the cells, the parasites undergo morphological changes. These are characterized by the acquisition of protoplasm, appearance of a flagellum, and separation of the nucleus-kinetoplast complex. At this stage, the parasite becomes motile, enabling the cell to move. The maturation process continues until the elasticity of the cellular membrane is overrun and the cell is disrupted, liberating the intracellular trypanosomes. The liberated parasites swim in the nutrient media until they penetrate the new cells, to repeat the cycle. The whole process of intracellular infection from the penetration of a single parasite into the cell, until the cell is disrupted and the parasite becomes free, lasts about four to five days.

An experiment was designed to determine whether the parasites reproduced in cultured medium irradiated or non-irradiated, kept their
infectivity for mice and cells. Monolayers of DC-II Cells were irradiated, and one non-irradiated monolayer group was used as a control. Both groups were inoculated with 1 ml of cultured trypanosomes and were left undisturbed during 4 days. The medium was changed and the cellular sheets washed three times with Hanks solution. The last washing was titrated in order to determine the infective activity, both in mice and in cells. From the fourth day, the medium was changed every 24 hours, the trypanosomes counted and infectivity tests in mice and cells were carried out. The infectivity of trypanosomes was not affected for at least a period of 32 days. (Figure 5).
### TABLE 1

Lethality of *T. cruzi* Infection on Mice Exposed to 400 Rads at Different Stages of Infection

<table>
<thead>
<tr>
<th>Day of Infection</th>
<th>LD$_{50}$ at 35 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td>860</td>
</tr>
<tr>
<td>-2</td>
<td>834</td>
</tr>
<tr>
<td>-1</td>
<td>700</td>
</tr>
<tr>
<td>0</td>
<td>440</td>
</tr>
<tr>
<td>+1</td>
<td>87</td>
</tr>
<tr>
<td>+2</td>
<td>620</td>
</tr>
<tr>
<td>+7</td>
<td>390</td>
</tr>
<tr>
<td>No Radiation</td>
<td>1,000</td>
</tr>
</tbody>
</table>

### TABLE 2

Cross Strain Protection

<table>
<thead>
<tr>
<th>Group</th>
<th>Corpus Christi 1.5x10$^7$ in mice i. p.</th>
<th>Parasitemia</th>
<th>Death Rate (7-21 Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td>19/20*</td>
<td>0-20**</td>
</tr>
<tr>
<td>2</td>
<td>60°C x 1 Hour</td>
<td>23/23</td>
<td>23/23</td>
</tr>
<tr>
<td>3</td>
<td>10$^5$ Rads</td>
<td>14/14</td>
<td>14/14</td>
</tr>
<tr>
<td>4</td>
<td>5 x 10$^5$ Rads</td>
<td>17/17</td>
<td>17/17</td>
</tr>
<tr>
<td>5</td>
<td>10$^6$ Rads</td>
<td>25/25</td>
<td>25/25</td>
</tr>
<tr>
<td>6</td>
<td>Medium Only</td>
<td>26/26</td>
<td>26/26</td>
</tr>
</tbody>
</table>

* Minimal and delayed (appearance in some up to 99 days).
** 2/20 at 35 days; 7/20 at 96 days.
### TABLE 3

**Mortality Rates in Irradiated* Mice Inoculated and Non-Inoculated With Coxsackie Virus**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Radiation</th>
<th>Not Inoculated Ratio Per Cent</th>
<th>Inoculated Ratio Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0/16***</td>
<td>0/14</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>0/14</td>
<td>0/12</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>0/15</td>
<td>0/14</td>
</tr>
<tr>
<td>IV</td>
<td>300</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>V</td>
<td>400</td>
<td>2/14</td>
<td>4/14 29</td>
</tr>
<tr>
<td>VI</td>
<td>500</td>
<td>4/14</td>
<td>4/14 29</td>
</tr>
<tr>
<td>VII</td>
<td>600</td>
<td>10/14</td>
<td>6/14 43</td>
</tr>
<tr>
<td>VIII</td>
<td>700</td>
<td>14/14</td>
<td>14/14 100</td>
</tr>
<tr>
<td>IX</td>
<td>800</td>
<td>14/14</td>
<td>16/16 100</td>
</tr>
</tbody>
</table>

* Mice (28-30 weeks old) were inoculated with $10^6.7$ suckling mice coxsackie virus type A10 immediately after irradiation with gamma ray from a cobalt$^{60}$ source of 1,510 curies.

** Animals were observed for 21 days.

** **Numerator is the number of mice dying; denominator is the number of mice exposed to risk.
TABLE 4

Effect of Irradiation* Upon the Average Day of Death of Adult Mice
Inoculated With Coxsackie Virus

| Groups+ | Rads | $10^6\cdot\text{LD}_{50}$ | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 21 | A.D.D.** |
| V       | 400  | +                        | 2 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|         | 400  | -                        | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| VI      | 500  | +                        | 2 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|         | 500  | 2                        |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| VII     | 600  | +                        | 2 | 2 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|         | 600  | -                        | 2 |   |   | 2 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| VIII    | 700  | +                        | 2 | 2 | 6 | 2 | 2 | 13.6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
|         | 700  | -                        | 2 |   |   | 4 | 2 | 2 |     | 2 |   |   |   |   |   |   |   |   |   |   |   |
| IX      | 800  | +                        | 2 | 2 | 2 | 2 | 2 | 2 |     | 4 |   |   |   |   |   |   |   |   |   |   |   |
|         | 800  | -                        | 4 | 8 | 2 |   |   |   |     |   |   |   |   |   |   |   |   |   |   |   |   |

* Mice (28-30 weeks old) were inoculated with coxsackie virus type A10 immediately after gamma irradiation from cobalt$^{60}$ source of 1,510 curies.
+ There were no deaths in the inoculated or inoculated groups receiving less than 400 Rads.
** Average day of deaths subsequent to irradiation.
**TABLE 5**

Viremia Found in Adult Mice Irradiated* and Inoculated
With Coxsackie Virus Type A10

<table>
<thead>
<tr>
<th>Number of Mice</th>
<th>Treatment Rads</th>
<th>DAYS AFTER VIRUS INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>200</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>300</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>400</td>
</tr>
<tr>
<td>VI</td>
<td>7</td>
<td>500</td>
</tr>
<tr>
<td>VII</td>
<td>7</td>
<td>600</td>
</tr>
<tr>
<td>VIII</td>
<td>6</td>
<td>700</td>
</tr>
<tr>
<td>IX</td>
<td>6</td>
<td>800</td>
</tr>
</tbody>
</table>

* Mice (28-30 weeks old) were inoculated with $10^6.7LD_{50}$ suckling mice Coxsackie virus type A10, immediately after irradiation with gamma rays from a Cobalt 60 source of 1,510 curies.

**Highest concentration of sample tested did not cause any death when tested in 2 litters of 8 mice each.

+ Animals were bled from the tail, the blood was pooled and titer given as $log LD_{50}$ per ml of blood.

++ Highest concentration of virus tested showed less than 50% mortality.
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rats</th>
<th>Treatment</th>
<th>Virus Inoculated</th>
<th>DAYS AFTER VIRUS INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rads</td>
<td>10^6.7LD50</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>0</td>
<td>+</td>
<td>&lt;2.23**</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>400</td>
<td>+</td>
<td>4.93+</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>400</td>
<td>-</td>
<td>0**</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>800</td>
<td>+</td>
<td>6.90</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>800</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>8</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

* Adults rats of weight from 105 to 207 grams and unknown age were inoculated immediately after irradiation with gamma rays from a Cobalt 60 source of 1,510 curies.

** Highest concentration tested showed less than 50% mortality.

+ The animals were bled from the tail, the blood pooled and titer given as log LD<sub>50</sub> per ml of blood.

++ Highest concentration of the sample tested did not showed any death when tested in 2 litters of 8 mice each.
TABLE 7

Viral Activity in Organs of Mice Irradiated* With Gamma Rays and Inoculated With Coxsackie Virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment of Rads</th>
<th>Number of Mice</th>
<th>Spleen</th>
<th>Brain</th>
<th>Heart</th>
<th>Liver</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>400</td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>500</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>600</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mice (28-30 weeks old) were inoculated with $10^6 \cdot 7LD_{50}$ suckling mice Coxsackie Virus A10 immediately after the first irradiation. A second dose of radiation 400 Rads was given 22 days after the first irradiation dose was given.

**Similar organs of the animals of a group were pooled and tested for viral activity in suckling mice.
### TABLE 8

**INVASION OF TISSUES BY Se\textsuperscript{75} TAGGED T. CRUZI (BER)**

<table>
<thead>
<tr>
<th>POST-INOC. ROUTE</th>
<th>INOC. ROUTE</th>
<th>1 HOUR</th>
<th>5 HOURS</th>
<th>10 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEIN</td>
<td>BRAIN</td>
<td>TONEUM</td>
<td>MUSCLE</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1,169</td>
<td>57</td>
<td>115</td>
<td>295</td>
</tr>
<tr>
<td>Kidneys</td>
<td>119</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass</td>
<td>1,068</td>
<td>492</td>
<td>2,268</td>
<td>1,661</td>
</tr>
<tr>
<td>Whole Body</td>
<td>2,585</td>
<td>683</td>
<td>2,962</td>
<td>1,760</td>
</tr>
</tbody>
</table>

*Radioactivity*
TABLE 9

Intracellular Multiplication of *T. cruzi* in Normal and Irradiated DC² Cells

<table>
<thead>
<tr>
<th>Generation</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>N</td>
<td>R/N</td>
<td>R</td>
</tr>
<tr>
<td>1</td>
<td>68</td>
<td>26</td>
<td>2.62</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>33</td>
<td>2.05</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>6</td>
<td>4.30</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>5</td>
<td>1.23</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>12</td>
<td>2.42</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1</td>
<td>10.00</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Above: Silvio Aristizabal, M. D., a radiotherapy trainee from Colombia, is taking a radiobiology course in the Medical Sciences and Radiobiology Division. Below: Medical School student Gualberto Borrero propagates sindbis virus into chicken embryo by inoculation. Observing are Dr. Julio I. Colon and Research Associate Mrs. Mirta Toro de Rodriguez.
Right: Control rod drives and operating platform for one kilowatt reactor. Below: Reactor control room.
REACTOR

The Reactor Division offers support and services to other divisions of PRNC which require neutron and/or gamma irradiation. It operates and maintains: (1) a one-megawatt, pool-type research reactor, (2) a ten watt, aqueous-homogeneous L-77 reactor, (3) a cobalt sixty gamma irradiation pool, and (4) high level hot cells.

The Reactor Division also operates and maintains all the auxiliary equipment associated with the reactor such as beam tubes, rabbit system, fuel element irradiator and gamma room, transfer port, etc., and all pool water cooling and purification equipment.

During the year, the one-megawatt reactor has been operating routinely two shifts per day, five days a week accumulating a total of 2939.176 megawatt-hours. During this same period, a total of 968 side-of-core irradiations were performed. In the gamma pool a total of 747 irradiations were carried out.

The L-77 reactor has been used intermittently for training and student experimentation. During the month of August, a pile oscillator was introduced in the L-77 reactor core. This oscillator is being used to carry out the experimental portion of the work of a graduate student.

Preparatory work to determine the worth and characteristics of the oscillator poison material and calibration of the associated electronic equipment was performed during the remainder of the year.

Installation of the one-kilowatt reactor facility in the north end of the one-megawatt reactor pool has been completed. Fuel grid plate, control rod drives and nuclear instrumentation have been mounted and tested. Installation of the ionization chambers is the only work remaining to be done.

A draft of the "Final Safety Analysis Report for the PRNC One-Kilowatt Reactor" has been prepared and submitted for AEC review and approval. Other documentation for which AEC approval is required prior to reactor startup is being prepared.

During the year, an experimental run with the one-megawatt reactor was carried out. This run was made at two megawatts and was of approximately three days duration. From the information gathered from this experiment, it was determined that the power
level of the present reactor can be increased to two megawatts with minor modifications to the present reactor operating limits. A recommendation to that effect has been submitted.

EDUCATIONAL ACTIVITIES

Mr. Fernando López-Carrasco, from México, finished his reactor supervisor course in June. He is now finishing the thesis work necessary for the completion of the requirement toward the M.S. degree.

During this year, the Reactor Division initiated a Reactor Operator Refresher Course. This course is designed to review all the material covered in the original reactor operator course and to bring up-to-date the operational aspects of the operator's duties which have changed during the past years. Course duration is expected to be two years. One operator trainee will become a qualified operator after successfully completing the course.

STAFF

Mr. Richard Brown-Campos, who served for several years as Reactor Supervisor, has been named Head of the Reactor Division. Mr. Pedro Cruz-González, formerly with the Health Physics Division, is now working with the Reactor Division as Associate Reactor Supervisor. Also, Mr. Fernando López-Carrasco is now working part time with the division as Associate Reactor Supervisor. Mr. Ernesto Guerra, Associate Reactor Supervisor, left the division in June, 1967.
Personnel monitoring. The developing of the films has just finished.
HEALTH PHYSICS

The Health Physics Division operates at both Río Piedras and Mayaguez with two main functions: (1) it provides the services needed for safe operation of the Puerto Rico Nuclear Center; (2) it contributes to the educational and research programs of PRNC. The responsibilities of Fire Protection and Industrial Safety were also added to the Division this year.

The services (see Table I) include consultation and supervision, as needed, in all matters concerning safety and especially in radiation safety. The Health Physics publications, PRNC-1, PRNC-2, PRNC-3, PRNC-4, PRNC-5, PRNC-6, and PRNC-89, establish the rules, regulations, and procedures to be followed by all PRNC personnel for safe operation, and by the Division in fulfilling their responsibilities.

In order to implement and enforce the safety regulations, the Division instituted monthly safety inspections and established safety committees with members within each Division. Indoc- trination on safety is offered to all PRNC personnel either through special courses or through the safety coordinating committee.

The education and research program includes: (1) courses in basic Radiation Protection at the graduate level for students not specializing in the field, (2) a program leading towards the M.S. degree in Health Physics, (3) advice and supervision of student research theses, (4) special training in applied Health Physics, and (5) basic research.

A new program leading towards the M.S. degree in Health Physics to be offered through the Department of Preventive Medicine and Public Health of the UPR, San Juan campus, is being considered. The program is expected to start in August 1968 pending approval of the Council of Higher Education of Puerto Rico, and the U.S. Atomic Energy Commission.
Table I

The following services are offered by the Division to any other project at PRNC:

a. Personnel monitoring*
b. Area monitoring
c. Calibration of radiation monitoring equipment
d. Radioactive materials handling
e. Environmental surveillance
f. Dosimetry*
g. Nuclear accident dosimetry*
h. Decontamination
i. Waste disposal
j. General laboratory safety
k. Industrial safety
l. Fire safety
m. Consultation on matters concerning all safety, especially on radiation and radioactive materials.
n. Indoctrination of staff members in Health Physics, Industrial Hygiene, Industrial Safety and Fire Prevention.

* This service is also offered to BONUS.

SERVICES

All service functions have been improved with emphasis on prompt service. Procedures have been revised and updated. The PRNC personnel exposure report, for example, is now distributed within two weeks after the collection of the film badges. Further improvements are expected when the computer program, now in development, becomes effective. Personnel monitoring films are now being supplied to the I. González Martínez Oncologic Hospital, University Hospital, the UPR School of Medicine, and the BONUS power plant, as well as to PRNC personnel (see Table II).

The environmental surveillance of soil, water, and vegetation in the vicinity of Mayaguez was discontinued. Only one sample of each is collected in addition to a water sample from the well of the India brewery, and these samples are processed monthly. The laboratory, however, is maintaining complete capability of performing a surveillance in case of an emergency.

The construction of the calibration facility was discontinued. New constructions in Mayaguez permitted the Division to hold the old calibration facility for lower range calibrations. The 20 Ci Cesium 137 source will be placed in one of the hot cells which will be modified into a calibration room for higher range calibrations.
The installations at Cornelia Hill will not be used at the present time. The film badge service and the Nuclear Accident Dosimetry (NAD) program will remain at the same place. A complete revision and updating of the NAD program is planned for the next year.

The radiation safety program at the Oncologic Hospital has greatly improved. Our aim is to adequately train hospital personnel so that they can implement the program under our supervision. The program, except for the film badge service, includes regular radiation surveys at the wards and rooms used for patients with implanted radiation sources, advice and recommendations on radiation protection, training in radiation protection of the nurses in charge of the patients carrying radioactive sources, and indoctrination in Health Physics to new hospital personnel.

All necessary Health Physics assistance was given to the Oncologic Hospital during the transfer of a Cobalt-60 Teletherapy unit from the San Juan City Hospital to the Oncologic Hospital.

A rough draft of an emergency plan relating PRNC problems and competencies to the surrounding community was prepared.

New procedures for the production and use of radioactive materials are now in effect.

Two committees were organized to deal with all safety problems. The committees have branches both in Mayaguez and in Río Piedras. The first consists of all division heads and one member from the Director's office. Through this committee, general safety policy and rules will be approved. The second consists of one member from each division who has supervisory or technical background. Through this committee, follow up of recommendations will be implemented. Indoctrination of personnel is offered in two ways: first, through safety institutes both in Mayaguez and in Río Piedras in conjunction with the Labor Department of the Commonwealth of Puerto Rico; and second, through lectures, personal contact and information pamphlets and posters supplied by the National Safety Council.
TABLE II

Health Physics Services 1967

Film Service to PRNC and BONUS

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Gamma</th>
<th>Neutron</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNC</td>
<td>5,411</td>
<td></td>
<td>853</td>
<td></td>
</tr>
<tr>
<td>BONUS</td>
<td>1,337</td>
<td></td>
<td>1,110</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6,748</td>
<td></td>
<td>1,963</td>
<td>8,711</td>
</tr>
</tbody>
</table>

Radiation survey meters calibrated:

- gamma: 149
- neutron: 23
- Total: 172

Area monitoring samples analyzed:

- smears: 896
- water: 27
- air: 10
- Total: 933

Environmental surveillance samples analyzed:

- water: 182
- air: 59
- soil: 45
- vegetation: 45
- Total: 331

Review of questionnaires for reactor experiments: 202

Review of requests for use of irradiation facilities other than reactor: 4

Medical Dispensary - Number of cases seen:

- minor accidents: 83
- physical exams: 74
- Total: 157
EDUCATION AND TRAINING

The education program in Health Physics has two main objectives. The first is to provide a graduate program leading to the M.S. degree and advanced training in Applied Health Physics for students who are planning a career in Health Physics. The second is to provide indoctrination in radiation safety and (general safety as well) for FRNC personnel and for students who will be working with radiation sources, but who do not intend to specialize in Physics.

Three students were accepted during the year in the Health Physics master's program: Amalia Vélez Paradís; Luis Martínez Bayrón; Efigenio Rivera.

One student was accepted in the Applied Health Physics Program: Ignacio Maldonado-Rico.

Three courses, one at the College of Arts and Sciences, Mayaguez, and the others at the School of Public Health, San Juan, have been given for non-specialists. Each course covered such topics as: basic nuclear physics, radioactivity, interaction of radiation with matter, the biological effects of radiation, instrumentation and methods of measurement, and the principles of handling of radiation in all its forms. Public health aspects of radiation were emphasized in the San Juan course since the students were either physicians or sanitary engineers. In addition, lectures related to radiation protection were given as part of the regular FRNC "Radioisotope Techniques" course offered four times this year (see Table 3).

TABLE III

List of Students

A. Biol. 601 (Health Physics): Aida Roca de Mari; Jorge Pérez Rivera; Efigenio Rivera; Michael Gileadi.

B. PM PH 231 (Radiation Hygiene): Luis D. Bernier Rivera; Francisco Fernández Trizarry; Francisco Folch Castañer; Juan Negrón Santiago; Rafael Orta Allende; Radamés Rodríguez Guzmán; Rafael Sánchez Valentín; Angel Santiago García.

C. Radiological Physics Course for Residence in Radiology:
Personnel monitoring. Reading the tracks produced on special film by neutron irradiation.
José A. Berlingery; Priscilla Díaz de Carau; Bernardo J. Marqués; José A. Nassar; Lidia D. Nieves de Reyes; César H. Soto; Santiago Vidal.

Thesis research status under the supervision of Dr. Paraskevoudakis is as follows:

**Determination of G-values of the Fricke Dosimeter for Monochromatic X-Rays in the Range of 5 to 15 Kev.** Francisco Jiménez. This work was completed and included a calibration of the calorimeter, measurements of dose rates of fluorescent monochromatic X-ray beams. The student received his M.S. degree in August 1967.

**Sensitivity Improvements of the X-Ray Calorimeter.** José Aguiar. This work was completed and included the construction of two new absorbers with reduced mass, use of new type thermistors, construction of a new Wheatstone bridge, noise reduction, calibration, and X-ray measurements. The student received his M.S. degree in August 1967.

**Gamma-Ray Spectra Around the FRNC Reactor.** Heriberto Cuebas. This work is incomplete. The purpose of this study is to determine the spectral distribution of gamma radiation around the reactor. The results so far indicate that the predominant gammas have an average energy of about 0.1 Mev. The student is not working at the present time.

**Study of Exposure Received by Patients During Chest X-Ray Examinations.** Amalia Velez. The purpose of this study is to determine the dose delivered to the patients during routine chest X-ray exposure in Puerto Rico. The age and the size of the patients, and the particular X-ray unit used, kVp, mA settings and the filtration used are considered. The entrance, the exit, and the gonadal dose are to be determined utilizing thermoluminescent dosimetry techniques.

**BASIC RESEARCH**

The calorimeter project. Substantial progress was made last year in the improvement of the sensitivity of the calorimeter. The purpose of this work is to measure the weak monochromatic X-ray beams produced by crystal diffraction. The work so far has demonstrated that this is feasible. Powers lower than 0.05 µW can be measured. A new design of a multiple bridge is completed.
and it is expected to reduce the noise level sufficiently to measure powers of the order of 0.01 uW. The power content of diffracted X-ray beams is considered to be within this range.

The neutron dosimetry project. This work is also in progress and it will be completed only if requested by another project, pending decision on the new reactor. The data collected so far at the beam tube suggested the redesign of the plug system with a new shutter and a new collimator four inches in diameter. The filter will be of pure bismuth and is expected to reduce the gamma field considerably. A new development in this project is the purchase of a solid state neutron spectrometer. This system is the best available today for measuring neutron spectra. Thus it will be possible to develop fairly accurate neutron dosimetry for chemical and biological studies using the neutron beam and the pool as well.

The enzyme inactivation project. This work has been reactivated recently. The purpose was to study the heat inactivation pattern or Horseradish Peroxide (HRP) in solution before and after irradiation, and to determine the radiation damage induced. It was found that a dose of 200 rads delivered in the solution was capable of inducing considerable damage which could be made manifest by heat inactivation but which has not been detected by other means. The new development includes reconfirmation of HRP results and use of other enzymes to check the same effect.

The transpiration of the tritiated water project. This work is also in progress. A series of measurements were performed at the rain forest. The transpiration of tritiated water from the rain forest soils was studied in cooperation with the Terrestrial Ecology Project. The Terrestrial Ecology Project is studying the movement of tritiated water in soils, and the Health Physics Division is studying the movement of tritiated water in the atmosphere. A cold trap technique using liquid nitrogen was utilized to collect tritium samples from the atmosphere at different heights and around the 1-square meter area sprinkled with 50 mCi of tritiated water diluted to 1 gallon of water. The results thus far indicate that the transpiration of water in the rain forest is a very slow process. Following an initial rise of a few hours, the activity persisted for several weeks at low rates. The relative humidity was 80 percent. The experiment will be repeated at a higher relative humidity level in the near future.
The phantom dosimetry program. This is a joint project with the Radiotherapy Division. Measurements are taken using the microrod teflon version of LiF thermoluminescent dosimeter in a plexiglass phantom. The advantage over the past technique is that one is able to place the dosimeters 0.5 cm apart. Dose distribution and depth dose measurements are being carried out.

* * *

Finally, it is worth mentioning a new effort in measuring the gonadal dose to patients undertaking abdominal X-ray exposures. It is felt that this kind of exposure may be more hazardous to the public in Puerto Rico than any other exposure. The work will consist of compiling data throughout the island and will include statistical variations. This work has just been initiated and there are no reportable results as yet.
10th anniversary symposium: U. S. Ambassador to the OAS Sol M. Linowitz reviews the day's activities with PRNC Director Henry J. Gomberg prior to Mr. Linowitz's symposium luncheon address on October 31. Below: Participants at one of the symposium's technical sessions, many of them using simultaneous translation "wands."
OFFICE OF THE DIRECTOR

The Office of the Director is responsible for planning and directing graduate level training and research activities and programs at PRNC, all of which are concerned with peaceful applications of nuclear energy.

The Office serves as the focus of internal and external communications for PRNC. Its staff prepares documents and reports on all organizational activities, assists in planning and managing special meetings and conferences related to PRNC's program, and participates in the teaching and research of several divisions of PRNC. The Office also handles the dissemination of public information.

MEETINGS

During 1967 the staff of the Director's Office participated in meetings outside of Puerto Rico, and assisted in planning and preparing for others held on the island. An account of participation in meetings not listed in the "Papers Presented" section of this Report follows:


In February, Dr. Henry J. Gomberg, Director of the Puerto Rico Nuclear Center, represented PRNC at a meeting convened by the Education Committee of Associated Midwest Universities at Argonne National Laboratory. The need for a conference on Nuclear Energy Education in Latin America, to be sponsored by Associated Midwest Universities, was considered at this meeting. Among others invited to participate in this meeting were: Dr. Carlos Vélez, Instituto Politécnico Nacional, Mexico; Dr. Milton Campos, University of Minas Gerais, Brazil; Professor José Emilio Amorós, Instituto Tecnológico y de Estudios Superiores, Monterrey, Mexico; and Mr. John C. Cera, Division of Nuclear Education and Training USAEC.

Following the Argonne meeting, Dr. Gomberg visited the mines of the Inspiration Consolidated Copper Company of Arizona to observe techniques, processes and problems related to copper mining and refining. This information is needed in connection with development of the PRNC program in "Flowshare."
From Arizona, Dr. Gomberg went to the Nevada Test Site as an official observer of the Cabriole shot in the Plowshare Program. Though the shot was delayed because of adverse weather, he observed other related activity at the site, including inspection of results of previous tests, the U.S. Public Health Service farm headquarters for study of fallout effects, and the Nuclear Rocket Test Station installations.

On February 6, a joint meeting of the Puerto Rico Radiation Control Commission and the Radiation Advisory Board was held at the offices of the Department of Health of the Commonwealth of Puerto Rico in San Juan. Dr. Amador Cobas, Deputy Director of PRNC, is a member of the Radiation Control Commission. The following PRNC personnel are members of the Radiation Advisory Board: Dr. Howard L. Andrews, Assistant Director for Health and Safety; Dr. Víctor A. Marcial, Associate Director for Medical Programs; and Dr. Sergio Irizarry, Head of the Clinical Applications Division. All PRNC members attended.

The purpose of this meeting was to initiate revision of legislation governing the use of ionizing radiation in Puerto Rico in accordance with Law 79 dated June 24, 1965. In this Law the Governor of Puerto Rico was given the authority to sign an agreement with the USAECC to assume responsibility for controlling the use of radioactive materials in Puerto Rico which is now controlled by the USAECC. This responsibility will be assumed jointly by the Departments of Health and Labor of Puerto Rico. This was the first meeting of the Commission and Board. At this meeting Dr. Howard L. Andrews from PRNC was named chairman of the Radiation Advisory Board.

Dr. Gomberg represented PRNC at the USAECC Bio-Medical Program Directors Meeting at the AEC Health and Safety Laboratory in New York City on February 13-14. The first day and a half of the meeting was devoted to the Health and Safety Laboratory program. Aspects of procedures on clinical research and investigation involving human subjects were discussed during the program of the USAECC Bio-Medical Division. The Congressional Sub-Committee Report on Food Irradiation was also discussed.

On February 16-17, the Advisory Committee to the Puerto Rico Nuclear Center met in San Juan. In keeping with the recommendations of the USAECC Oak Ridge Operations Office, the Committee was expanded and broadened to cover the physical sciences and engineer-
ing, in addition to biology and medicine. Two outstanding scientists accepted invitations from University of Puerto Rico President Jaime Benítez to join the Committee: Dr. Michael Ference, Vice President for Research and Development at Ford Motor Company, and Dr. Frederick Seitz of the National Academy of Sciences. Also, Dr. John C. Bugher, who served as PRNC's Director from July 1960 to November 1966, accepted an invitation to return to the Committee, on which he had served before becoming Director.

Dr. Gomberg presented a paper at the Seminar on Applications of Nuclear Energy in Latin American Development, held at UCLA on March 27-28. During the trip, Dr. Gomberg also made arrangements for a future PRNC teaching program in "Flowshare" technology.

On April 14, Dr. Andrews, who is a past President of the Health Physics Society, officially installed the Florida Chapter of the Health Physics Society, in Jacksonville. Dr. Andrews also attended a special meeting of the National Academy of Sciences Advisory Committee to the Federal Radiation Council in Washington, D.C. The meeting was held to consider the problem of radiation exposure of uranium miners.

Dr. Gomberg attended the Conference of Presidents and Rectors of Caribbean Universities at the Crown Point Hotel, Tobago, on April 27-30. He served as a delegate from Puerto Rico at this meeting, together with UPR President Benítez, and participated in a discussion on research program planning for developing countries.

On May 1, Dr. Gomberg went to Washington, D.C. to attend the presentation of the Atomic Energy Commission Citation to Dr. John C. Bugher, former PRNC Director, by Dr. Glenn Seaborg, Chairman of the USAEC. The Citation included mention of Dr. Bugher's "outstanding leadership and foresight in developing and directing the training, education and research programs of the Puerto Rico Nuclear Center."

From Washington, Dr. Gomberg traveled to Mexico City for the International Conference on the Utilization of Research Reactors and Reactor Mathematics Computation, sponsored by the Reactor Group of the Centro Nuclear de Mexico and by the Mathematics and Computation Division of the American Nuclear Society. At this meeting, Dr. Gomberg met with Dr. Graef Hernández, Chairman of the Mexican Atomic Energy Commission; Dr. Albert Barajas, Chairman of the Ad-
visory Board of the Centro Nuclear de Mexico; and with staff members of the Centro, of the Instituto Politécnico, and of the Universidad Nacional Autónoma de Mexico. Preliminary plans were made to train Mexican students and staff at PRNC.

Dr. Victor Marcial, Associate Director for Medical Programs, attended the USAEC Bio-Medical Program Directors Meeting at the Lawrence Radiation Laboratory in Livermore, California.

On May 3, the Puerto Rico Chapter of the Health Physics Society was inaugurated by Dr. Walter Snyder, President-elect of the Health Physics Society, during a meeting in Mayaguez. Dr. Peter Paraskevoudakis, Head of the Health Physics Division, was installed as President of the new Chapter; Mr. Carlos Jiménez, an engineer at the BONUS Power Plant, was installed as President-elect; Miss Heidi Pabón, Research Associate in the Health Physics Division, was installed as Secretary; Miss Rosa Julia Santiago, Research Associate in the Marine Biology Program, was installed as Treasurer. Approximately 50 persons attended the meeting.

The 15th Annual Meeting of the Radiation Research Society was held at the San Jeronimo Hilton, San Juan, on May 7-11. Over 800 persons registered for the meeting, establishing a new attendance record. PRNC's staff prepared an exhibit for the meeting, organized a Symposium on Tropical Radiation Ecology, and presented papers at many of the sessions. Dr. Bugher served as chairman of the symposium. The local committee, responsible for coordinating meeting activities, was staffed entirely by PRNC scientists: Dr. Howard L. Andrews, chairman; Dr. José A. Ferrer Monge; Dr. Francis K.S. Koo; Dr. Robert A. Luse, and Dr. Peter Paraskevoudakis. Mr. Teodoro Moscoso, Board Chairman of Commonwealth Oil Refining Company and former Coordinator for the Alliance for Progress Program of the U.S. State Department, presented the address at the Society Banquet.

On May 3, the Technical Sub-Committee of the Puerto Rico Committee for Bilharzia, held its quarterly meeting at PRNC in San Juan. Personnel from the following institutions attended: Puerto Rico Nuclear Center, U.S. Public Health Service, Puerto Rico Department of Agriculture, Fisheries Commission, Department of Health, General Clinical Research Center, UPR School of Medicine, Walter Reed Army Research Institute, and the San Juan Veterans Administration Hospital.
On February 25, approximately 1,785 high school students visited PRNC in Mayaguez in conjunction with the annual Open House sponsored by the University of Puerto Rico at Mayaguez. PRNC staff members prepared exhibits in Nuclear Engineering, Agriculture, Physical Sciences, Marine Biology, and International Nuclear Energy for the Open House. A documentary film, "Atoms for the Americas" was shown at regular intervals. The photos on this page show various aspects of the Open House at PRNC.
On June 11-15, Dr. Gomberg attended a meeting of the Committee on Honors and Awards during the annual meeting of the American Nuclear Society in San Diego, California. On June 16, Dr. Gomberg visited the U.S. Department of Agriculture and the USAEC Headquarters in Washington, D.C. to confer with officials on insect control and other FRNC programs.

On September 28-29, the FRNC Advisory Committee met at FRNC, Mayaguez.

On October 16-20, Dr. Wheeler attended the Study Group Meeting on Research Reactor Utilization organized by the International Atomic Energy Agency at the Japan Atomic Energy Establishment, Tokai, Japan.

Tenth Anniversary Symposium

The UPR and the USAEC organized a "Symposium on Nuclear Energy and Latin American Development" to mark the tenth anniversary of the Puerto Rico Nuclear Center. The Symposium was held at the San Jerónimo Hilton Hotel in San Juan, Puerto Rico during the period of October 29 to November 1, 1967.

Among the distinguished participants in the Symposium were: Dr. Glenn Seaborg, Chairman, USAEC; Mr. James T. Ramey, Commissioner, USAEC; Honorable Sol M. Linowitz, U.S. Ambassador to the Organization of American States; Honorable Ben S. Stephansky, Deputy U.S. Representative to the OAS; Mr. Teodoro Moscoso, former Director of the Alliance for Progress; Mr. Sam S. Sapir, Manager, Oak Ridge Operations, USAEC; Mr. Jaime Benitez, President of the University of Puerto Rico; and Dr. Sigvard A. Eklund, Director General of the International Atomic Energy Agency. Mr. John T. Conway, Executive Secretary of the Joint Congressional Committee on Atomic Energy, read a "Statement" by Congressman Chet Holifield, Vice Chairman of the JCCAE, to the participants.

Puerto Rico's Governor Roberto Sánchez Vilella and his wife attended the cocktail and dinner held on Monday, October 30, 1967, during which Dr. Glenn Seaborg spoke on "Nuclear Energy in Latin America." All participants in the Symposium were invited by the Governor to a cocktail in his home, La Fortaleza, which was held on Tuesday evening.

Approximately 113 persons participated in the Symposium, including 25 from South and Central America, 49 from Puerto Rico,
and the remainder from continental United States and Europe.

On November 1, 1967, tours were arranged for the participants to visit PRNC facilities in either Río Piedras or Mayaguez.

From November 6-8, 1967, Dr. Gomberg attended a meeting of the advisory group for program planning in nuclear energy activities (OAS Regional Scientific and Technological Development Program), as one of two representatives from the United States. The other representative was Dr. E.S. Friedman from the Division of International Affairs of the U.S. Atomic Energy Commission. The advisory group prepared a preliminary report outlining an international nuclear energy program. The meeting was held in Oaxtepec, Mexico.

On November 18, 1967, the Puerto Rico Chapter of the Health Physics Society held its quarterly meeting in the Conference Room of the PRNC Bio-Medical Building.

The following PRNC staff members presented brief papers at the meeting:

a. María Palacios de Lozano, "Radiological Protection Problems Associated with Interstitial and Intracavitary Therapy"

b. Heidi Pabón, "Radiologic Protection in X-Ray Technology"

c. Peter Paraskevoudakis, "Health Physics in Space"

In addition, Dr. Victor Marcial, Dr. Owen H. Wheeler, and Dr. Robert A. Luse participated from PRNC. Dr. Charles Meinhold of the Brookhaven National Laboratory spoke on "Health Physics Training in Brookhaven National Laboratory."

Dr. Gomberg departed from Puerto Rico on November 24, 1967, in response to an invitation to join an IAEA Panel of Experts on Research Reactor Utilization which met in Vienna from November 27 through December 1, 1967.
Following the meeting in Vienna, Dr. Gomberg visited the facilities of the Greek Atomic Energy Commission in Athens to review its program and met with the President of the Commission, Dr. F. Demopoulos and members of the staff. Also at the invitation of the IAEA, Dr. Gomberg visited the IAEA Monaco Laboratory to discuss possible cooperative programs related to problems arising from discharge of radioactivity into the sea.

Dr. Marcial was in South America during the period of November 23 to December 16, 1967, participating in the following activities:

a. the Latin American Regional Conference of Cancer Control which was organized by the International Union Against Cancer in Santiago, Chile during the period of November 25 to 29.

b. the IV Latin American Cancer Congress in Buenos Aires, Argentina during the period of November 30 to December 5.

c. the IX Inter-American Congress of Radiology in Punta del Este, Uruguay during the period of December 6 to 12 where he served as a representative of Puerto Rico in the capacity of Counsellor. Dr. Marcial presided at the session on "Fractionation in Radiation Therapy."

d. The National Cancer Institute in Rio de Janeiro, Brazil, on December 13 where he met with Dr. Adayr Erias de Araujo, Head of the National Cancer Service.

Mr. Héctor Barceló, Assistant Director for Operations, attended the IAEA Study Group Meeting on Research Reactor Utilization in Bogotá, Colombia, on December 11-15, 1967. Mr. Barceló attended this meeting as a representative of the U.S. Government.

The American Society of Agricultural Sciences ("Sociedad Americana de Ciencias Agrícolas," or SACA) held its 27th Annual Meeting in the PRNC Conference Room, Mayaguez, on December 1. Dr. Robert A. Luse, Head of the Agricultural Bio-Sciences Division, presided over the meeting as President of the Society. Mr. José Cuevas, also of the Division, assisted as Secretary-Treasurer. Fifteen scientific papers on Puerto Rican Agriculture were presented at the two simultaneous sessions in the morning. At this meeting Mr. Cuevas presented a paper entitled "Radiation Preservation of Tropical Fruits." This meeting marked the end of the term of office for Dr. Luse and Mr. Cuevas, though both continue on the Board of Directors of SACA through 1969.
Dinner on the first night of the symposium. Seated, left to right: Roberto Sanchez Vilella, Governor of Puerto Rico; Dr. Glenn T. Seaborg, Chairman, US AEC; Sol M. Linowitz, U. S. Ambassador to the OAS. Standing: UPR President Jaime Benitez, PRNC Director Henry J. Gomberg.

Left: UPR President Benitez; John Conway, Secretary of the Joint Congressional Committee on Atomic Energy; James T. Ramey, Commissioner, US AEC. Right: During one of the technical sessions. PRNC Deputy Director Dr. Amador Cobas; Dr. Sigvard E. Eklund, Director General of the International Atomic Energy Agency; Dr. Shields Warren, Cancer Research Institute, New England Deaconess Hospital, Boston.
EDUCATION

Dr. Gomberg participated in the doctorate thesis examination of Mr. Baltazar Cruz-Vidal at Harvard University on May 30, 1968. His dissertation, entitled "F Center Formation at 78°K in KBr During Exposure to Monochromatic X-Ray Energies Around the Bromine K Edge," was prepared in Puerto Rico under the supervision of Dr. Gomberg. Mr. Cruz was granted a Ph.D. degree in Physics by Harvard.

Dr. Owen H. Wheeler submitted a proposal in May to the Dean of Arts and Science of the UPR at Mayaguez for the participation of PRNC (Mayaguez) in a doctorate program in Chemical Physical Sciences.

STAFF

From May 29 to June 10, Dr. Howard L. Andrews served as a Temporary Adviser for the Pan American Health Organization, Pan American Sanitary Bureau, Regional Office of the World Health Organization in Venezuela, Trinidad, and Guyana. In August, Dr. Andrews resigned his position as Assistant Director for Health and Safety to accept an appointment as Professor of Physics in the University of Rochester Medical School.

Miss Vivian Taylor terminated her appointment in November as a Technical Assistant to the Director.

Mr. Hector Barceló, who has served as Head of the Reactor Division and Technical Services, was appointed Assistant Director for Technical Operations in October. His responsibilities in this new position include supervision of reactor operations, supervision of Technical Services, coordination of planning and conceptual design preparation for general plant projects and capital projects, development of technical training programs, and provision of logistic support for the International Exhibit Program.
"ATOMS IN ACTION" IN LATIN AMERICA

Since 1965, the Puerto Rico Nuclear Center has assumed responsibility for the research activities conducted in conjunction with the "Atoms in Action" exhibits in Latin American countries.
Above: Inauguration day at the "Atoms in Action" exhibit in Quito, Ecuador. Below: Otto Arosemena, President of Ecuador, speaking at the inaugural ceremony.
PRNC PARTICIPATION IN THE US AEC "ATOMS IN ACTION" EXHIBIT IN LATIN AMERICA

Since 1965 the Puerto Rico Nuclear Center has been responsible for research conducted in conjunction with the Atoms in Action Exhibits in various countries of Latin America. The exhibits provide information on peaceful applications of nuclear energy to persons of varying backgrounds. The general public is given guided tours of special displays which demonstrate the nature, applications, and developments of atomic energy. Students, scientists and physicians are offered demonstration lecture courses and supervised participation in research involving applications of radiation to problems of regional origin and interest, with emphasis on the use of gamma and neutron sources available at the Exhibit.

In May and June, the exhibit visited Panama City, Panamá. In November and December it visited Quito, Ecuador.

RESEARCH ACTIVITIES IN PANAMA

A preliminary visit to Panama indicated that the research emphasis should be directed to the biological sciences because of the existence of the Gorgas Memorial Laboratory and the well-established Department of Biology at the National University. Projects were selected according to local needs, similarity of current research, and availability of PRNC personnel. Most of the projects were designed to be of service to graduate students working on their theses at the College of Pharmacy and Natural Sciences of the National University. The 5000-curie cobalt-60 gamma irradiation facility, the principal research tool, was used in projects related to mathematical statistics, radiation chemistry, food preservation, parasitology, bacteriology, and radiation effects in biological systems. A 6-curie Pu-Be neutron source was utilized primarily for neutron diffusion theory experiments. Phosphorus-32 was utilized for rice-soil fertilization studies. The exhibit's program in Panama received special cooperation from the Ministry of Agriculture, Commerce, and Industry; the University of Panama (College of Engineering, Natural Science and Pharmacy, Agricultural Engineering, Specialized Laboratories for Drug and Food Analysis) and the Gorgas
Memorial Laboratories. The National Association of Engineers and Architects cooperated in a special lecture program on Plowshare.

LECTURES IN PANAMA In conjunction with the research program, PRNC personnel gave the following lectures during the "Atoms in Action" Exhibit in Panama, which were attended by 645 persons.

Dr. Rupert Lee, PRNC Nuclear Science Division: Radiation Chemistry and Gas Evolution; Degradation of Polymers with Gamma Radiation.

Dr. Jorge Chiriboga, PRNC Assistant Director for Scientific Programs: Radiobiology in the Tropics I and II.

Dr. Robert A. Luse, PRNC Agricultural and BioSciences Division: Actual Status of the Food Preservation Program; The PRNC Projects in Food Preservation by Radiation.

Dr. Donald Sasscer, PRNC Nuclear Engineering Division: Neutron Cross Section; Neutron Diffusion Theory I and II; Neutron Activation Analysis.

Mr. Fausto J. Muñoz-Ribadeneira, International Exhibits Program: Introduction to Radiation Chemistry; Applied Statistics in Chemical Analysis; Calculation Formulas in Nuclear Homogeneous Reactors I and II; Chemical Dosimetry of Gamma Radiation.

A series of lectures on Plowshare using slides and films were also offered to the Association of Engineers and Architects of Panama. Mr. Harold Curtis from the AEC's San Francisco Operations Office, and Mr. Peter Myget from the Interoceanic Canal Studies Commission helped prepare the lectures and answered questions pertinent to the Panama Canal.

The PRNC representative, Mr. Fausto J. Muñoz-R. (M.S.) was in charge of this special lecture program on Plowshare requested by the AEC Division of Peaceful Nuclear Explosives (PNE). One hundred and forty people attended lectures on: Characteristics of the Nuclear Explosives and Nuclear Explosions; Safety Considerations in the Uses of Nuclear Explosives; Application of Nuclear Explosives in Large Scale Civil Engineering Problems; Industrial Applications of Nuclear Explosions.
A list of the graduate student theses already accepted by the University is indicated below:

A. PROJECTS AT THE UNIVERSITY OF PANAMA


- "Application of the Least Square Theory to Chemical Analysis". Person in Charge: Mr. Rubén Rivera.

- "Statistics and Nuclear Disintegration." Persons in Charge: Mr. Rolando Carvajal and Miss Delia Falcón.

- "G Value Determination in Fricke-Copper Solutions" I. Sulfuric Acid Concentration. Persons in Charge: Mr. Carlos R. Córdova and Mr. Brajil Morán.

- "Quantitative Determination of Fe+++ by the Prussian Blue Complex". Persons in Charge: Miss Jacinta Him and Miss Gladys Ortiz.

- "Dosimetry by Gas Evolution." Persons in Charge: Mrs. Nitza de Díaz and Miss Myrna Barreto.

- "Depolymerization of a Polymer of Methyl Methacrylate by Gamma Radiation." Persons in Charge: Miss Angela Tellos and Mr. Francisco Flores.

- "Binding Energy of the Deuteron" (H2). Persons in Charge: Mr. Gonzalo Molina and Mr. Francisco Flores.

- "Determination of Diffusion Length of Neutrons in Water Using Foil Detectors." Persons in Charge: Miss Elvira Cabada and Miss Irma Ríos.

- "Determination of Thickness of Water for Moderation of Neutrons from a Neutron Source." Persons in Charge: Mr. Plutarco Ramos and Mr. Félix Cuevas.
• "Pitting of a Thermal Neutron Flux in a Cylindrical Arrangement." Persons in Charge: Mr. Bolívar Vázquez and Mr. Jorge Lee.

• "Neutron Activation Analysis." Persons in Charge: Miss Analida Ardila and Miss Teresita Jaén.

• "Determination of the Range of Beta Particles." Persons in Charge: Mr. Silvio Candanedo and Mr. Bladimiro Castillo.

• "Orange Preservation by Gamma Radiation." Persons in Charge: Miss Blasina Saavedra, Miss Amanda Monge, Miss Elizabeth Rodríguez, and Mrs. Mercedes de León.

• "Radiobiology of Rhodnius prolixus." Persons in charge: Miss Geneva Luciani and Miss Carolina Windehake.

• "Special Project in Rhizobium Sp." Persons in Charge: Miss Alina Torres, Miss Mitzi Marenga and Mr. Carlos Delgado.

• "Radiation Effects on Daphnia Sp". Persons in Charge: Miss Sabina González and Mr. Alberto Cruz.

• "Radiation Effects on Frogs' Pigmentation." Person in Charge: Mr. Raimundo Husband and Mr. Domingo López.

• "32P in Soil-Rice Plants Fertilization Studies." Person in Charge: Mr. José M. Chaverri.

B. PROJECTS AT OTHER INSTITUTIONS

• At the Ministry of Agriculture, Commerce, and Industry

The research divisions of this Ministry participated in radiation preservation studies of potatoes and onions. The main objective is to control sprouting and extend product shelf life. The projects are being satisfactorily continued.

• At the Gorgas Memorial Laboratories

The Gorgas Memorial Laboratories are sponsoring the project on the radiobiology of Rhodnius prolixus where radiation effects were carried out in eggs, fifth pupae stage, and adult insects, by two students of the university. This institution has already completed studies of radiation effects on the development of infection by Tripanosoma rangeli in Rhodnius prolixus and Rhodnius pallenices. A third part of the project, initiated in cooperation with the PRNC Division of Medical Sciences and Radiobiology, consisted of studying the virulence of infection caused by Tripanosoma cruzi under the parameter of radiation.
RESEARCH ACTIVITIES IN ECUADOR

The same procedures used for the establishment of the FRNC program in Panama were followed, although the scientific development of the universities in Ecuador and the interest of government institutions in introducing the nuclear energy parameter in their current research problems was taken into account. This made it possible, for the first time in the AEC International Exhibits Program, for institutions located in Guayaquil (400 kilometers from Quito), Cuenca (600 kilometers from the capital), and Portoviejo (350 kilometers from Quito) to actively participate in the program.

The research program at the Exhibit in Ecuador had the cooperation of the Franco-Ecuadorian Institute of Agronomic Investigations (IFEIA, Guayaquil), the National Commission of What (CNT); the Central University of Ecuador (College of Pharmacy, College of Engineering, College of Agronomy and Veterinary Medicine); Polytechnic Institute (EPN); Ministry of Agriculture (Division of Agronomic Institute), the Catholic Institute (Institute of Natural Sciences), and the University of Guayaquil (College of Agronomy).

With the coming of the "Atoms in Action" Exhibit to Quito, for the first time studies in problems related to the agricultural economy of Ecuador were initiated using nuclear techniques. These included studies on the preservation by radiation of "naranjilla" (Solanum quitensis); the radiation effects on "Picudo negro" (Cosmopolite sordidus); and third, the first shipment of irradiated bananas was exported to the United States. For the first time also in South America, experiments on radiation effects on Anastrepha fraterculus Wied, were carried out. Due to the special cooperation received from Dr. Luis A. Romo (Ph. D. University of Wisconsin), a professor at Central University, it is anticipated that the number of publications produced will be the largest ever generated from an Exhibit research program.

LECTURES IN ECUADOR

In conjunction with the research program, personnel of the Puerto Rico Nuclear Center gave the following lectures during the "Atoms in Action" Exhibit in Ecuador, which covered the cities of Quito, Guayaquil, and Cuenca; registering a record attendance of 1731 people. Lectures offered outside of Quito are
indicated.

Dr. David Walker: Nuclear Techniques in Agricultural Research; The PRNC Research Project in the Irradiation of the Sugar Cane Borer; Nuclear Techniques in Agricultural Research (Guayaquil).

Dr. H. Harry Szmant: Mechanism of Chemical Reactions Using Radioisotope I and II; Mechanism of Radiation Induced Reactions.

Mr. José Cuevas Ruiz: Food Preservation by Radiation; The PRNC Research Projects in Food Preservation by Radiation (Guayaquil).

Mr. Juan Silva Parra: Nuclear Power and Desalinization; Radiation Sources and Their Applications.

Dr. Eddie Ortiz: Neutron Cross Section; Neutron Diffusion Theory I and II.

Mr. Fausto Muñoz-R: Research Programs of Atoms in Action Exhibit in Central America; Possibilities of Research at the Exhibit Laboratories; Principles of Radiation Chemistry (Guayaquil); Atoms in Action Exhibit in Ecuador (Cuenca); Theory of the Free Radical Formation in Radiation Chemistry; Chemical Dosimetry of Gamma Radiation; Research Programs of Atoms in Action Exhibit (Guayaquil); The Development of Atomic Energy and its Importance in Modern Technology.

Mr. Muñoz-R. also offered several lectures on "Flowshare" which are listed under the PRNC Peaceful Nuclear Explosive Program.

ACCOMPLISHMENTS OF THE RESEARCH PROGRAM IN PANAMA AND ECUADOR

Results are gratifying, since several scientific papers are being prepared for publication and some graduate students from the Universities will receive degrees through theses prepared under the PRNC research program at the Exhibits. A list of continuing and completed research projects is presented.
FOOD PRESERVATION

Studies were carried out on the radiation preservation of bananas (Ecuador's most important export product), "naranjilla," a typical fruit of the Ecuadorian highlands, and potatoes.

- **Studies on Banana Preservation** - The Franco-Ecuadorian Institute of Agronomy Research (IFEIA) carried out this project. Banana fruits from the Pichilingue area were irradiated with doses from 10 to 60 kilorads; the most favorable doses in "Gross Mitchell" variety were found between 30 to 40 kilorads, and the retardation on the maturation time was 8 days when bananas were kept at 65°F. A refrigeration chamber of the National Institute of Fishery and Hunting was adapted for this experiment.

- **Radiation Effects and Mycology Studies on Bananas** - The objective of the project was to study the behavior of the fungus infection in bananas which were irradiated for preservation studies. The fungus species which were used were *Cleistotrichum musanena*, *Fusarium solani*, *Botrodiploclados theobromae*.

The procedure was as follows:

a) a series of fruits were irradiated before the inoculation; b) a series of fruits were irradiated after the inoculation.

Doses of 30 to 40 kilorads, were found to be the most favorable in the preliminary studies.

- **Export of Irradiated Bananas to the United States** - Through direct action of the Exhibits' Office in Washington (DTI), Dr. E. C. Maxie of the College of Agriculture, University of California, at Davis, California, agreed to study the irradiated bananas (35 kilorads) from Ecuador, which were shipped from Puerto Bolivar (Pais de El Oro) on November 27 to Long Beach, California. The daily newspapers "El Comercio" from Quito and "El Universo" from Guayaquil published a first page article titled "First Exportation of Irradiated Bananas to the United States."

- **Preservation of Potatoes** - This project was located at the College of Pharmacy, Central University, and will form a base for a thesis project. In addition to physical tests on the acceptability of the product, the following biochemical tests related to dose and time parameters are being carried out: total sugar,
reducing sugar, and vitamin C.

- **Radiation Effects on "Naranjilla" Preservation - "Naranjilla" (Solanum quitoense) is a delicious fruit which has a wide internal market but foreign markets have not yet been found due to the fast deterioration of the fruit. This project was located at the Polytechnic Institute where a special maturation chamber with controlled temperature was built. The project, being carried out under the direction of Marcelo Coronel, has thus far shown that doses higher than 200 kilorads produce a physical damage in the fruit as well as destruction of flavor and aroma. Near ripe fruits show the most favorable aspect for irradiation treatment.

- **Entomology -** Studies on insects attacking valuable food stuffs and export products in Ecuador were carried out in cooperation with the Exhibit.

- **Radiation Effects on the Highlands Fruit Fly -** Fruits of sweet pulp that are cultivated in the highland valleys are suffering great damage from infestation by a fruit fly (Anastrepha fraterculus Wield). Taking as a base the studies of its life cycle performed by researchers from the College of Agronomy, Central University, preliminary studies on the radiation effects on this insect were carried out by Mr. Cristóbal Barba and Mr. César Wanderaberg from the Research Division, Ministry of Agriculture. Two thousand insects in pupal stages were irradiated with different dosages.

- **Work on "Picudo Negro" (Cosmopolite sordidus) -** For the first time in Latin America, studies on the radiation effects of Cosmopolite sordidus were carried out, thanks to the interest of IFEDA. Using special precautions, about 1000 insects were brought from Prebilingue to Quito. The study showed that doses of the order of 100 kilorads killed the insects in one day. If these studies are a success, they may constitute a real contribution to Ecuador; although much effort should be dedicated to studying the appropriate diet in rearing this insect, first in the laboratory and later in massive form.

- **Disinfection of Wheat and Flour -** Due to the interest of the National Commission of Wheat (CNT), a project on radiation effects in Platiedra gossipeda was initiated with the Exhibit in Ecuador; this insect is causing great damage in Quito's flour mills. CNT hired Miss Beatriz Hidalgo and asked Mr. Jaime Flores, employee of CNT, to work on a full time basis for the project whose main objective was to determine the ovoposition rate and
fertility of the eggs of *Platiedra gossypiela* in relation to the dose parameter. The Ministry of Agriculture also cooperated in this project and Mr. Flores will use the data when submitting his thesis to the College of Agronomy, Central University.

**RADIATION EFFECTS IN ORE DRESSING OPERATIONS**

Studies on the radiation effects in the flotation of sulfur and copper ores (Chalcopyrite type) were initiated at the Exhibit in cooperation with the Polytechnic Institute and the College of Engineering of Central University. Preliminary flotation trials in both projects indicated the most significant parameters that should be taken into account for the success of the operation. Based on this information, a factorial experiment was designed. Analysis of variance indicated the influence of the effects of the different factors by themselves, and in their combinations, on the yield of the operation. In both projects the equation of yield is being calculated by taking into consideration the replications and factors used in the experimentation. Correlation of data done by using the reciprocal matrix concept.

A second part of the project consisted of irradiated sulfur ore and chalcopyrite ore samples at different dose levels; the yield on the valuable component will be correlated to the dosage received by the examples.

The third part consisted of selecting sulfide and sulfur crystals and submitting them to an intensive gamma radiation field. Possible effects in crystalline structure are being studied at the laboratories of the National Bureau of Mines and also at the PRNC, using X-ray analysis diffraction techniques. Data from X-ray analysis will be correlated with the yield of flotation due to radiation effects, if any.

Two graduate students from Central University (Mr. Eduardo Vega and Mr. Rafael Pazmiño) and one from the Polytechnic, Mr. Trajano Ramírez, participated in these projects and will use the data when submitting theses. The thesis is a prerequisite for a professional degree.

**GENETICS**

IFEIA and the Catholic University at Quito initiated the following projects at the Exhibit.
- **Banana Buds - IFETA** decided to study the radiation effects on banana buds of the "Cross Mitchell" variety, which is very susceptible to fungus infection. The objective was to study the possible development through radiation of a mutation resistant to the fungus infection called "Panama sickness."

- **Work in Chalceonaria crenata** - Chalceonaria is a plant cultivated in the Ecuadorian highlands for ornamental purposes. Brother Clemente Armas (M.S., Biology, University of California at Berkeley), now a professor at the College of Science, Catholic University in Quito, irradiated seed samples of Chalceonaria for studies of chromosomes.

**Special Research Program at the College of Pharmacy, Central University**

Due to the special interest of the Dean of this College of Central University, and through the special cooperation of Dr. Luis A. Romo (Ph.D. University of Wisconsin), the following projects were carried out during the "Atoms in Action" Exhibit in Quito:

- **Adsorption of Cobalt-60 in Silicates** - Several samples of Ecuadorean clays activated by different procedures were used in this project. Mr. Claudio Carrillo cooperated with Dr. Romo and will use the data for a professional thesis at the university.

- **Radiation Effects in the Crystalline Structure of Kaolin as Measured by Phosphate (P³⁻) Adsorption and X-Ray Diffraction Studies.**

- **Depolymerization of Polysaccharides by Gamma Radiation** - Pectine samples were irradiated and viscosity measurements were used to evaluate the depolymerization rate.

- **Radiation Effects on the Degradation of Vitamin C and Stabilizing Effects of Polysaccharides.** An experiment was designed to study the possible stabilizing mechanism of polysaccharides in Vitamin C under gamma irradiation conditions. Thin layer chromatography techniques were used to evaluate the results.

- **Investigation of the Minimum Dosage of Gamma Radiation for Breakdown of Vitamin B 6 and B 12**
Dimerization of Acenophtylene - The dimerization of Acenophytene was achieved under a variety of experimental conditions and the mixture of trans and cis dimers is being separated by chromatographic means. This project, suggested by Dr. Harry Szmant, is being continued; all the other projects were completed in their experimental part.

Other Projects in Radiation Chemistry

The Exhibit's gamma irradiation facility was also used to study the decomposition of bipyridine, and in a polymer chemistry project.

Radiation Effects on Bipyridine - Mr. Guillermo Romero, a graduate student from the Chemical Engineering Department, Central University, has been studying the copolymerization of vinyl acetate and crotonic acid using the concentration of this acid and the radiation dose as parameter. The copolymerization rate and reactivity ratios have been evaluated and the molecular weight of the polymer will be determined at the PRNC laboratories. Mr. Romero will use this data for a thesis at Central University.

GRADUATE STUDENT THESIS

Graduate students from the Central University and the Polytechnic Institute participated in different research projects and will use the data for submitting a thesis, prerequisite for a professional degree. The students and project titles are:

Radiation Effects on the Flotation of Chalcopryite. Mr. Rafael Pazmiño and Mr. Eduardo Vega (U.C.)

Radiation Effects and Copolymerization. Mr. Guillermo Romero (U.C.)

Adsorption of Cobalt-60 in Silicates. Mr. Claudio Carrillo (U.C.)

Radiation Effects on Platiedra gossypiela. Mr. Jaime Flores (U.C.)

Radiation Effects on Bipyridine. Mr. Edwin Acosta (E.P.N.)
Radiation Effects and Flotation of Sulfur. Mr. Trajano Ramírez (E.P.N.)

Radiation and Preservation of Naranjilla. Mr. Bolívar Izurieta (E.P.N.)

Preservation of Potatoes Through Radiation. Miss Rosario Jacho (U.C.)

Consultant services have been rendered to several students of the College of Education, Catholic University, in relation to their thesis.

Special Program, Department of Physics, Central University

Due to the interest of this Department, a special demonstration program in neutron diffusion theory was presented. It was attended by all the professors of physics from the University and also by three professors from the College of Science, University of Cuenca, a city located about 600 kilometers from Quito. The Director of the Department of Physics (U.C.), has written a letter of acknowledgement to Dr. Eddie Ortiz indicating the benefit received by Central University from this lecture and demonstration program.

Acknowledgement from the College of Pharmacy
to PRNC Personnel

The Dean of the College of Pharmacy, Dr. Gerardo Armendariz, and the Faculty of the same College of Central University, held a reception for PRNC personnel who participated in the "Atoms in Action" Exhibit in Quito. He gave Diplomas of Acknowledgment to Dr. H. Harry Szmont, Mr. José Cuevas, and Mr. Fausto J. Muñoz. The diplomas clearly show the impact made by the lectures and research projects initiated with "Atoms in Action" in Quito.

Other Activities and Services

Irradiation Services - Irradiation services were offered to high school students for projects which were included in the First National Service Fair of Ecuador. Several seed irradiations were also performed for people in the flower business interested in observing the possible changes in color of ornamental flowers, as well as for agricultural people interested in observing the radiation effects on barley and wheat seeds. Irradia-
Above: President Arosemena and other Ecuadorian government officials hear explanation of the uses of the gamma radiation facility in agricultural-economy research programs initiated at the time of the "Atoms in Action" exhibit in Ecuador. Below: President Arosemena and Education Minister Dr. Fabian Jaramillo with the "Atoms in Action" staff in Quito, Ecuador.
tions were performed, too, for several small fruit and vegetable packing companies interested in observing the radiation effects on the preservation of their products.

- **Attendance of Special Groups from the Universities of Ecuador** - Due to direct action taken by the PRNC representative, professors and students from the College of Engineering, University of Manabí, came to Quito and attended a special seminar on Flowshare and other lectures related to the nuclear engineering field. Four of the young assistant professors have already written to the University of Puerto Rico requesting application blanks for graduate studies. This program took place during November 27 - November 29, 1967.

Professors and students from the College of Pharmacy, University of Guayaquil, also visited the Exhibit during December 4-5, 1967, and a lecture-demonstration in radiation chemistry and nuclear instrumentation was offered to them. Two students from this College attended the Exhibit courses offered by personnel of the Oak Ridge Associated Universities (ORAU).

By request of the religious order of the Christian Brothers (HH, CC) a seminar was arranged on November 26, 1967. For twelve brothers of the order, and for professors of physics and mathematics from the La Salle, Carlos María de la Torre and Noviciado Schools in Quito, and San José, in Guayaquil.

PRNC personnel participated in the special "VIP" seminar prepared for The National Assembly, the Junior Chamber of Commerce, the Rotary and Lions Clubs.

- **Consultant Services** - PRNC personnel rendered the following consultant services:

  a) **National Institutes of Health, (NIH), Guayaquil.** Dr. Jorge Chiriboga stopped in Guayaquil on his way to Perú, and discussed with members of the NIH of Ecuador the implementation of nuclear techniques in parasitology studies. NIH sent four people to attend the Exhibit courses and is initiating radiobiology studies on several parasites using an X-ray source from the Cancer League (SOLCA) in Guayaquil.

  b) **Sugar Exporters Association and IFEIA, Guayaquil.** Dr. David W. Walker discussed the uses of radiation on the control of insects, and advised these organizations on the steps to
follow in order to correctly apply the radiation parameters.

c) **College of Science, University of Cuenca, and Central University, Quito.** Dr. Eddie Ortiz gave advice on the improvement of a curricula in Physics to professors of these universities who attended his seminar on neutron diffusion theory.

d) **Central University of Ecuador.** At the request of the Vice-Chancellor, Mr. Fausto Muñoz R., scientific coordinator of the FRNC Program at the International Exhibits, presented a memorandum indicating the need to establish a Nuclear Science Institute at this University.

We think the success of the FRNC Program at the International Exhibits has opened a very wide field of possible applications of atomic energy in problems related to the agricultural economy, public health and scientific development of the universities in these countries. Several publications from work initiated during 1965 and 1966 have appeared during 1967.

One, by Dr. Pedro Solé of Guatemala, titled "Gamma Radiation Sterilization of Canned Pineapple," has been submitted to Intern J. App. Radioisotopes. Another, by Dr. Ricardo Bressani (INCAP) and Mrs. Aurea de Monzón of Guatemala, titled "Radiation Effects in Incaparina," was also submitted to the same journal.

The other three papers, prepared in collaboration with FRNC personnel, will appear in the Appendix of the annual report after publication.

Four other papers are ready and will be presented at the 10th Latin American Congress in Chemistry in February, 1969. Another paper has been accepted for publication by the magazine of the Entomological Society of America, Washington, D.C.

Several young university professors from the countries which the Exhibit visited have been or are presently enrolled in graduate studies at UPR and are doing their research work at FRNC, (Table 1). Some students received their professional degrees in 1967 through thesis work initiated with the Exhibits in their countries. This was made possible by the continuous association of FRNC scientists who had participated in the Exhibits and the local personnel who participated in the projects (Table 2).
TABLE 1
List of Persons Who Have Continued Graduate Studies Through Assistance Given by the PRNC Exhibits Program

<table>
<thead>
<tr>
<th>Names</th>
<th>Original Institution</th>
<th>Studies</th>
<th>Origin of Fellowship</th>
<th>Time (Mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dr. Alicia Pineda</td>
<td>Instituto Salvadoreño de Investigaciones del Café</td>
<td>M.S. Agriculture</td>
<td>AID</td>
<td>(1)12</td>
</tr>
<tr>
<td>2. Dr. Armando Bustamante</td>
<td>Ministry of Public Health El Salvador</td>
<td>Post-graduate studies in Radiology (UPR)</td>
<td>AID</td>
<td>(1)18</td>
</tr>
<tr>
<td>3. Prof. Mario Saca</td>
<td>Dept. of Physics, Univ. of El Salvador</td>
<td>M.S. Physics</td>
<td>UPR</td>
<td>24</td>
</tr>
<tr>
<td>4. Mr. Roger Landaverde</td>
<td>Ministry of Agriculture El Salvador</td>
<td>M.S. Agriculture</td>
<td>AID</td>
<td>(1)*6</td>
</tr>
<tr>
<td>5. Mr. Virgilio Cruz</td>
<td>Ministry of Agriculture El Salvador</td>
<td>M.S. Agriculture</td>
<td>AID</td>
<td>(1)*6</td>
</tr>
<tr>
<td>6. Prof. Alfonso Mata</td>
<td>Dept. of Chemistry Univ. of Costa Rica</td>
<td>Ph.D. Chemistry</td>
<td>UPR</td>
<td>24</td>
</tr>
<tr>
<td>7. Prof. Delia Ortega</td>
<td>Dept. of Chemistry Univ. of Nicaragua</td>
<td>M.S. Chemistry</td>
<td>UPR</td>
<td>24</td>
</tr>
<tr>
<td>8. Prof. Luisa Benedet</td>
<td>Dept. of Chemistry</td>
<td>M.S. Biology</td>
<td>&quot;</td>
<td>24</td>
</tr>
<tr>
<td>9. Prof. Irma Zea</td>
<td>Dept. of Chemistry Univ. of San Carlos, Guatemala</td>
<td>M.S. Chemistry</td>
<td>IAEA</td>
<td>12</td>
</tr>
</tbody>
</table>

* Six month extensions were obtained in order to permit them to complete their graduate work.

(1) Returned already to their country.
### TABLE 2

List of Students Who Have Received Their Professional Degrees Through Thesis Work Initiated with the Atoms in Action Exhibit Program During 1965-1967

<table>
<thead>
<tr>
<th>Names</th>
<th>Thesis Title</th>
<th>Graduation Date</th>
<th>Degree Received</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr. Jaime Cáceres</td>
<td>Effects of the Formic Acid in the Fricke Dosimetry System</td>
<td>Nov. 1965</td>
<td>Chemist</td>
<td>El Salvador</td>
</tr>
<tr>
<td>Mr. Rafael Granados</td>
<td>Radiation Effects on Beans (<em>Phaseolus vulgaris</em> L)</td>
<td>July 1966</td>
<td>Agronomic Engineer</td>
<td>El Salvador</td>
</tr>
<tr>
<td>Mr. Max Sheel</td>
<td>The Fermi Acid-Fricke System I. <em>H₂SO₄</em> Concentration</td>
<td>Dec. 1965</td>
<td>Chemist</td>
<td>Guatemala</td>
</tr>
<tr>
<td>Mrs. Pauline de Rodríguez</td>
<td>Radiation Chemistry of Titanium Sulfate Solutions</td>
<td>June 1967</td>
<td>Chem. Engineer</td>
<td>Guatemala</td>
</tr>
<tr>
<td>Mr. Mario López</td>
<td>Kinetic Interchange Between Cyclo Alkine Chloride &amp; Radioactive Chlorine Ion</td>
<td>Mar. 1967</td>
<td>Chem. Engineer</td>
<td>Guatemala</td>
</tr>
<tr>
<td>Mr. Roberto Gutierrez</td>
<td>Radiation Effects on Canned Pineapple (See Table 1)</td>
<td>June 1967</td>
<td>Chem. Engineer</td>
<td>Guatemala</td>
</tr>
<tr>
<td>Mrs. Aurea de Monzón</td>
<td>Radiation Effects in <em>Inca-parina</em> (See Table 1)</td>
<td>Apr. 1967</td>
<td>Chem. Engineer</td>
<td>Guatemala</td>
</tr>
<tr>
<td>Miss Juanita Freer</td>
<td>Sulfur-35 Exchange Reactions in Triphenylphosphine Sulfide (See Table 1)</td>
<td>Nov. 1967</td>
<td>Chemist</td>
<td>Costa Rica</td>
</tr>
<tr>
<td>Mr. Carlos Rodríguez</td>
<td>Preservation of Bananas Cavendish variety through gamma radiation</td>
<td>Nov. 1967</td>
<td>Chemist</td>
<td>Costa Rica</td>
</tr>
<tr>
<td>Mr. Luis Guillén</td>
<td>Nuclear Energy &amp; Law</td>
<td>Dec. 1966</td>
<td>Lawyer</td>
<td>Costa Rica</td>
</tr>
</tbody>
</table>
The FRNC International Exhibits Program was visited in Ecuador by Dr. Henry J. Gomberg, FRNC Director, and Dr. Amador Cobas, FRNC Deputy Director. We express our gratitude for their comments, suggestions, and support of the Program. Mr. Juan Silva Parra also attended the exhibit in Ecuador. He is taking over the responsibilities of the field operation of the program. The special support in logistics from the USAEC Division of Technical Information is acknowledged. A résumé of the Exhibits in Panamá and Ecuador is presented in Table 3.

**TABLE 3**

Résumé of the FRNC Program at
The Atoms in Action Exhibit in Panama and Ecuador

<table>
<thead>
<tr>
<th></th>
<th>Panamá</th>
<th>Ecuador</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRNC Participating Scientists</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total Number of Research Projects</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Research Projects at Government Institutions</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Research Projects at Private Institutions</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Student Participation</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>Lectures Offered by FRNC Scientists</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Attendance at Lectures</td>
<td>705</td>
<td>1731</td>
</tr>
<tr>
<td>Expected Publications During 1968</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>
BIOLOGICAL AND MEDICAL RESEARCH
PROGRAMS

The research activities in biology and medicine (also called the 06 Program) are sponsored by the US AEC Division of Biology and Medicine, and comprise approximately one-third of PRNC's total program.
Right: Preparation of an orange-peel-grab for use in collecting a sample of marine bottom sediment off the west coast of Puerto Rico.

Left: A technician separates infaunal polychaetes from a sample of marine bottom sediment for trace element analysis on individual organisms.

Right: The Caloric content of biological samples is measured by bomb calorimetry. Trace elements are reported on the basis of ash, wet and dry weight, per gram of carbon and nitrogen and 10,000 calories.
MARINE BIOLOGY

The aims of the research in the Marine Biology Program have not changed during the past year although the methods of approach and points of emphasis have, in a few instances, been altered.

The work includes field and laboratory investigations with the major emphasis directed toward measurements under natural conditions.

The field area on the west coast of Puerto Rico includes a 20-mile stretch of coast which encompasses the watersheds and offshore marine areas of three rivers. The northernmost river, the Culebrinas, drains a watershed with large amounts of limestone. The central Añasco River has its origin in volcanic terrain with hydrothermal deposits of copper. The Guanajibo River, to the south, drains an area with extensive outcroppings of serpentinite. Each river carries a unique assemblage of trace elements into the marine waters; the influences of these additives upon the productivity of the marine areas, and the trace element composition of the marine organisms are being studied.

Investigations of trace element distributions in the sea is of interest in the study of oceanographic chemistry and in allied areas including those concerned with biological productivity, the influence of organisms upon the chemical and physical forms of elements in sea water, the geochemical histories of elements introduced into marine waters by natural processes, and the distribution of man-made radionuclides deposited in the sea.

A knowledge of the biogeochemistry of trace elements is of critical importance to man insofar as radioactive isotopes of the same elements may become incorporated into food webs from which human food is derived. The distribution patterns of many of the trace elements in marine waters, organisms, and sediments have not been measured. Little is known of the relative influence of physical, chemical, and biological mechanisms which control the transport and distribution of these elements. Thus, little is known of the relationships between rates of photosynthesis by marine plankton and the rates of incorporation of trace elements into marine food webs. Even less is known of the relative rates of loss of trace elements with the death and decomposition of marine organisms.
Investigations designed to measure and define the relative and individual influences of the physical, chemical, and biological mechanisms on the distribution of radionuclides in a given marine environment may be better planned if they are based upon a prior knowledge of the natural distribution patterns of stable trace elements in the same environment. In the Marine Biology Program, analyses are being made for cadmium, calcium, carbon, cobalt, copper, hydrogen, iron, lead, manganese, molybdenum, nitrogen, phosphorus, rubidium, scandium, silver, strontium, sulfur, and zinc in a limited number of minerals, rocks, soils, river waters and river sediments from the landmass which contributes trace elements to the neighboring marine waters, the pelagic, littoral and benthic organisms, and sediments. For all biological samples, micrograms of trace elements are reported per gram of wet, dry and ash weight, per gram of carbon and nitrogen, per 10,000 calories, and per microgram of phosphorus.

In addition to the distribution studies of stable elements, other areas of investigation being carried out off the west coast of Puerto Rico include basic studies in marine ecology and taxonomy; measurements of biological productivity; background observations in physical, chemical, and biological oceanography; analyses for rare earth distribution patterns in the watersheds and the neighboring marine waters, organisms, and sediments; and laboratory measurements of the rates of accumulation and loss of selected organisms for given radionuclides.

**DEVELOPMENT OF ANALYSIS METHODS**

A significant part of the work is concerned with the development of methods for stable element analysis in a variety of sample types. Among the methods used in the Marine Biology Program are destructive and non-destructive neutron activation analysis, x-ray emission spectrophotography, atomic absorption and flame spectrophotometry, colorimetry, fluorescence analysis, arc spectrography, polarography, gas chromatography, and automated chemical analysis using, primarily, colorimetry. During the past year a non-destructive neutron-activation method has been developed for the analysis of aluminum in sediments, rocks, minerals, and modern biogenic carbonates. A method is under development for the analysis of the same element in river and sea water. A rapid separation for neutron-activated scandium in rocks, minerals, and biogenic carbonates by liquid-liquid extraction has been developed to replace a slower precipitation method. An ion
Technicians measure gamma radiation dose from a rabbit with samples irradiated in PRNC's research reactor. Several elements, including manganese, aluminum, copper, silver, iron, scandium, mercury, cobalt, zinc, iodine, strontium, lanthanum, tungsten and chromium, are analyzed by this method.

Dr. R. Gordon Pirie removing a sediment sample from the orange-peel-grab.
exchange method has been tested and used for the determination of Cu, Fe, Zn and Sc, and rare earths by neutron activation in samples of marine organisms. A method for measuring zinc and manganese in sea water samples by coprecipitation and liquid-liquid extraction followed by atomic absorption spectrophotometry has been developed. In sea water samples containing large amounts of silica from phytoplankton, the samples must further be purified by an ion-exchange step using a chelating resin.

During the past year a Technicon Autoanalyzer has been put into operation for the analysis of phosphate, nitrate and silicate in samples of river and sea water and marine organisms. The equipment performs 20 analyses per hour. Methods are being developed to analyze for Ca, Sr, Fe and Mo.

**INVESTIGATIONS**

Studies have been started on the influence of age and size upon carbon-nitrogen ratios and trace element content in zooplankton. Analyses of 150 individual specimens of the marine copepod, *Pontella mimoberani*, have been completed. In conjunction with these analyses, a study is in progress of the total CHN content in specimens which have been subjected to lipid extraction. The fatty acid distribution patterns in these lipids are being analyzed by gas chromatography of the methylesters.

Field investigations, related to uptake studies of Sc, Cu, Fe, Sm and Zn by populations of the intertidal clam, *Donax denticulatus*, and the nine-armed starfish, *Luidia senegelensis*, were completed. Natural populations of the starfish were collected from areas under the direct influence of two river outflows. An analysis of their stable element content demonstrated that, of the elements analyzed, the levels of Fe and Zn were different between the two areas. These elements varied in relation to feeding activity in previous studies.

Levels of the stable elements Zn, Sc, Fe, Sm, and Cu have been compared between inshore-offshore populations of benthic fauna in an area adjacent to the Añasco River. Levels of Cu and Sm in the fauna are enhanced by proximity of the landmass, while levels of Zn, Sc, and Fe are not. In both areas the sediment-water interface, and the feeding activities of the fauna that live above and below it, proved to be important in the partitioning of Zn, Fe, and Cu in the organisms. Change in the taxonomic
composition of faunal groups does not appear to influence the
stable element composition of the group, but feeding types seem
to have a major role in stable element distribution.

Two studies of diurnal variation in zooplankton abundance,
species composition, and elemental content were completed. In
the first study, marked changes in the species composition of
the surface-water plankton were accompanied by fluctuations in
concentration in several of the 15 elements analyzed. In the
second investigation changes were again observed at the surface
and also at 100 meters depth.

A survey was begun of the concentration of plant nutrients
important in primary production off the mouths of the three
rivers on the west coast. In conjunction with this study, sali-
nity measurements are being made in order to better understand
the magnitude and direction of flow of the rivers.

Studies were made on the frequency distributions of Ca, Ca,
Co, Cu, Fe, Pb, Mn, Ni, Sc, Sr and Zn in the shells of Donax denti-
culatus and the queen conch, Strombus gigas, and in the soft tissues
of thread herring, (Ophisthomonema oglinum), and mullet, (Mugil curema).
Although the frequency distributions for the trace elements approxi-
mate a log-normal shape, statistically significant differences do
not occur in the concentrations of the elements within a population
from one location.

Preliminary investigations on uptake of a wide variety of
elements by the invertebrate, Artemia, show that the elements
in sea water are concentrated in patterns by chemical groups.
In general, the "B groups" of elements are concentrated to a
higher degree than the "A groups."

Studies on the taxonomy and distribution patterns of fora-
miniferans in the marine sediments off the west coast of Puerto
Rico have been continued. Over 160 species and subspecies
have been identified to date. Seven living foraminiferal as-
semblages have been found including near shore, reef, inner
shelf, middle shelf, outer shelf, muddy sediments and deep
water.

The topography and bottom sediments of an offshore-onshore
transect in Añasco Bay have been investigated. Samples were
analyzed for percent sand and gravel and for mean diameter and
standard deviation. Carbonate was determined by gasometric ana-
lysis and the amounts of organic carbon and nitrogen were meas-
ured. The parameters vary in a regular pattern which may be
related to sedimentary processes and sediment types along the transect. Four sediment facies have been identified.

VISITING INVESTIGATORS

In addition to work conducted by the staff, a group of visiting investigators worked in the Marine Biology Program during the summer of 1967.

Dr. Tudor Davies and Mr. Ian Evans of the University of South Carolina did the field work for an investigation on the microarchitecture of living molluscan shells, their contained trace elements, and the changes which occur in the structure and compositions of the shells after death of the organism.

Dr. R. Gordon Pirie, Mr. Robert Kattman, and Mr. John Comer of the University of Wisconsin investigated the sedimentology and petrology of the carbonate sands on the Cabo Rojo platform, a submerged reef off the west coast of Puerto Rico; the clay mineralogy of the Añasco River sediments and the marine sediments of Añasco Bay; and the distribution of trace elements in the waters and sediments of Joyuda Lagoon.

Dr. James Zischke, an Oak Ridge fellow, from St. Olaf College in Minnesota, completed a study of calcium deposition in the shells of marine molluscs.

PANAMA-COLOMBIA STUDY

As an extension of the work in Puerto Rico, field collections and measurements were made in Panama and Colombia as part of a feasibility study for the possible use of nuclear explosives for digging an isthmian sea-level canal. The marine biology group at PRNC conducted the estuarine and marine phase of the project. The research ships were in the isthmian region for seven months, returning to Puerto Rico September 6, 1967. Samples of water, sediments, phytoplankton, zooplankton, fish, crustacean, and other benthic invertebrates, including molluscs, were taken for analysis of H, C, N, P, Mn, Fe, Ca, Sr and Sc. In addition, a limited number of analyses for I, rare earths, and U are being made.
The distribution patterns of the trace elements in the sediments and waters of the Gulf of Panama and the Pacific Coast conform to the patterns expected as a result of runoff from the land and upwelling of deep waters of the sea. The results from the survey will be utilized to evaluate, by a specific activity approach, the possible hazards provided by the incorporation of radionuclides into food webs leading to humans.

Foster anchor dredge with a sample of sediment collected for infaunal analysis.
Injecting radioisotopes into tree for tracer.
TERRESTRIAL ECOLOGY PROGRAM I
THE RAIN FOREST PROJECT

The Rain Forest Project is a series of studies on one small area of the montane rain forest 1500 feet up the side of El Yunque mountain in Eastern Puerto Rico. It has three objectives: 1) to study the effects of gamma radiation on the tropical ecosystem; 2) to study mineral cycling and dispersion in the system; 3) and to study the basic biological functions of this ecosystem, such as respiration, transpiration, and photosynthesis, to better understand phenomena related to the first two objectives.

The project is in its fifth year. A section of the forest has been irradiated and many follow-up studies have been completed. (For details of the radiation experiment see PRNC-82, Annual Report 1965). Present effort is being directed to long term studies on recovery and succession of vegetation in the irradiated area, and to detailed investigations of mineral cycling and distribution in the tropical ecosystem. Studies are carried out at both the PRNC Río Piedras Laboratories and at the El Verde field laboratory, on the Northwest slope of El Yunque.

RECOVERY AND SUCCESSION STUDIES

Vegetation Indices. The objective of the recovery and succession study is to determine whether gamma radiation produces long-term specific effects on the tropical ecosystem after the irradiation has terminated. The study is carried out by comparing various indices of vegetation quality and quantity in the irradiated center with similar indices from areas in the rain forest which have been damaged by mechanical clearing or by application of herbicides. Measurements in the irradiated area include a detailed annual survey of plant species and size in addition to the various comparative indices. Details on the radiation center measurements are given in a previous annual report (PRNC-102 p. 141-142).

Indices being used to compare the irradiated area with other damaged areas include species diversity, importance values, similarity index, and optical measurement of leaf area index. The irradiated center has an area of well drained soils and an area
of poor drainage. Since this may influence recovery, the above indices were computed separately for both areas.

Species diversity for each area was determined by counting individuals and species. Diversity lines were plotted using standard regression analysis techniques, and differences between lines were calculated by analysis of covariance. Species diversity for seedlings in the irradiated area in March 1964, was measured by McCormick, a former consultant to the project. Species diversity did not differ significantly between the two herbicide areas, nor between the well drained and the poorly drained portions of the irradiated area. Differences between all other areas existed at greater than the 99 percent level of confidence (Fig. 1).

Diversity after the irradiation was greater than before in the irradiated center. Differences in diversity between one area and another are probably related to the size of the canopy opening. The herbicide damaged areas had the greatest canopy opening while the radiation center was intermediate and the cut center the least after regenerative canopy sprouting. Thus the greater penetration of light may have produced a greater diversity in the herbicide areas. There is no reason at this time to believe that the diversity differences are primary treatment effects.

To compare quality of vegetation in the experimental areas, the importance values of all species were calculated, and the most important values are presented (Table 1). Psychotria herbertiana and Palicourea riparia are woody species which become established in open areas near El Verde. They are sub-canopy trees, and can survive, at least for a while, beneath the shade of canopy trees. Tabebuia heterophylla, Didymopanax morototoni, and Cecropia peltata are canopy trees, and also commonly become established in open areas. Heliconia bihai is a semi-herbaceous plant similar to the banana tree. Phytolacca icosandra is an herb having a niche similar to Phytolacca americana of the Northeastern United States which is that of an early colonizer of forest openings. Phytolacca icosandra had a low importance value in the irradiated area at the time of sampling. Most of the plants were decaying, and there were no new seedlings. However, P. icosandra was the most conspicuous plant in the irradiated area several months after radiation ceased. Although there is a considerable amount of natural variation, the data generally show that the same species are invading all four areas of study.
1. Herbicide Area No. 2, 1/67
2. Herbicide Area No. 1, 1/67
3. Total Irradiated Area, 3/64
4. Irradiated Area, Poorly Drained Soil, 1/67
5. Irradiated Area, Well Drained Soil, 1/67
6. Cut Area, 1/67

Figure 1

Light at two wavelengths, and the ratio between the two, above the canopy, on Nov. 16, 1967.

Figure 2
To quantify differences between plots, percentage similarities between all pairs of plots were calculated (Table 2). The well drained area of the irradiated plot was approximately as similar to all other plots as the two herbicide plots were to each other. This indicates that differences between the irradiated plots and other plots is not a function of treatment. Radiation recovery studies are continuing although at this time it appears that recovery and succession is not primarily related to the original treatment. Within the natural variation of small plots the recoveries of vegetation are similar for all damaged areas.

Optical Measurements. For many ecological studies, the leaf area index (square meters of leaf surface per square meter of ground) must be known. Optical density has been used as a measure of canopy thickness. However, there are several problems with optical density as a measure of leaf area index. One is that the optical density method, which compares photocell readings above and below the canopy, assumes that all light is either transmitted or absorbed. Actually, much of the light is scattered as it moves downward through the canopy. Another problem is that photocells must be read simultaneously above and below the canopy.

Leaf area index can be calculated by measuring the infrared-red light intensity ratio (800 μm/675 μm) on the forest floor, thus eliminating the difficulties of traditional optical density measurements. The theoretical basis of this measurement is as follows. Light at a wavelength of 675 μm is absorbed in proportion to the amount of chlorophyll in the canopy, and thus its attenuation is a better measure of leaf area index than light at all wavelengths. However, the amount of 675 μm light is independent of time of day, at least between 9:00 a.m. and 3:30 p.m. (Fig. 2), and also time of year. Since there is very little attenuation of infrared light as it passes through the canopy, the infrared-red ratio appears to be a good measure of leaf area index, at least on sunny days. Calibration of leaf area index with the 800 μm/675 μm ratio was repeatable within limits of instrument error and sampling variability (Fig. 3), using direct measurement of leaf area index from a tower.

Insect Studies. Dr. Elizabeth McMahan surveyed termite survival during the summer of 1967. It was found that 7 out of 13 termite nests in the 80 meter circle around the radiation center were abandoned since the end of irradiation. Four of these nests were in the region between 30-80 meters from the position of the
Figure 3

Dr. J. Kline adjusts a plastic bag used for measurement of metabolism in leaves.
gamma source. Three of these were alive during the summer of 1966 but were abandoned sometime during the following year. The lowest calculated radiation dose to an abandoned nest was 100 R near the 80 meter circle. By contrast only one abandoned nest out of 11 studied was found in the 80 meter circle of the control center. The long delayed abandonment of termite nests (radiation ceased in April 1965) may be due to sterilization of reproductives in the nest without actually killing the existing insects.

MINERAL CYCLING STUDIES

The objectives of the mineral cycling projects are to measure existing distribution of macro and micro elements in the rain forest, to study pathways of movement of these elements, and to measure rates of movement along these pathways. These studies are expected to provide in the short run an empirical basis for the prediction of the fate of radionuclides which may be released in tropical communities, and may lead in the long run to a theoretical understanding of material handling mechanisms in tropical ecosystems. The mineral cycling studies are divided for convenience into four categories of activity. These are: 1) Fallout Measurements; 2) Radioactive Tracer Experiments; 3) Stable Element Analyses; 4) Water Budgets.

FALLOUT MEASUREMENTS

$^{137}$Cs Residence Time. Measurement of radionuclide residence times in the El Verde forest is a continuing effort in which the half time estimates in various compartments are revised approximately at six month intervals. The estimates are based on a group of samples collected monthly at the field site from canopy, understory, and litter. The samples are oven-dried after collection and counted in a Marinelli breaker by the method of gamma scintillation spectrometry, utilizing a shielded NaI (TI) crystal connected to a 400 channel pulse height analyzer. The complex spectra are resolved into their individual components by computer solution of simultaneous equations. The data for each nuclide are then plotted as a function of time on a semilogarithmic scale and regression lines are fitted by the method of least squares.

Residence times for $^{137}$Cs in canopy, fresh leaf litter, and understory leaves are given in Figure 4. Understory leaves have
Effective residence times for $^{137}$Cs in freshly fallen litter, canopy leaves, and understory leaves in the rainforest at El Verde.

Figure 4

Figure 5
Retention of $^{134}$Cs Against Extractions with Ammonium Acetate Solutions in Soil of El Verde.

Figure 6

El Verde field laboratory and living quarters.
the greatest burdens, followed by canopy leaves and fresh leaf litter. The low values in leaf litter indicate a possible loss of $^{137}$Cs by leaching since these leaves are collected only once each month. The computed residence times for each forest compartment are given in Table 3 along with deposition data. Understory leaves have the longest residence half time while canopy leaves have the shortest. The values of environmental half life for $^{137}$Cs are shorter than those for $^{90}$Sr and imply a less effective retention mechanism for $^{137}$Cs. Similar measurements have been made for $^{144}$Cs, $^{95}$Sr, $^{54}$Mn, and $^{90}$Sr.

**TRACER EXPERIMENTS**

**Behavior of $^{134}$Cs in Soil.** A tracer experiment described in a previous report was terminated with the conclusion that very little cycling of this radionuclide from soil occurred in understory vegetation. (PRNC-102, p. 146). After the conclusion of the vegetative phases of the experiment, soil and litter experiments continued.

$^{134}$Cs was leached by rain from the organic surface litter of the plots with an environmental half life of approximately 15 days. Upon reaching the soil, however, nuclide movement became extremely slow. Figure 5 shows the depth of penetration of the nuclides into the soil after 18 months of leaching. The relationship is exponential and shows that activity is reduced by about half in the soil for every inch of depth. This behavior is unexpected for highly weathered soils in a high rainfall area. A partial explanation for this behavior is given by the results of laboratory extractions of soil (Figure 6).
The results show that $^{134}$Cs reacts with some component of the soil more or less irreversibly and cannot be removed by successive extractions with a neutral salt. When extractions of the soils with HCl were done, similar amounts of the nuclide remained in the soil.

The results of the field and laboratory analyses of soils suggest that $^{134}$Cs became irreversibly bound to, or fixed by, clays of the soil. Fixation of alkali metals is well known in soil chemistry when expanding lattice clay minerals are present. X-ray diffraction analyses of rain forest soils are planned to determine whether such minerals are in fact present.

Reactions of Nuclides With Epiphyllae. Epiphyllae are mixtures of organisms which grow on the surfaces of leaves in the rain forest. Their populations may include algae, fungi, lichens, liverworts, mosses or bacteria. These organisms have been suspected of playing a role in the nutrient cycling mechanisms of the forest because they were found to contain large amounts of fallout radionuclides. Experiments were carried out in cooperation with Dr. Raymond Henzlik, an Oak Ridge Research Participant from Ball State University, to examine some reactions of these organisms with radionuclides.

Leaves from four species of trees were contaminated with radioactive solutions containing $^{134}$Cs and $^{85}$Sr for a period of 20 minutes, after which the leaves were washed in tap water for 6 minutes. All species had leaves both with and without epiphytic growth. The results are shown in Table 4. Epiphyllae were from 4 to 7 times more efficient in retaining radionuclides than were leaves which had no surface growth. This indicates that these organisms may be adapted to deriving their mineral nutrient requirements by interception of rain water or canopy leachate.

Minerals which are intercepted by epiphyllous leaves might be transferred to the leaves by foliar uptake. If this happens it could be an important source of nutrients for the higher plants of the area. An experiment was done to determine whether leaves received nutrients from labeled epiphyllae. Excised leaf sections containing labeled epiphyllae were placed firmly against the surfaces of leaves in the field and held there for 24 hours. Leaves in the field were matched for those having surface growth and for those which did not. After up to 10 days the leaves were harvested, sectioned and counted.
The results (Table 5) show that epiphyllae took up more radioactivity from the labeled overlays than did clean leaf surfaces. The activity in the tip portions of the leaves probably indicates surface translocation since the leaves are adapted to shedding water in this direction. The activity in the basal portion of the leaves may indicate some uptake and translocation by the leaf. It is apparent, however, that most of the activity remained in the region of the leaf which was originally contaminated. It is concluded that epiphytes of this type are not efficiently adapted to furnishing their higher plant hosts with minerals through foliar uptake. The strong binding adaptation exhibited by these organisms for mineral elements suggests that the opposite may be true. Minerals leached from leaves by rain water may be the sources of nutrients for the epiphyllae.

**Nitrogen Fixation.** The source of biologically available nitrogen in the tropical rain forest is a question of long standing interest. This is because there are no significant geochemical sources of nitrogen and also because the environmental conditions favor rapid nitrification of biologically released nitrogen which may be lost from the system due to leaching. One possible source of nitrogen in this system is fixation of atmospheric nitrogen by epiphyllae which grow in abundance on practically all leaf surfaces in the forest. An experiment to test for nitrogen fixation by these mixed communities was carried out in cooperation with Dr. Joe Edmisten (University of Georgia) using $^{15}$N$_2$.

Five leaves of a grapefruit (Citrus paradisi) tree were enclosed in plastic bags with an airtight seal. The normal atmosphere was withdrawn from the bags through hypodermic needles and the atmosphere was replaced with an artificial one consisting of 75% argon, 20% oxygen, and 5% nitrogen-15. The leaves were allowed to remain in this atmosphere for 48 hours and were then harvested. Epiphyllae were scraped from the leaves immediately, and pooled into one sample. Leaves and pooled epiphyllae were sent to a commercial laboratory for $^{15}$N ratio analysis.

Results are shown in Table 6. The epiphyllae had 9.67% of their total N in the form of $^{15}$N. The leaves from which they had been scraped were also enriched above the natural level of $^{15}$N which is 0.3%. It is apparent that N fixation took place and that some transference to the host leaf also occurred. This suggests a possible symbiotic relationship in which N may be furnished
directly to forest plants by these organisms. The epiphyllae may in turn receive inorganic nutrients from the leaves of the host trees. Whether or not the relationship is truly symbiotic, the epiphyllae certainly add to the pool of available N in the forest through leaf fall and cell turnover. It is apparent that a potentially important new source of biologically available N in the tropical rainforest has been found.

Radionuclide Distribution in Animals. This study was designed to evaluate the utilization by forest animals of lower plants growing on tree trunks. Three tree trunks were tagged by spraying each with 1 mCi of carrier free $^{65}$Zn solution. Absorption of the tracer nuclide was effective and similar to that previously discussed for leaves. The three trees were spaced in the form of a triangle. Collections of snails, lizards, frogs, spiders and insects are made weekly from within and outside of the triangle by various methods. Animals are counted live by gamma ray spectrometry when possible and released near the area of capture. All of the groups of animals mentioned have shown measurable levels of $^{65}$Zn.

Herbivorous insects have failed to show levels of $^{65}$Zn that would indicate direct feeding on epiphytes, even when they are taken from labeled trunks. High levels of tracer in vertebrate and invertebrate carnivores, however, suggest that a herbivorous link exists. Relative levels of tracer indicate that snails may be the principal herbivores utilizing this source of food.

Lizards, frogs, and snails have been captured, marked, counted and released through several repetitions. This procedure has allowed the acquisition of information on homing behavior and territoriality as well as feeding habits. This behavior is well developed in snails and lizards.

Figure 7 shows an example of homing and territorial behavior for 4 snails of the species Caracola caracola. Data for a total of 137 snails of this species are available and indicate that there are 2 categories of behavior following release at the point marked X. Some snails leave the study area in apparently random directions after remaining in one place, usually a tree, for several days. These are called transients. Others, designated as residents, move at once to other locations, which, when plotted over several releases, delineate a territory, or home range. There seem to be two distinct types of residents, one with a small home range
RECAPTURE POINTS

- Snail 028, wt. 32.0 gm
- Snail 095, wt. 24.4 gm
- Snail 085, wt. 30.4 gm
- Snail 086, wt. 30.2 gm

Scale 1 cm = 1 meter
averaging about 10 square meters in area, whose range seems independent of snail size; the other type has a larger range that increases roughly with snail size to about 200 square meters. Both types of range are illustrated in the figure and the snails are classified as narrow-ranging or wide-ranging individuals. The full significance of this difference is not yet understood. Some transient snails entered the study area voluntarily, while others were carried in experimentally. None of the latter established home ranges in the study area.

Overall recapture percentages for snails has been 79%, for 646 recaptures. The number of recaptures has ranged from one to 16 per individual, with no indication of a limit of tolerance for this treatment. There is no evidence that snails develop either evasive tactics or increased susceptibility to capture. The mean recapture interval is 13 days based on weekly collections with extremes up to 106 days.

Most of the snails have low levels of $^{65}$Zn, which is probably due to foraging in contaminated litter that now surrounds the base of the contaminated trees. It is obvious, however, when a snail actually grazes on one of the treated trees, because of a sudden large increase in body burden of $^{65}$Zn. Such jumps in radioactivity are termed a "high-level uptake" (HLU) and are useful for describing snail behavior. Table 7 gives a description of snail behavior for various categories of snails. It is apparent that the transient snails contact and feed on the contaminated trees with much greater frequency than the residents. Among the residents the wide-ranging individuals contact the treated trees with greater frequency than the narrow-ranging individuals in spite of the fact that their greater range should lower the probability of contacting a radioactive tree. The tree climbing behavior of transient snails is so dependable that it has been utilized intentionally to obtain a group of labeled snails for a bio-elimination study.

Sixty snails were made radioactive by the above method for a bio-elimination study. Thirty were released into their home ranges while the remainder were retained in cages. The biological half-life of $^{65}$Zn in snails released in their home range was estimated to be in the range of 18 to 24 days, with an indication that there was more rapid early release. Dissection of individuals revealed that within one week 80% of the radioactivity was confined to the liver while the remainder was in muscles and other soft parts and almost none in the shell.
STABLE ELEMENT ANALYSES

The project received a Perkin Elmer Model 303 Atomic absorption spectrophotometer during the past year and put it into service immediately upon arrival. The first analytical project involved the determination of Ca, Mg, Mn, Sr, and Co in canopy, understory, and litter leaves to determine whether stable elements in natural mineral cycles follow the same pathways as the fallout nuclides. While this project has not yet been completed or summarized, preliminary results indicate that the stable elements are enriched in understory vegetation, as was found with the fallout nuclides. The results also indicate that there are significant differences in mineral content among species and that these contents do not undergo seasonal variation.

A second project was initiated to measure Ca, Mg, Mn, and Na in water collected from various points in the forest. Sources of water include rainwater, canopy leachate, stem flow, and soil water. More than 150 samples of water have been analyzed for the above elements thus far, on monthly composites. Significant amounts of the above elements are found in all phases of the forest water although tentative conclusions would be premature at this time. The project will continue for at least one year, at which time a calculation of mineral budget based on water flow volumes for each compartment, stand densities, and leaf turnover will be computed.

A third project is being initiated at this writing to study mineral contents of secondary successional vegetation. The project will continue for at least one year and will attempt to characterize mineral cycling in successional communities.

WATER BUDGET

Tritium Experiments. Tritium in the form of tritiated water could be released to the tropical environment through thermo-nuclear detonations of either a peaceful or military nature. The behavior of such releases in the high rainfall tropical ecosystem is not known. An experiment was carried out to measure some aspects of this behavior in soil of the rain forest.

Tritium (HTO) was applied to a small soil plot in the rain forest. The plot was equipped with a lysimeter (see FRNC-102, p. 147) and a runoff collecting device, both of which drained
into plastic water collection vessels. Water samples were collected from the plots at first after every rainstorm and later at weekly intervals for a period of seven months. Samples were counted by standard methods of liquid scintillation counting.

Results of the experiment are shown in Figure 8. Tritium reached a peak of activity at the depth of the lysimeter (18 cm) in approximately 16 days. The environmental residence time of tritium in the soil profile was calculated by least square analysis to be 16.3 days after reaching the peak. Tritium loss in the soil litter was shown to be a 2-compartment system. The first phase of loss had an environmental residence time of 2.9 days; the second had a residence time of 35.6 days.

The results for the soil profile and surface litter indicate that there must be compartments which do not have rapid exchange by self diffusion of water. Restricted self diffusion could be the result of the tortuosity of clay pore spaces, absorbed water films on clay surfaces, or exchangeable hydrogen. Regardless of the reason for restricted self diffusion, it is apparent that tritiated water has a considerable persistence in soils and litter of the tropical rain forest. This persistence must be considered in predicting the effects of thermonuclear detonations in a tropical environment because it is evident that plant roots growing in contaminated soil will be exposed to this nuclide for considerable periods after even a single input pulse. Crops grown in forest clearings for human or animal consumption will be correspondingly contaminated.

Water Balance. Measurements of water flow through the forest environment continue. Compartments measured include total input, water reaching ground level, soil flow through, surface runoff, and tree trunk runoff. A preliminary water budget based on 8 months of measurement at one location is given in Table 8. The results show that 72.6% of the total rainfall reaches the ground at this location and that 36.9% of the total rainfall moves through the soil. Only 4.5% of the total water runs off the soil surface. The difference between input to the ground and bulk loss must be a measure of evapotranspiration. Computations from Table 8 indicate that this quantity is of the order of 2.65 mm/day. Odum (personal communication) has previously measured evaporation from a free water surface at the top of the canopy and has given an average value of 1.8 mm/day. A computer program is being written to process the data from more than 30 lysimeters and 10 rain
gages. It is expected that this will allow more precise evaluation of the water budget.

Stem flow is an important means by which chemical elements are transported from the canopy to the forest floor. It is also an important segment of the water budget of the forest.

Twenty-two stem flow collectors were put out in the summer of 1967. The total amount of stem flow per tree collected from August through October 1967 is shown in Figure 9. Quantity of stem flow is independent of species, for the five species measured. Amount of stem flow increases with increasing size of tree, up to the intermediate size trees, and then drops off sharply with the larger trees. This may be caused by the shape of the crowns. The large trees tend to have rounded crowns, whereas the smaller ones have V-shaped crowns.

SOIL METABOLISM

Metabolism of decomposer organisms and roots in the soil is difficult to measure, because the respiration rate is influenced by the rate at which the air being analyzed for change in CO₂ content is passed over the soil. However, by putting a relatively large box over the soil surface, and measuring CO₂ buildup inside the box, soil respiration can be calculated. An objection to this approach is that the buildup of CO₂ in the box inhibits further respiration. However, in a large box, inhibition begins only after about 20 minutes.

The results (Table 9) are based on the rate of CO₂ increase in the box during only the first 10 minutes of measurement. They show a respiration rate of 0.4 gm. CO₂/m²/hr. These results are lower than have been previously reported (PRNC-82, p.143), but may be more realistic since they agree more closely with known rates of leaf fall and decomposition.

STAFF

Dr. George Drewry completed requirements for the Ph.D. in Zoology at the University of Texas. He has initiated new projects on the cycling of radionuclides through some animal populations of the rainforest.
Mr. Douglas Krom joined the project as an instrument technician to operate the weather station. He also has skills in computer programming and has written a program to process water budget data.

Dr. Raymond Henzlik (Ball State University) spent June, July, and August working with project staff as an Oak Ridge Research Participant. His work consisted primarily of studies on reactions of radionuclides with epiphytic plants.

Dr. Elizabeth MacMahan (University of North Carolina) received support during July and August 1967 to continue studies on the effects of radiation on termite populations.

Dr. Joe Edmisten (University of Georgia) visited the project during September 1967, to participate in nitrogen fixation studies using $^{15}$N.

Dr. Joe Koranda and Mr. John Martin (Lawrence Radiation Laboratory) visited the project in September 1967, to discuss the possibility of doing a cooperative tritium experiment in the rain forest. It was decided to attempt to study the kinetics of tritium behavior in mature canopy trees early in 1968. Dr. Jerry Kline visited Dr. Koranda's Laboratory in November 1967, to study tritium analytical techniques. At that time Dr. Koranda agreed to loan the project some specialized equipment for tritium analyses to be installed in the forest laboratory at El Verde. The equipment has been received and installed.
Table 1. Importance values of secondary successional species in a tropical rain forest.

<table>
<thead>
<tr>
<th>Species</th>
<th>Irradiated Center</th>
<th></th>
<th>Cut Center</th>
<th>Herbicide 1</th>
<th>Herbicide 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well drained</td>
<td>Poorly drained</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychotria berteriana</td>
<td>118</td>
<td>16</td>
<td>106</td>
<td>112</td>
<td>40</td>
</tr>
<tr>
<td>Palicourea riparia</td>
<td>27</td>
<td>65</td>
<td>10</td>
<td>52</td>
<td>182</td>
</tr>
<tr>
<td>Tabebuia heterophylla</td>
<td>36</td>
<td>89</td>
<td>3</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Phytolacca icosandra</td>
<td>9</td>
<td>0</td>
<td>17</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Didymopanax morototoni</td>
<td>45</td>
<td>33</td>
<td>25</td>
<td>47</td>
<td>45</td>
</tr>
<tr>
<td>Cecropia peltata</td>
<td>44</td>
<td>7</td>
<td>12</td>
<td>92</td>
<td>33</td>
</tr>
<tr>
<td>Heliconia bihai</td>
<td>0</td>
<td>0</td>
<td>61</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Indicates the most important species in the area.

Table 2. Percentage similarities¹ between pairs of experimental plots.

<table>
<thead>
<tr>
<th>Pairs of Plots</th>
<th>Percentage similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well drained - Poorly drained</td>
<td>51.3</td>
</tr>
<tr>
<td>Well drained - Cut</td>
<td>45.7</td>
</tr>
<tr>
<td>Well drained - Herbicide plot no. 1</td>
<td>61.0</td>
</tr>
<tr>
<td>Well drained - Herbicide plot no. 2</td>
<td>43.9</td>
</tr>
<tr>
<td>Herbicide plot no. 1 - Herbicide plot no. 2</td>
<td>49.4</td>
</tr>
<tr>
<td>Poorly drained - Cut</td>
<td>30.3</td>
</tr>
<tr>
<td>Poorly drained - Herbicide plot no. 1</td>
<td>42.0</td>
</tr>
<tr>
<td>Poorly drained - Herbicide plot no. 2</td>
<td>39.2</td>
</tr>
<tr>
<td>Cut - Herbicide plot no. 1</td>
<td>42.5</td>
</tr>
<tr>
<td>Cut - Herbicide plot no. 2</td>
<td>31.3</td>
</tr>
</tbody>
</table>

¹ A percentage similarity of 100 means that the plots are identical; one of 0 means they are entirely different.
Table 3. Residence times of $^{137}$Cs of fallout origin in various rainforest compartments.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Environmental half life (days)</th>
<th>Leaf Biomass (gm/m$^2$ dry)</th>
<th>$^{137}$Cs Burdens (nCi/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy</td>
<td>588</td>
<td>859</td>
<td>5.8</td>
</tr>
<tr>
<td>Understory</td>
<td>937</td>
<td>ND*</td>
<td>-</td>
</tr>
<tr>
<td>Leaf fall</td>
<td>710</td>
<td>40.5**</td>
<td>0.2**</td>
</tr>
<tr>
<td>Leaf litter</td>
<td>-</td>
<td>124</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* ND = Not determined. Included in canopy estimate.

** Average per month.

Table 4. Adsorption of $^{134}$Cs and $^{85}$Sr on epiphyllous and non-epiphyllous leaves of the tropical rain forest.

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>$^{134}$Cs CPM/sect</th>
<th>$^{85}$Sr CPM/sect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphyllous</td>
<td>9134</td>
<td>5363</td>
</tr>
<tr>
<td>Non-epiphyllous</td>
<td>1405</td>
<td>1269</td>
</tr>
</tbody>
</table>

Table 5. Uptake of radionuclides by epiphyllous and non-epiphyllous leaves and translocation of the nuclides within the leaves.

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>Region</th>
<th>$^{134}$Cs CPM/sect</th>
<th>$^{85}$Sr CPM/sect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphyllous</td>
<td>tip</td>
<td>171</td>
<td>498</td>
</tr>
<tr>
<td></td>
<td>overlay</td>
<td>1062</td>
<td>2604</td>
</tr>
<tr>
<td></td>
<td>base</td>
<td>42</td>
<td>180</td>
</tr>
<tr>
<td>Non-epiphyllous</td>
<td>tip</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>overlay</td>
<td>117</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>base</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 6. Amounts of total nitrogen in leaves and epiphyllae and percent of total N as $^{15}$N after 48-hour exposure to $^{15}$N$_2$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. of Material Kjeldahled (g)</th>
<th>Total N$_2$ (g)</th>
<th>$%^{15}$N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled epiphyllae</td>
<td>0.2259</td>
<td>0.0114</td>
<td>9.67</td>
</tr>
<tr>
<td>Scrapped leaf #1</td>
<td>1.1703</td>
<td>0.0234</td>
<td>5.65</td>
</tr>
<tr>
<td>Scrapped leaf #2</td>
<td>1.7533</td>
<td>0.0085</td>
<td>0.99</td>
</tr>
<tr>
<td>Scrapped leaf #3</td>
<td>1.2940</td>
<td>0.0280</td>
<td>0.70</td>
</tr>
<tr>
<td>Scrapped leaf #4</td>
<td>1.1351</td>
<td>0.0220</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table 7. Utilization of $^{65}$Zn trees by various groups of snails.

<table>
<thead>
<tr>
<th></th>
<th>Number of snails</th>
<th>Number of releases</th>
<th>Number of HLU*</th>
<th>Releases HLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Involuntary</td>
<td>24</td>
<td>190</td>
<td>35</td>
<td>2.57</td>
</tr>
<tr>
<td>Voluntary</td>
<td>14</td>
<td>26</td>
<td>6</td>
<td>4.33</td>
</tr>
<tr>
<td>Residents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wide ranging</td>
<td>21</td>
<td>139</td>
<td>20</td>
<td>6.95</td>
</tr>
<tr>
<td>Narrow ranging</td>
<td>59</td>
<td>362</td>
<td>35</td>
<td>10.34</td>
</tr>
<tr>
<td>Inner Zone</td>
<td>13</td>
<td>88</td>
<td>11*</td>
<td>8.00</td>
</tr>
<tr>
<td>Transition</td>
<td>35</td>
<td>210</td>
<td>20</td>
<td>10.50</td>
</tr>
<tr>
<td>Outer Zone</td>
<td>11</td>
<td>64</td>
<td>4</td>
<td>16.00</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>617</td>
<td>96</td>
<td>6.43</td>
</tr>
</tbody>
</table>

*HLU = High level uptake, an indication that a snail has grazed on a labeled tree trunk.

Table 8. Water balance at one location in the rain forest at El Verde.

<table>
<thead>
<tr>
<th>Location</th>
<th>Water Flux cm/day</th>
<th>Percent of total input %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tower rain gage</td>
<td>0.847</td>
<td>-</td>
</tr>
<tr>
<td>Ground rain gage</td>
<td>0.616</td>
<td>72.6</td>
</tr>
<tr>
<td>Lysimeters</td>
<td>0.333</td>
<td>36.9</td>
</tr>
<tr>
<td>Runoff</td>
<td>0.038</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Table 9. Results of soil metabolism experiments.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of box</td>
<td>249.6 liters</td>
</tr>
<tr>
<td>Area of soil covered by box</td>
<td>625 sq. meters</td>
</tr>
<tr>
<td>Change in CO₂ concentration in 10 minutes (2 trials)</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Rate of CO₂ production</td>
<td>0.40 g/m²/hr.</td>
</tr>
<tr>
<td>Rate of carbon release</td>
<td>0.11 g/m²/hr.</td>
</tr>
<tr>
<td>Rate of oxidation of organic matter</td>
<td>0.24 g/m²/hr.</td>
</tr>
</tbody>
</table>

Dr. Drewry prepares the gamma ray spectrometer for the measurement of radioactive samples from a field experiment.
Miss Mirtha Trujillo, a student from Cuba, with Dr. Alec Grimison and Dr. George Simpson, using the equipment for the detection of thermoluminescence of gamma irradiated samples.
RADIATION CHEMISTRY PROJECT: MATRIX ISOLATION STUDIES OF PRODUCTS OF GAMMA-RADIOLYSIS OF HETERO CYCLIC MOLECULES

The project aims at the trapping and subsequent characterization of the species formed by $\gamma$-radiolysis of heterocyclic molecules of possible biological importance. Emphasis is therefore placed on direct observation of the usually labile intermediates formed after the absorption of high energy radiation. This is made possible by utilizing the matrix isolation technique, in which the molecule is irradiated in some form of rigid matrix, normally at low temperatures. Under appropriate conditions, radicals and molecular ions can be stabilized by this technique for extended periods of time, and studied by spectroscopic techniques. An important part of the program involves the quantum-mechanical calculation of electronic properties of heterocyclic radicals and ions. These results are then used in conjunction with the experimentally measured properties to help identify unknown intermediates.

A description of current research projects follows:

1. Absorption Spectra of Radiolytic Intermediates at $77°C$.
   The work carried out in previous years with heterocyclic solutes in methyltetrahydrofuran (MTHF), 3-methylpentane (3MP), and Freon glasses has been extended considerably. In particular, the characterization of the properties of the Freon mixture under gamma-radiolysis has been reported, demonstrating the importance of positive-charge migration in halogen glasses. This has led to the assignment of the absorption maxima for irradiated pyrrole, in Freon at 800 $\text{m}_{\mu}$ to the pyrrole radical cation, that of pyridine at 380 $\text{m}_{\mu}$ to the pyridine radical cation, and that of thiophene at 320 and 830 $\text{m}_{\mu}$ to the thiophene radical cation. This represents the first characterization of these species.

A list of the new absorption maxima obtained from gamma-irradiated heterocyclic in MTHF, 3MP, and Freon glasses during the past year is given in Table 1. The results obtained for pyridine, pyridazine, and pyrazine in MTHF are particularly interesting. The radical anions of pyridine, pyridazine, pyrazine, and pyrimidine have previously been prepared by Dr. Hush, at the University of Bristol, England, by chemical means. He reported absorption maxima of pyridine anion at 335 $\text{m}_{\mu}$, pyridazine anion at 352 $\text{m}_{\mu}$, and pyrazine anion at 364 $\text{m}_{\mu}$, all measured in liquid THF. These results are obviously in close accord with the species formed by gamma-irradiation in Table 1, especially for pyridazine. This represents the first demonstration of the importance of ionic intermediates in the radiolysis of heterocyclic molecules. The
**TABLE 1**

NEW ABSORPTION MAXIMA FROM GAMMA-IRRADIATED HETEROCYCLIC MOLECULES IN RIGID GLASS AT 77°K

<table>
<thead>
<tr>
<th>Substance</th>
<th>Glass</th>
<th>( \lambda ) max (m( \mu ))</th>
<th>Effect of bleaching solvent band</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>Silica cell</td>
<td>256</td>
<td>increase (uv bleach)</td>
</tr>
<tr>
<td>Indole</td>
<td>Freon</td>
<td>1000, 620, 600, 565, 550</td>
<td>--</td>
</tr>
<tr>
<td>Imidazole</td>
<td>Freon</td>
<td>580</td>
<td>decrease</td>
</tr>
<tr>
<td>Pyrazine</td>
<td>MTHF</td>
<td>340, 500</td>
<td>--</td>
</tr>
<tr>
<td>Pyrazole</td>
<td>MTHF</td>
<td>321</td>
<td>--</td>
</tr>
<tr>
<td>Pyridazine</td>
<td>MTHF</td>
<td>354</td>
<td>increase</td>
</tr>
<tr>
<td>Pyridine</td>
<td>MTHF</td>
<td>350</td>
<td>increase</td>
</tr>
<tr>
<td>Pyridine</td>
<td>3MP</td>
<td>360, 1200</td>
<td>--</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>MTHF</td>
<td>400</td>
<td>decrease</td>
</tr>
<tr>
<td>Thiazole</td>
<td>MTHF</td>
<td>330</td>
<td>increase</td>
</tr>
<tr>
<td>Thiophene</td>
<td>MTHF</td>
<td>670, 370</td>
<td>increase</td>
</tr>
<tr>
<td>Thiophene</td>
<td>3MP</td>
<td>830, 320</td>
<td>decrease</td>
</tr>
</tbody>
</table>
results for indole in Freon glass are in partial agreement with flash-photolysis studies by Grossweiner. Thus he reported intermediates with absorption maxima in the region 600-400 m\textmu, and tentatively assigned this to a neutral radical in early papers. Our results suggest that the intermediate observed by Grossweiner may have been the indole radical cation.

2. Luminescence of Irradiated Glassy Solutions. The purpose of the luminescence experiments recently initiated is two-fold. The existence of new emitting species formed by gamma-irradiation, and having different excitation and emission characteristics from the original solute, can be demonstrated. Also, recombination luminescence of charge-separated states formed by gamma-irradiation can be stimulated by infrared or thermal excitation of the matrix. This causes the breakdown of trapping sites in the matrix, and under suitable conditions gives rise to the characteristic luminescence of the original solute molecule, subsequent to its regeneration. Table 2 shows some of the preliminary results obtained for a range of irradiated solutes in 3MP, including some aliphatic and homocyclic compounds previously studied by other groups; and for which excellent agreement is obtained. In some cases, extremely long-lived (10 minutes) luminescences are observed which may be due to radical cation-anion recombinations.

3. Electron Spin Resonance Studies. The ESR spectra of the irradiated Freon mixture have been measured on a Varian E3 spectrometer. The resonance associated with the degassed, irradiated sample and tube is complicated, extending over 500 gauss with unresolved and overlapping structures. The analysis is further complicated by hydrogen atom resonances which change on exposure to visible light. The hydrogen atom resonances are produced from water molecules adsorbed on the silica sample tubes. The sensitivity of light suggests that an intermediate adsorbing in the visible region, such as the trapped electron or adsorbed H$_2$O, may be present. ESR spectra of irradiated frozen Freon droplets, without the use of a sample tube, were therefore studied. Some experimental difficulties remain, but an ESR resonance at 2800 gauss and 9.2 gigacycles has been shown to decrease on bleaching with ultraviolet light. This is concomitant with the decrease in the 585 and 330 m\textmu u.v. absorption bands of irradiated Freon. It can therefore be assigned in the same way to positively charged species produced in the Freon matrix by gamma-radiation.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Glass</th>
<th>Luminescence max before irradiation</th>
<th>Excitation</th>
<th>Warm up emission max</th>
<th>Radiation induced emission band same excitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica tube</td>
<td>3MP</td>
<td>---</td>
<td>230</td>
<td>450</td>
<td>None</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>3MP</td>
<td>390,415</td>
<td>300</td>
<td>500,530,570</td>
<td>None</td>
</tr>
<tr>
<td>DNA</td>
<td>3MP</td>
<td>450</td>
<td>290</td>
<td>450</td>
<td>None</td>
</tr>
<tr>
<td>Pyridine</td>
<td>3MP</td>
<td>---</td>
<td>---</td>
<td>330</td>
<td>---</td>
</tr>
<tr>
<td>Pyrazine</td>
<td>3MP</td>
<td>380,405</td>
<td>300</td>
<td>375,400</td>
<td>---</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>3MP</td>
<td>425</td>
<td>276</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>MTHF</td>
<td>425,485, 590</td>
<td>300</td>
<td>500,530,570</td>
<td>None</td>
</tr>
<tr>
<td>Thiophene</td>
<td>3MP</td>
<td>460</td>
<td>290</td>
<td>550</td>
<td>None</td>
</tr>
<tr>
<td>Toluene</td>
<td>3MP</td>
<td>300 f1 400 phos</td>
<td>280</td>
<td>530</td>
<td>None</td>
</tr>
<tr>
<td>Thymine</td>
<td>3MP</td>
<td>400,425</td>
<td>270</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Triphenyl amine</td>
<td>3MP</td>
<td>425</td>
<td>330</td>
<td>440</td>
<td>---</td>
</tr>
</tbody>
</table>

*λ<sub>max</sub> are reproducible to ± 25 mp due to positioning in A-B spectrometer.
4. Photochemistry of Heterocyclic Molecules in Rigid Matrices at 77°K. As an aid to the identification of radiolytic intermediates, a series of experiments on the ultra-violet irradiation of heterocyclic compounds and aromatic amines have been made. The generality of biphotonic ionization of a large class of compounds via the triplet state has recently been demonstrated. This therefore provides an alternative route to the production of the radical cations of heterocyclic molecules. A large number of aromatic amines were first photolyzed, since many of these are already known to photoionize. This provided a test for the suitability of the present experimental setup for observing photoionization, and a confirmation of the earlier published work. The results (Table 3) were in excellent agreement with earlier work in almost all cases, and the first steady-state (as opposed to flash-photolysis) photoionization of ethylaniline and diethylaniline was observed.

Of fifty heterocyclic compounds screened in preliminary testing, only fifteen gave significant production of intermediate absorptions. One particularly interesting result was the production of a band at 420 mμ from photolysis of indole. This corresponds closely to the spectrum assigned to the indole radical cation in our gamma-radiation experiments and the flash-photolysis work of Grossweiner, described earlier. The fact that the photoionization of heterocyclic molecules appears to be an inefficient process is not entirely unexpected. It is already known that the triplet lifetimes of many N-heterocycles are much shorter (1/1000) than those for corresponding homocyclic molecules. Modifications in future experimental procedure should permit more effective production of photoionization.

5. Self-Consistent Field Calculations on Heterocyclic Radicals and Radical Ions. A computer program has been completed which utilizes the input Hückel wave-functions to carry out Pariser-Parr-Pople self-consistent field calculations on open and closed shell systems. This program calculates wave-functions and eigenvalues for up to 10 pi-electron systems. The Pariser-Parr-Pople program is then linked to a second program which computes the molecular repulsion integrals, and thus the singlet-singlet and singlet-triplet electronic transition energies and oscillator strengths for neutral molecules, and the doublet-doublet electronic transition energies and oscillator strengths for radicals. This produces a theoretical absorption spectrum of a given radical, which can be compared to the experimental spectrum assigned to that species.
TABLE 3
PHOTOLYSIS OF AROMATIC AMINES USING AN OSRAM HBO500 LAMP AT 77°K
VISUAL OBSERVATIONS OR λMAX (μm)

<table>
<thead>
<tr>
<th>Solute</th>
<th>Freon</th>
<th>MTHF</th>
<th>3 MeP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylaniline</td>
<td>Colorless</td>
<td>Tan-yellow</td>
<td>Colorless (525)</td>
<td>-----</td>
</tr>
<tr>
<td>Dimethylaniline</td>
<td>Yellow-green</td>
<td>Faint yellow-green</td>
<td>Faint yellow-green (459)</td>
<td>J. Am. Chem. Soc. 65, 2424 (1943)</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>Light green (670)</td>
<td>yellow-green</td>
<td>Light yellow-green (680)</td>
<td>J. Am. Chem. Soc. 64, 2801 (1942)</td>
</tr>
<tr>
<td>Diphenylmethylamine</td>
<td>Colorless</td>
<td>Colorless</td>
<td>Colorless (650)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Ethylaniline</td>
<td>Pale yellow</td>
<td>Strong yellow (410, 450)</td>
<td>---</td>
<td>-----</td>
</tr>
<tr>
<td>N,N,N&lt;sup&gt;1&lt;/sup&gt;, N&lt;sup&gt;1&lt;/sup&gt;-Tetramethylbenzidine</td>
<td>Strong yellow-green</td>
<td>Strong yellow-green (360, 390, 440, 447, 550**)</td>
<td>Strong yellow green</td>
<td>J. Am. Chem. Soc. 64, 2801 (1942)</td>
</tr>
<tr>
<td>Triphenylamine</td>
<td>Brown-pink (480, 640)</td>
<td>Yellow green</td>
<td>Colorless (656)</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

*Intense color resulted after 2 minutes exposure to lamp; all others required up to 30 minutes exposure.

**Spectra beyond 650 μm not examined. Visual color and existence of one band near 550 suggest similarity of these absorptions and those referenced.
The greatest uncertainty in such calculations lies in the choice of the best values of semi-empirical parameters for radical species. The approach taken here is to first carry out an extensive set of calculations on the parent heterocyclic molecule to find the values which best reproduce its known spectrum. These parameters are then used in calculating the spectra of the derived radicals. This approach is extremely time-consuming, since no general search method can be implemented. However, the results obtained in this way for the lowest transition of the pyrrole molecule are:

<table>
<thead>
<tr>
<th>Transition</th>
<th>Theoretical Energy</th>
<th>Experimental Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 → 4 (1B₁)</td>
<td>5.62</td>
<td>5.88</td>
</tr>
<tr>
<td>3 → 4 (3B₁)</td>
<td>4.19</td>
<td>4.32</td>
</tr>
</tbody>
</table>

These calculations now yield an absorption maximum for the pyrrole radical cation of 1.2 eV, against the experimental value assigned in this laboratory of 1.5 eV. In spite of this encouraging result, anomalies have appeared in calculations on some systems, particularly radical anions, in which spin correlation effects may be very large. In view of this, the matrix elements for a limited configuration interaction treatment of the Pariser-Parr-Pople wave functions have been derived. A program using this is being tested now. It will link with the earlier programs and produce configuration-interaction improved wave functions and energy values which should be more reliable.

6. Valence-Bond Calculations on Heterocyclic Systems. These calculations are being carried out in cooperation with Professor C. Zauli, of the University of Bologna, Italy, and his group. They aim at calculating the electronic structure of triplet states of simple heterocyclic systems, and doublet states of derived radicals, using a non-empirical valence-bond method. The preliminary calculations are being made on cyclopentadiene as a model compound.

The formal expression of integrals among determinantal basis functions in terms of integrals among atomic orbitals is completed. During a two month visit by Dr. A. Grimison to the University of Bologna, some 500 atomic integrals were computed on an IBM 7094, for a net of exponent values, and using a very accurate self-consistent field potential for the ring carbon atoms. These values are now being inserted into a program at PRNC to produce the singlet, triplet, and quintuplet energy levels of cyclopentadiene. An advantage of the approach used in this project is the ease of computing radical species by setting certain sets of integrals equal to zero in a closed shell molecule calculation.
Dr. Lawrence S. Ritchie counting radioactive fish with cercariae of *Schistosoma mansoni* which have been labeled with $^{75}$Se.
SCHISTOSOMA MANSONI PROJECT

This research program is directed toward a better understanding of the mechanism of the host-parasite relationship that could eventually lead to the control of schistosomiasis, a disease which affects over 200 million people throughout the world.

During 1967, a special agreement with Walter Reed Army Institute of Research enabled us to secure the assistance of Dr. Lawrence S. Ritchie, an authority in the schistosomiasis field. Dr. Ritchie's participation in the project since July 1967 made possible the standardization of snail culture and the infection of the definitive host used by us (the mouse). Several experiments were also started and others completed under his supervision. Some were presented to scientific meetings; others have been submitted for publication, some of which have been approved by such journals as Experimental Parasitology.

Cooperation was greatly increased with a group at Minas Gerais from Belo Horizonte, Brazil, headed by Dr. Jose Pellegrino. Last year we were visited by Dr. Celio Murilo de Carvalho Valle and Dr. Pellegrino, who worked with us for four months and one month, respectively. Collaboration begun last year with the U.S. Public Health Service Laboratory (directed by Dr. Frederick Ferguson) was continued and various results were obtained, especially in relation to labeling of snails and cercariae. Accomplishments warranted extending this project to include field observations. Cooperation continued with the group from the U.S. Veterans Administration Hospital under Dr. Menendez Corrada with reference to certain immunological investigations; some results are ready to be submitted for publication.

The following abstracts describe the major projects carried out during 1967.

The Maintenance of Schistosoma mansoni at the Puerto Rico Nuclear Center. Several changes have been made in maintaining the life cycle of Schistosoma mansoni. They are concerned primarily with snail culture and standardizing the exposure for mice.

Tap water in this laboratory was found to be toxic for snails, presumably due to copper ions from the piping. Water was then obtained from a river source for snail culture. Since this limited the size of the stock that could be maintained, an attempt was made to condition the tap water by passing it through sand and charcoal. This proved effective and 15 shallow aquaria (30 x 18 x 6 inches)
were constructed in tiers of three. These were provided with a continuous flow (fast drip rate) of conditioned water. This improvement afforded a stock of about 2,500 uninfected and 800 infected snails. Young snails are cultured in small aquaria until they are 3-5 mm in diameter and are then transferred to the aquaria. They normally reach a size of 12 mm in about 2 months. All snails are fed a formula of ground alfalfa, pulverized dry dog food, wheat germ and powdered milk (4:2:2:1 parts, respectively). The alfalfa was substituted for Cerophyl recently, since it proved equally satisfactory and is sold locally as dairy feed at much less cost.

The sample of cercariae for mouse exposure is provided by means of a semi-automatic pipette. Before the multiple samples are taken for a group of mice, each of five samples is dispersed in a serological spot-plate. A drop of iodine is added to each spot and the cercariae are counted. For this series of 5 counts, the standard deviation is determined; a standard deviation of ± 5 is deemed acceptable for a sample of 100 cercariae.

Exact worm counts have been made for 10 mice from each of 2 series of mice exposed weekly. The mean worm counts have been between 40 and 50, with a standard deviation of ± 10-15. The number of cercariae recovered as worms represents a recovery rate of 40-50%.

Effect of Radiation on the Reproductive Potential of Biomphalaria Glabrata. During 1967 groups of laboratory-reared Biomphalaria glabrata were irradiated with a 60 Co gamma source to provide exposures ranging from 125-4000 Rads. These results complemented data collected in 1966 which involved exposures ranging from 4,000-64,000 Rads (See 1966 PRNC Annual Report, p. 165). With 16,000 Rads, marked damages were noted in all parameters, including 100% mortality after 60 days, no movement or ingestion of food subsequent to exposure, termination of egg laying within two weeks, and no hatching of the eggs that were laid. With 8,000 Rads, results were intermediate, while with 4,000 Rads near-normal results were obtained in all the above parameters. However, the development of eggs showed abnormalities even with 4,000 Rads, and it was deemed advisable to determine the effects of lesser dosages (125-4,000 Rads). Five hundred (500) Rads marked the threshold of irradiation effects and reference will not be made to the lower doses used.
Irradiation with 500-4,000 Rads did not cause significant deaths among mature *B. glabrata*. The mean number of clutches laid per snail per day was reduced for snails exposed to 4,000 Rads, but not with 2,000 and 1,000 Rads. With increase in radiation from 500-4,000 Rads the number of eggs per clutch and eggs produced per snail per day declined somewhat. Egg development was not affected except for eggs laid by snails exposed to 4,000 Rads. Even with this radiation, eggs laid during the first month developed in numbers equalling the non-irradiated controls. During the second month, development of eggs laid was reduced about one-third. Hatching of eggs was the aspect of snail development most sensitive to irradiation. Whereas 43% of the eggs from non-irradiated snails hatched, the corresponding figures for 500, 1,000, 2,000 and 4,000 Rads were 42, 25, 14, and 2%, respectively (Table 1).

In comparing the results of the two experiments, it was noted that egg production was reduced more by 4,000 Rads in the experiment carried out in 1967. The most important finding for the radiations within the range of 500 to 4,000 Rads was the marked reduction in hatching.

Snails exposed to 0, 4,000, 8,000, 16,000, 32,000, and 64,000 Rads (60Co) were sectioned serially for histopathologic studies. Three snails from each of the above categories were sectioned weekly for 7 weeks. After exposure to a dosage of 16,000 Rads, the snails were permanently sterile. The corresponding histopathological picture and the histology of a normal snail are shown in Figures 1 and 2.

**Labeling of Biomphalaria Glabrata with Radioselenium.** The objective of this study was to evaluate radioselenium for labeling *B. glabrata*. Both 75Se (H2SeO3 in HCl) and 75Se-methionine proved very effective for this purpose; these isotopes should be effective for studying a wide range of parameters in relation to the population dynamics of *B. glabrata*. Since these observations were made in conjunction with attempts to label cercariae, the snails used were infected.

In each of two trials, 20 infected snails were exposed to 10 μCi of 75Se-methionine by mixing the isotope with 250 mg of food formula. Seven weekly feedings with a total of 70 μCi were administered in both the first and second trial, but in the case of the latter, 3 additional feedings were given during the 8th week for a total of 100 μCi. In the first trial, radiations were
<table>
<thead>
<tr>
<th></th>
<th>Weeks after irradiation</th>
<th>Amount of Irradiation (Rads)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Death rate (%)</td>
<td>1-8</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Mean clutches/day</td>
<td>1-8</td>
<td>.45</td>
<td>.78</td>
</tr>
<tr>
<td>Eggs/Clutch</td>
<td>1-8</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Eggs/Snail/Day</td>
<td>1-8</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>% Eggs with Embryos</td>
<td>1-4</td>
<td>96</td>
<td>99</td>
</tr>
<tr>
<td>% Embryos Hatched</td>
<td>1-8</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>
started 2 weeks after snails were exposed to miracidia. In the second trial, the infections were patent at the onset of radiation. A group of 20 infected specimens without the isotope were kept as controls.

Snails were rinsed thoroughly before determining radioactivity. Counts were made with a Model 186-A Nuclear Chicago Counter. At the end of the second experiment, 4 snails were killed by immersion in water at 70°C for 30 minutes, after which soft tissues were removed and radioactivity was determined for shell and soft tissues separately. This was also done for a group of 5 snails exposed one week to 25 μCi of 75Se (H275SeO3).

Two days after the first feeding of 75Se-methionine (10 μCi), the radioactivity exceeded 200,000 cpm. There was no increase after 12 days, but some occurred after 26 days. The counts exceeded 400,000 cpm after 40 days and increased after 54 days to more than 850,000 cpm in one trial and to over 672,000 cpm in the other (See Table 2).

Separate counts of soft tissues and shell from infected snails given 100 μCi over 8 weeks in food showed that 75Se-methionine was incorporated chiefly by the soft tissues, the ratios ranging from 6.77 to 17.78 (Table 3). For 5 snails exposed to 25 μCi 75Se for only one week, higher tissue and shell counts were obtained but the differences between activity in soft tissues and shell were less.

The mortality rate for infected B. glabrata exposed to 75Se-methionine was found to be a little less than for infected snails not exposed to the isotope.

Feces accumulated during the final week were found to be highly active (156,624 cpm) while the water had only 2,179 cpm/ml. This indicates that, at least in part, 75Se-methionine was assimilated through food consumption.

Evidence that B. glabrata incorporated 75Se-methionine was obtained by precipitating homogenates of soft tissue with 10% trichloracetic acid and determining the radioactivity of the supernatant and residue after centrifugation. The activity of the precipitate was about 7 times that of the supernatant.

Since the half-life of 75Se is 127 days, relatively long-term experiments on dispersion and population dynamics of B. glabrata should be possible when they are labeled with radioselenium. Studies on radio-damage will be pursued.
### TABLE 2

Labeling of *Biomphalaria glabrata* with $^{75}$Se-methionine

<table>
<thead>
<tr>
<th>Days after Exposure</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Cpm per Snail</td>
<td>No.</td>
<td>Cpm per Snail</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>225,570</td>
<td>19</td>
<td>211,246</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>211,228</td>
<td>19</td>
<td>214,625</td>
</tr>
<tr>
<td>26</td>
<td>16</td>
<td>231,358</td>
<td>19</td>
<td>363,274</td>
</tr>
<tr>
<td>40</td>
<td>14</td>
<td>414,029</td>
<td>19</td>
<td>410,413</td>
</tr>
<tr>
<td>54</td>
<td>10</td>
<td>856,985</td>
<td>12</td>
<td>672,531</td>
</tr>
</tbody>
</table>

### TABLE 3

Radioactivity determination on the shell and soft tissues from snails exposed to $^{75}$Se-methionine.

<table>
<thead>
<tr>
<th>Snail No.</th>
<th>Soft tissues cpm</th>
<th>Shell cpm</th>
<th>Soft tissues shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>476,192</td>
<td>70,337</td>
<td>6.77</td>
</tr>
<tr>
<td>2*</td>
<td>384,766</td>
<td>21,644</td>
<td>17.78</td>
</tr>
<tr>
<td>3*</td>
<td>399,863</td>
<td>36,040</td>
<td>11.09</td>
</tr>
<tr>
<td>4*</td>
<td>513,647</td>
<td>43,788</td>
<td>11.73</td>
</tr>
<tr>
<td>5**</td>
<td>183,651</td>
<td>38,547</td>
<td>4.76</td>
</tr>
<tr>
<td>6**</td>
<td>173,999</td>
<td>38,653</td>
<td>4.50</td>
</tr>
<tr>
<td>7***</td>
<td>197,851</td>
<td>9,199</td>
<td>21.51</td>
</tr>
<tr>
<td>8***</td>
<td>134,225</td>
<td>12,397</td>
<td>10.83</td>
</tr>
</tbody>
</table>

* Infected snails (Experiment 3)
** Control snails (8-week exposure)
*** Control snails (4-week exposure)
Labeling of *Marisa cornuarietis* with Radioselenium and Strontium. We attempted to label *Marisa cornuarietis* (a competitor-predator snail against the vector of *Schistosoma mansoni*), to provide a means of studying its distribution when introduced into a colony of unlabeled snails. Adult *Marisa* exposed to 2 µCi of 89Sr acquired a radioactivity of about 3,000 cpm/snail. With 60 µCi of 75Se, juvenile *Marisa* were much more active, giving about 3,000,000 cpm. Further tests will be required to compare the relative merits of these two isotopes for labeling *Marisa*.

The time necessary for tagged *Marisa* to achieve a distribution coincident with an untagged population will be computed from observations on the snails in an artificial canal at the Tropical Disease section of the U.S.P.H.S. This information will be useful in planning field assessments of snails by the tag and recovery method.

Periodicity of Cercarial Emergence (Circadian Rhythm). It is known from the literature that the emergence of *S. mansoni* cercariae from infected snails occurs chiefly during the day and is dramatically reduced at night (Faust & Hoffman, 1934, Puerto Rico J. Publ. Health & Trop. Med. 10: 1-19; Giovannola, 1936, Proc. Helm. Soc. Wash. 3: 60-61; Maldonado, 1959, Bol. Assoc. Med. Puerto Rico 51: 336-339; Rowan, 1965, Bull. Wld. Hlth. Org. 33: 63-71). In field experiments conducted in Puerto Rico it was found that cercarial densities vary during the day and a consistent sharp peak was noted between 11 a.m. and 12 noon (Rowan, 1958, Am. J. Trop. Med. & Hyg. 7: 374-381).

Determinations of the numbers of cercariae shed by laboratory infected *B. glabrata* were performed every two hours for 4 consecutive days in Belo Horizonte, Brazil and in San Juan, Puerto Rico. Each snail was placed in a beaker containing 50 ml of dechlorinated water. The experiments were conducted outdoors under indirect sunlight. The cercariae that emerged within the 2-hour periods were killed with formalin and counted. Practically all cercariae are shed within a 6-hour period of the day. In Puerto Rico the peak of emergence was found to be between 9 a.m. and 11 a.m. whereas in Belo Horizonte the peak was between 1 p.m. and 3 p.m. This fact is clearly shown in Figure 3. The seasonal fluctuations of cercarial shedding are being investigated in Belo Horizonte, Brazil as well as in San Juan, Puerto Rico.
Labeling of Cercariae of Schistosoma Mansoni with Radioisotopes. The immediate goal of the present study was to label S. mansoni cercariae with radioselenium. This is desirable as a means of studying a wide range of parameters in relation to cercariae dispersion under field conditions, and the role of cercariae predators in natural control of the infection.

The study was divided into three parts.

Exp. 1 - Snails with patent infections of S. mansoni were placed in a glass aquarium containing one liter of dechlorinated water. $^{75}$Se was added to the water (specific activity 5.5 Ci/mg) for a final concentration of 200 $\mu$Ci/liter. Water containing radioselenium was changed weekly for 4 weeks.

Exp. 2 - Snails exposed 2 weeks previous to S. mansoni miracidia were placed in a glass aquarium containing 5 liters of dechlorinated water. $^{75}$Se-methionine (sp. activity 4 Ci/mg) was mixed with the powdered snail food. A total of 70 $\mu$Ci were administered (for details see the preceding abstract).

Exp. 3 - Snails which had started to shed cercariae 2 weeks before exposed to $^{75}$Se-methionine through the food. A total of 100 $\mu$Ci were administered with the diet.

Labeled cercariae were observed as early as 3 days after exposure of the snails to radioselenium. The counts per minute increased slowly, reaching the maximum after 3 weeks (6.99 cpm per organism). Although labeling of cercariae was also observed with $^{75}$Se-methionine, the activity per organism was lower than for $^{75}$Se (Table 4). A control experiment indicated that feeding the radioisotope gave about the same results as mixing it in the water.

As soon as the cercariae began to emerge from infected snails they were already tagged. Figure 4, an autoradiograph of the labeled cercariae, clearly indicates that radioselenium was incorporated by them with a concentration occurring particularly in the penetration glands. Cercariae labeled with $^{75}$Se-methionine retained the ability to infect mice. About 20% were recovered as adult schistosomes, in comparison with about 35% for normal cercariae. The worms were not radioactive.

Preliminary trials have shown that cercariae labeled with radioselenium are quite reliable for conducting studies on cercarial predators.
### Figure 3

<table>
<thead>
<tr>
<th>HOURS</th>
<th>AM</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>5</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.RICO</td>
<td>P.M.</td>
<td>100</td>
<td>60</td>
<td>20</td>
<td>100</td>
<td>60</td>
<td>20</td>
<td>100</td>
<td>60</td>
<td>20</td>
<td>100</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>BRAZIL</td>
<td>P.M.</td>
<td>50</td>
<td>30</td>
<td>10</td>
<td>50</td>
<td>30</td>
<td>10</td>
<td>50</td>
<td>30</td>
<td>10</td>
<td>50</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

**Average Percentage of Cercariae Shed by Infected Snails**

**P.RICO** --- **BRAZIL**

Daily periodicity for shedding of *S. mansoni* cercariae in Brazil and Puerto Rico.

### Figure 4
TABLE 4

Mean counts per minute for cercariae of
*Schistosoma mansoni* exposed to radioselenium

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>75Selenium</th>
<th></th>
<th>75Selenium</th>
<th></th>
<th>75Selenium</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.Cerc.</td>
<td>Mean Cpm</td>
<td>No.Cerc.</td>
<td>Mean Cpm</td>
<td>No.Cerc.</td>
<td>Mean Cpm</td>
</tr>
<tr>
<td>7</td>
<td>131</td>
<td>0.90</td>
<td>363</td>
<td>1.10</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>12</td>
<td>227</td>
<td>2.14</td>
<td>835</td>
<td>1.20</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>19</td>
<td>172</td>
<td>3.13</td>
<td>1,423</td>
<td>0.73</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>21</td>
<td>71</td>
<td>6.99</td>
<td>--</td>
<td>--</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>26</td>
<td>84</td>
<td>5.15</td>
<td>1,038</td>
<td>1.51</td>
<td>520</td>
<td>1.74</td>
</tr>
<tr>
<td>29</td>
<td>64</td>
<td>4.06</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>33</td>
<td>--</td>
<td>--</td>
<td>853</td>
<td>2.49</td>
<td>618</td>
<td>1.50</td>
</tr>
<tr>
<td>40</td>
<td>--</td>
<td>--</td>
<td>843</td>
<td>1.98</td>
<td>903</td>
<td>0.46</td>
</tr>
<tr>
<td>47</td>
<td>--</td>
<td>--</td>
<td>1,215</td>
<td>1.63</td>
<td>880</td>
<td>0.57</td>
</tr>
<tr>
<td>54</td>
<td>--</td>
<td>--</td>
<td>1,185</td>
<td>0.98</td>
<td>315</td>
<td>1.70</td>
</tr>
</tbody>
</table>

-- not done

* prepatent period
Effect of Irradiation (\(^{60}\)Co) on the Host-Parasite Relationship in Snails Infected with Schistosoma mansoni. Preliminary experiments at the P.R.N.C. by Mr. Cello Murilo of Brazil were made using 1,000 to 6,000 Rads (\(^{60}\)Co). The number of cercariae that emerged from the snails was determined every 3rd day for two weeks. A temporary inhibition of emergence occurred with 2,000 to 4,000 Rads, while 6,000 Rads were lethal for the infected snail.

Radiation of the infected snails did not influence the periodicity of cercarial emergence.

Effects of Irradiation (\(^{60}\)Co) on the Host-Parasite Relationship in Mice Infected with Schistosoma mansoni. Acquired resistance against Schistosoma mansoni has been conclusively demonstrated, but the mechanism has not been established. Experiments have failed to show that any of the numerous antibodies are involved, but the possibility of unidentified protective antibodies should not be abandoned. A second possible mechanism is that resistance is mediated by cells, or that it is a complex of both humoral and cellular mechanisms. In order to disassociate these two possible mechanisms, whole-body radiation of the host was used as a means of depressing humoral antibody formation, whereas cellular components such as macrophages and reticular cells are relatively more radioresistant.

In a preliminary experiment, mice were exposed to wholebody radiation with \(^{60}\)Cobalt and then infected with 100 cercariae. The death rate among these animals was compared with mice that were infected but not irradiated. In a second experiment, mice were exposed to 100, 200, 400, 800 and 1,600 Rads, and each of these groups was infected with 16, 32, 64, 128 and 256 cercariae.

The results showed that 400 Rads was the optimal dosage, since it did not kill significant numbers of normal mice and provided a difference in the death rate among infections with 64 and 128 cercariae; no differences were noted with 8, 16, 32 and 256 cercariae. Moreover, the differences in mortality occurred after 3 - 4 weeks following exposure to infection, as well as later when the disease was clearly manifested. One group of mice, irradiated a second time after 4 weeks of infection, showed a significantly higher death rate than control animals, but only when the infections were 60 days old.
A pilot experiment involving whole-body radiation of mice as a means of suppressing acquired resistance induced by irradiated cercariae gave results that warrant further trials.

Correlation Between Worm Burden and Schistosome Pigment in the Liver of Mice Experimentally Infected with Schistosoma mansoni. It is known that red blood cells are ingested by schistosomes and that hemoglobin is actively metabolized inside the lumen of their gut and finally transformed into a granular pigment. This pigment is swept back mainly to the liver of the vertebrate host where it is taken up by phagocytic cells. Kloetzel & Lewert (1966, Am. T. Trop. Med. & Hyg., 15:28) claim that the pigment produced by Schistosoma mansoni is not a free porphyrin but a complex compound soluble in 70% ethanol, containing 0.4% potassium hydroxide. A proteolytic enzyme with a marked substrate specificity for hemoglobin has been found in ground-up preparations of S. mansoni adult worms (Timms & Bueding, 1959, Brit. J. Pharmacol., 14, 68).

In mice harboring bisexual and male S. mansoni infections, it was observed that a linear relationship exists between the schistosome pigment accumulated in the liver and the duration of infection. Daily pigment production, expressed in terms of hemin, was estimated to be 1.19 micrograms per pair of schistosomes and 0.2 micrograms per male in unisexual male infections. (Kloetzel & Lewert, loc. Cit).

In order to investigate the relationship between the schistosome pigment present in the liver and the worm burden, 5 groups of 10 mice, each weighing 20 grams, were infected with 25, 50, 75, 100 and 125 cercariae of S. mansoni (Puerto Rican strain maintained at the Puerto Rico Nuclear Center, San Juan) per animal, using the tail immersion method. The mice were sacrificed 8 weeks after cercarial exposure and the worm burden determined by perfusing the liver and mesenteric vessels with normal saline and by a thorough examination of these vessels for remaining schistosomes. The liver of each animal was then weighed and minced with fine scissors. The whole liver was homogenized with a sonifier and an acetone powder prepared. Hematin was extracted from a 25 mg sample of the acetone powder according to the technique of Kloetzel & Lewert (loc. Cit). Hematin concentrations were determined spectrophotometrically at 400 m\(\upmu\) by comparison with a hematin standard under the same conditions and the total liver pigment was then estimated. Determination of hematin content in livers of 10 control mice showed that it ranged from 0.174 to 0.343 mg, with a mean value of 0.255 mg.
Although these values were rather low when compared to the figures obtained from infected mice, it was found advisable to correct the data from infected animals by subtracting 0.255 mg.

The results obtained in 46 surviving infected mice are shown in Figure 5. The worm burden varied from 4 to 60 schistosomes, with an average of 28 worms and with a sex ratio of 1.0 male:0.83 female. The regression curve, determined by the least-square method, obeys the equation \( y = 23.59 + 1.15x \), \( x \) representing the worm burden and \( y \) the total schistosome pigment in the liver, as expressed in mg of hematin. The coefficient of correlation (\( r = 0.80 \)) was statistically significant at the 0.01% probability level.

Our data demonstrates that a linear relationship exists between the total pigment in the liver of infected mice and the worm burden and confirms the observation of Kloetzel & Lewert (loc. Cit.) that no "crowding effect" is apparent as far as pigment formation is concerned.

An Attempt to Induce Immunity in Mice to Schistosoma mansoni Using the Millipore Diffusion Chamber. Levine and Kagan (J. Parasit 46:787, 1960) were able to induce some protection in mice against Schistosoma mansoni by inoculating them with metabolic products from the cercariae of this parasite. In order to test this concept further, we introduced live worms into the peritoneal cavities of mice, in Millipore diffusion chambers (pore-size, 0.45). We assumed these mice would be exposed to large amounts of antigen in the form of excretions and secretions, and that high titers of antibodies would be produced.

The experimental design was as follows: a Millipore diffusion chamber containing 5 pairs of live adult worms of S. mansoni was surgically introduced into the peritoneal cavity of each of 25 Swiss albino mice (Group I). They were removed after one week and the liquid that they contained was collected and used for inoculating a second group of 25 mice (Group II). These animals were inoculated twice, over a period of two weeks. A third group of mice received a Millipore chamber without worms (Group III). All three groups were exposed to one hundred bisexual S. mansoni. This was done two weeks after the capsules were removed from mice in Group I and one week after immunizations were complete in Group II. Seven weeks later the mice were perfused in order to
Correlation Between Worm Burden and Schistosome Pigment in the Liver of Infected Mice

Figure 5

Tentative Classification of Haemolymph Antigens of Biomphalaria glabrata, small vector of Schistosoma mansoni
determine worm recovery rates.

The slide flocculation test, the Ouchterlony double diffusion method, and immunoelectrophoresis were used to detect serological antibodies. Sera were taken from the animals in Group I one week after Millipore chambers were removed, and from Group II one week after the last injection (1 day before challenge). Sera were again collected at necropsy.

At the time of challenge, Group I and II had a positive slide flocculation test, 4+ and 1+, respectively; the control group was negative. At the time of sacrifice all three groups had 2-3+ reactions. For Groups I and II, gamma G and gamma M globulin components were elevated at the time of challenge, especially Group I as demonstrated by the immunoelectrophoretic method (Hillyer, G.V., L.P. Frick, Exp. Parasit., 20:321-25 1967).

Worm counts revealed that no resistance had been induced against the challenge exposure in each of the two experimental groups. The worm burdens for Groups I, II and III were 32, 28, and 29 mature worms.

This experiment provides no evidence of resistance to S. mansoni infections. In spite of the presence of circulating antibodies, protection against schistosomiasis could not be correlated (Hillyer, G.V., L.S. Ritchie, Exp. Parasit., 20:326-33, 1967; Jachowski, L.A., R.I. Anderson, E.H. Sadun, Am J. Hyg., 77:137-45, 1963). If specific antibodies do confer resistance to Schistosomiasis, be it humoral or cellular, one must determine the type in order to use it as an index of resistance. We are still confronted with the need to demonstrate this relationship.

The Antigens of Biomphalaria glabrata Haemolymph. I. Characterization and Enumeration of Bands by Immunoelectrophoresis. This study was concerned with immunoelectrophoretic analysis of the haemolymph of normal Biomphalaria glabrata. The objective was to provide a base-line of comparison for changes that might occur when B. glabrata are exposed to ionizing radiation. By means of the latter, attempts will be made to produce strains of snails that are resistant to Schistosoma mansoni. Several investigators have studied the protein components of tissue extracts, haemolymph,
and eggs of *B. glabrata* using electrophoretic and immuno-electrophoretic (IEP) techniques. Only Michelson (1966, *Ann. Trop. Med. & Parasit.*, 60:280) has analyzed haemolymph by means of IEP, and he reported 5-7 antigenic components in the haemolymph of these snails.

**Preparation of antisera:** High titers of *B. glabrata* antisera were prepared in rabbits by means of six injections of 0.5 ml of haemolymph mixed with 0.5 ml Freund's adjuvant ("complete" for the first inoculation and "incomplete" thereafter).

**Immunological tests:** Ouchterlony double diffusion and IEP were performed as reported by Hillyer and Frick (1967, *Exp. Parasit.* 20:321). Control slides using human serum were used as an aid in designating the relative electrophoretic mobilities of the haemolymph antigens.

**Column chromatography:** Haemolymph was fractionated using *Sephadex G-75, G-100, and G-200*. The fractions were concentrated by water dialysis and lyophilization.

**Haemolymph labeling:** Adult snails were exposed to $^{59}$Fe for one week, and haemolymph was then collected and submitted to IEP. The slides thus obtained were processed by autoradiography.

**Results:** Agar electrophoresis of haemolymph revealed a strong band having α-2 mobility. Unstained slides showed this region to have red pigmentation, indicating the heme fraction. Heavily stained slides using a protein triple stain (Crowle, 1961) showed trace components in the α-1 and β electrophoretic positions. Weak haemolymph antisera showed two very strong components in the α-2 region, one of which presumably is the heme fraction. On the other hand, higher titer antisera showed a dramatically different immuno-electrophoretogram. When compared to the human serum IEP pattern, one observes haemolymph antigens from the fast α-1 region to the slow γ (β 3).

*Sephadex G-100* was found to be preferable to *Sephadex G-200* for fractionating haemolymph. Three or four significant peaks were found, the fastest always containing the heme portion. Further analysis will be required to detect fractions in each of the peaks.

A tentative classification of haemolymph antigens is proposed.
in Figure 6. Two $\alpha_1$, five $\alpha_2$, eight $\beta_1$, and one $\beta_3$ antigens have been identified as the minimum number representing the haemolymph antigen structure of B. glabrata. Several other antigens in the $\beta_1$ and $\beta_2$ regions still must be identified. Presently, twenty antigens can be consistently identified.

Autoradiography was used to identify the heme antigens. One component picked up the $^{59}\text{Fe}$ but cannot be conclusively identified. Further trials will be required for clarification.

Congeneric and heterogenic studies: Haemolymph from Marisa snails was reacted with anti-B-glabrata serum by IEP. No precipitin lines were found. Haemolymph obtained from B. glabrata maintained in another laboratory in Puerto Rico was found to have an extra band in the $\beta_3$ region. A comparison was made between Albino and normally pigmented B. glabrata. The Albino appeared to have fewer bands, both qualitatively and quantitatively, in all areas of the immunoelectrophoretic picture. Further study is in progress on this comparison. Haemolymph of mature snails (12-weeks old) was compared with young snails (6-weeks, 8-10 mm). The young snails had no precipitin bands corresponding to $\alpha_1$-I and $\alpha_1$-II. This, however, could be quantitative, not qualitative.

The results of this study are highly significant, at least on two points. The number and complexity of the antigens in B. glabrata haemolymph exceeds considerably what has previously been reported. These results, along with those of Dusanic and Lewert (1963, J. Infect. Dis., 112:243), also give evidence that invertebrates may have the capacity to produce specific antibodies, which is contrary to previous reports. It is noteworthy that such a large number of antigens with $\beta$ and $\gamma$ mobilities were found. Lewert (1968, in preparation) discusses the possibility of immune mechanisms occurring in B. glabrata.

The Study of the Effectiveness of Schistosomicidal Drugs with the Aid of $^{59}\text{Fe}$. The need for a rapid and simple method for testing new schistosomicidal drugs is evident. At present, the only method for testing drugs is the oogram method developed by Dr. Pellegrino and J. Paria (1965, Am. J. Trop. Med. & Hyg, 14:363).

Because S. mansoni parasites are fed on the host's blood, $^{59}\text{Fe}$ may be helpful to measure the degree of sickness caused to the parasites when schistosomicidal drugs are administered to infected animals.
Right: Irradiating cercariae of Schistosoma mansoni with a special cobalt source. Below: technicians take care of mice infected with Schistosoma mansoni.
Experimental Procedure: Forty mice, infected with 150 cercariae, were used after eight weeks for the preliminary experiment to test the effectiveness of Ambilhar. One tablet containing 500 mg was ground in a mortar and a few drops of "gum arabic" added to help homogenize the suspension that was diluted to 333 ml. The final concentration of this solution was 1.5 mg/ml.

The mice were divided into four groups of 10 each. The first group was used as a control and the rest of the mice were treated with 0.15 mg, 0.3 mg, and 0.6 mg, respectively.

The mice were fed the drug orally through a cannula for 7 days and simultaneously given 5 μCi of 59Fe intraperitoneally to tag the blood. At the end of the 7 day period, five controls and five treated animals were killed with Nembutal and their livers perfused. The worms were separated from the porta, mesentery and the liver. Males and females were separated and washed twice with saline. The worms were assayed for radioactivity in a well scintillation counter. It was found that the radioactivity in the schistosomes from the control animals was 2 1/2 to 3 times higher than the radioactivity found in the schistosomes from the treated animals (Table 5.). This suggests that it may be possible to evaluate the effectiveness of schistosomocidal drugs using this technique. However, the technique is long and, in an effort to simplify it, worms were chosen at random and placed on an X-ray film. The film was exposed for seven days. When developed, no differences in the radioactivity could be detected with the methods available to us.

In an effort to investigate this further, another group of 20 infected mice was chosen for a second experiment. This time, the animals were treated with Ambilhar for 2 days before the 59Fe was injected. Two animals from each group were killed with Nembutal and the livers perfused. The worms were separated and washed three times with saline containing a small amount of hydrochloric acid to get rid of any 59Fe that may have been coating the schistosomes.

Worms were selected at random from each of the groups and placed on an X-ray film. It is difficult to evaluate the data using densitometric techniques because the worms vary in size and particularly the schistosomes from the treated animals seemed to have morphological changes. The sensitivity of this technique must be evaluated further and work is in progress for testing other drugs.
<table>
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<td>Female</td>
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</tbody>
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* Average of 5 animals

Drug used: Ambilhar

Host: Mice
Victor Quintana transferring larvae by aspirator.

Aurea Lugo removing pupae from artificial food.

Sugarcane borer moth on a corn stalk leaf.
SUGARCANE BORER PROJECT

The sugarcane borer, Diatraea saccharalis (Fab.) (Lepidoptera, Crambidae) causes loss of two to three million dollars annually in Puerto Rico. It is a serious pest throughout the Caribbean, in the United States, Mexico, Central America, and the northern and western sugar-producing areas of South America. This project began in 1963 to determine if it was feasible to sterilize this species by radiation. We have found that the adult stage can be sterilized by radiation exposure to 30 K. of $^{60}\text{Co}$ gamma without adverse effects on longevity or mating behavior.

We first measured the radiation effect on different life stages of the organism. The egg stage and all of the larval stages are extremely radio-sensitive. The late pupal stage, fifth day and older, and the adult stage are considerably more resistant. Adults have survived exposure to 240 K. However, these adults do not mate normally.

The normal mating behavior has been described, and comparisons have been made of the mating behavior of treated individuals. Other aspects of the development of this species have also been studied intensively.

A sterilizing radiation exposure (30 K.) given to the adult does not reduce the oviposition rate. The average egg production for normal fertile females is 350, although some females may lay as many as 700 eggs. Over 90 percent of the eggs laid are fertile. A high percentage (usually over 90 percent) of the fertile eggs hatch, but there is a variation in the hatching rate throughout the year.

Adult longevity was observed in 1966 with individuals reared on corn in the field. Average lifespan of adult males was in excess of 5 days. Average life of adult females was 7 days. Adults reared on the artificial diet developed at PRNC live 1 1/2 to 2 times as long as adults reared in nature.

Field tests have been carried on in a large cage at the nearby UPR farm. Population change of the borer has been measured in the plant hosts (corn) growing in the cage when the sterile adults were released. Different test series have been conducted involving the release of sterile males and normal males with
normal and sterile females; and the release of sterile males and sterile females simultaneously with normal adults, and with normal adults without sterile adults. When only normal adults were released, the next generation was fifteen times the greater than the released population. In all tests where sterile individuals have been released, there has been a decrease in population. This series of experiments has not been completed. However, it appears that the sterilized individuals behave in nature in a similar manner to their behavior in the laboratory. On the basis of our preliminary information, we have evidence that population suppression will take place under natural field conditions.

The next major objective of the project is the development of our capacity to mass-rear the species. The FRNC diet was developed in 1965. Many modifications of this basic diet have been tested since it was developed. We are presently producing from 40 to 60 adults each day on one of these modified diets. Cost per adult is 0.1 to 0.2 cents, or $1,000 to $2,000 per million adults.

Several basic diets have been tried with this species. Early diet tests were based upon the inclusion of the natural food, or other gramineous products in the food. Corn stalk, cane stalk, grass stem, leaves, and other plant parts were included in the diets being tested. Our objective is a diet that is cheap, easy to prepare, and easy to use, that produces a high yield of vigorous adults in the shortest time possible. The present modification produces a 30-to-40 percent yield under mass-culture conditions within 25 to 30 days.

The adults that we are producing live longer than adults produced in nature, they mate more times, and they lay more eggs than those collected from natural host plants.

After hatching, the larvae are collected and placed in artificial food. This food contains carrot powder corn stalk extract, brewer's yeast, ascorbic acid, vitamin supplement, hydrochloric acid and mold inhibitor. A technique for self-transfer is being used, so that the number of larvae per vial varies from 15 to 400. Larvae are manually transferred to fresh food when necessary. At the time of transfer only five larvae are placed in each 30 ml. vial. As many as 25 pupae per vial have been produced with this method.
Above: Life stages of the sugarcane borer: eggs and adults in and near hatching cups, larvae and pupae in food vials and on egg hatchability recording sheet, and pupae removed from vial on table. Left: Victor Quintana harvesting larvae from the egg incubator.

Right: Josefa Torres and Victor Quintana examining larvae in the laboratory insectary.
Tests were made to select a strain of this species that is well-adapted to laboratory rearing. Desirable traits that were sought include short larval life span and long adult lifespan. Approximately two-hundred lines were inbred over five successive generations. There was a great variation in the length of larval lifespan among individuals within strains as well as between strains.

Further work on diet improvement is in progress at this time. Handling techniques are also under study to provide the optimum conditions for survival, with minimum labor equipment. We are testing other methods for self-transferring of larvae after hatching. These are based on the attractive substance produced by the natural host plant that stimulates feeding. In addition, improved methods of cleaning vials, transferring mature larvae, and removing pupae from food are under development.

During the earlier phases of the project, occasionally it was necessary to use the surviving offspring from sterility tests for laboratory tests. It was observed that these offspring were partially sterile. On the basis of this knowledge, further tests were made to measure the inheritance of lethal factors over more than one generation. Recently we completed a series of tests where sterility was observed in several different lines over five generations after irradiation. Two main types of lines were established. The first included a series where the irradiated lines were inbred over successive generations, and the second where survivors were outbred with mates from non-irradiated lines. The lethal effect, as measured by egg hatch, larval survival, and pupal survival was observed through the third generation. This data is to be published in the near future.

The U.S. Department of Agriculture has established a program of investigation on the Island of Vieques, eight miles east of Puerto Rico. There are approximately 1,000 acres in cane production as well as several thousands of acres in guinea grass, which is also a host plant of Diatraea saccharalis. Experiments are planned to measure the effect of an integrated control program for suppressing and eventually eradicating this pest from Vieques. This program will involve the study of parasites as well as the release of sterile adults. This cooperative program includes personnel of the Entomology Research Division of the USDA and the PRNC.

The field operation is expected to begin early in 1968 by entomologists of the USDA. PRNC will produce the sterile insects to release on Vieques after the initial study phase has been completed.
PHYSICAL RESEARCH PROGRAMS

Research in physical sciences, sponsored by the US AEC Division of Research, represents approximately one-tenth of PRNC's program.
Dr. M. I. Kay (left) and Dr. K. Okada check the final mounting of a crystal on the goniometer installed on PRNC's automatic neutron spectrometer.
NEUTRON DIFFRACTION

The neutron diffraction group at the Puerto Rico Nuclear Center is working on two types of problems: (1) the chemical binding of atoms in crystals and molecules; (2) the nature of ferromagnetism. Both are related to the spatial arrangement of atoms in molecules.

If either X rays or neutrons are scattered from crystals, patterns can sometimes be analyzed that show the arrangement of atoms in the crystal. Since the amplitude of X rays diffracted is proportional to the atomic number of the scattering atom, if both light and heavy atoms occur in the same compound, the contribution of the light atom is very weak and its position can be determined only with great difficulty. Neutrons, however, are scattered by the nuclei of the atoms. Diffraction of neutrons by light elements compares favorably with that from heavier elements, and the coordinates of the lighter atom may be determined with greater precision than with X rays. In compounds having atoms with unpaired electrons, a neutron-electron spin interaction is also present. Since the magnetic properties of substances are related to the way the electron spins are arranged within the crystal, determination of such spin arrangements by neutron diffraction provides information about magnetic structures.

Overall Progress. The last two progress reports listed several completed problems. In the past year and a half several new problems were initiated and new work on different phases of the old problems extended. Some of these problems are now nearing completion and enough results are available to make preliminary reports.

I. Phenantrene. Damask and Arndt have reported that phenan-threne undergoes a phase transition about 72°C as detected by heat capacity, electrical conductivity, and polarization measurements. Since the effect was found to be uniform throughout the crystal, the authors consider it possibly due to some type of ordering effect in the crystal, perhaps to hydrogen atom motion, since no change in X ray pattern was noted on heating phenantrene through its transition.
We have not yet done any work at high temperature, and the room temperature data collection has just been completed. However, some analysis was carried out when about half the neutron diffraction data now available was taken. These preliminary results which confirm previous conjectures about the structure of phenanthrene are presented in Fig. 1a and 1b, and in Fig. 2.

The structure of phenanthrene was solved in the 1950's by Trotter who reported the three rings (see Fig. 1) to deviate slightly from planarity. When we undertook the neutron work, Okaya at IBM laboratories took new X-ray data to obtain more accurate carbon positions. His results confirmed Trotter's on ring puckering in that the hydrogen positions determined from the neutron diffraction data show that it is the close H4 - H5 approach of 1.96 Å that causes the deviation from planarity as surmised by Trotter.

The nature of this deviation is shown in Fig. 2 in which the distances from the average plane of the central six carbons are given for all atoms. (It should be noted that at this point the hydrogen atom positions are known to only ca. 0.1 Å). The top ring is bent below the plane of the center ring; the bottom ring is bent up from the plane of the center ring. The three six-member carbon rings are each independently planar ± 0.01 Å, and the top and bottom ring are about 45° from the central ring.

It would be extremely interesting to compare final neutron and X-ray positions to see if the electron density has any effect on the position of the carbon atoms. However, it is doubtful if the neutron data will be of sufficient accuracy to make such a comparison meaningful because of the high incoherent hydrogen background and the low PRNC reactor flux. Another possible procedure that could help shed light on the electrical properties would be scattering electron densities above and below the transition.

II. Spiral Oscillator Problem in Na Al(304)2.12H2O. The structures of the Alums were reported in PRNC-94. On page 9 of that report we note that three of the sulfate oxygens in sodium Alum (Na Al(304)2.12 H2O) seem to undergo a translational motion down the threefold axis which is correlated with a rotation about the threefold axis to avoid a near hydrogen neighbor. The scattering from this "spiral oscillator" is really not well approximated by the usual form of the Debye-Waller factor which assumes
Fig. 1(a). Phenanthrene: Carbon atoms are at the intersection of 3 solid lines. Hydrogen atoms are at the ends of solid lines. Atom numbering is denoted by integers. Bonds are denoted by solid lines with distances adjacent to the lines.

Intramolecular H-H distances are denoted given in dashed lines. Note the short H4-H5. The standard deviations of the hydrogen atoms are about .05 Å at the current stage of refinement.

Phenanthrene Bond Angles.
a gaussian distribution of scattering density about an equilibrium position.

It is easy to write the expression for scattering from a given distribution. Classically, the average time that an atom spends at point \( r \) is dependent upon potential \( V(r) \) through the Boltzmann distribution. If \( \mathbf{H} \) is the reciprocal lattice (scattering) vector and the scattering power of the atom is given by \( b \), then the average scattering amplitude from an atom is given by:

\[
\oint b \exp (2\pi i \mathbf{H} \cdot \mathbf{r}) \cdot \exp (-V(r)/kT) \, d\mathbf{r}
\]

The above integral is rather difficult to evaluate in closed form, unless \( V(r) \) is rather simple. It and its derivatives may, however, be evaluated numerically.

The numerical values of the scattering, as evaluated from estimated positional and potential function parameters, and their derivatives is the information needed for a least squares fit of those parameters to the measured scattering data. We are now in the process of trying to evaluate the constant for the sulfate group in sodium alum. If this approach is successful, it could be generally used to evaluate and interpret the Bragg scattering from many types of molecules undergoing varied types of large torsional and other anharmonic oscillations.

III. Ferroelectrics.

A. The ferroelectric, sodium nitrite, has been studied a great deal in the last nine years, at least partly because the molecular motions are large and therefore more likely to be easily interpreted in terms of the electrical properties than many other compounds. In spite of all this work there is still some doubt about the direction of rotation of the \( \text{NO}_2 \) groups during the transition.

To try to clear up this point, three-dimensional neutron diffraction data was taken at 150°C, about 15° below the transition. The results were ambiguous, i.e. the r.m.s. amplitudes of motion perpendicular to the a axis of the crystal were about equal to those perpendicular to the c axis.

Further three-dimensional data will be taken at about 185°C and 225°C to see if one type of motion predominates.
Fig. 2. Distances of atoms from the average plane of the central carbon atom ring (C9 through C14). Note the top ring is bent down and the bottom ring bent up from the central ring. Hydrogens have standard deviations of about .05 Å.

Fig. 3. Sodium Trihydrogen Selenite projected down the b axis. Y coordinates (in fractions of a unit cell) are given for the asymmetric unit. The results are for a preliminary refinement and should be regarded as demonstrating the general molecular geometry rather than details.
B. The room temperature (paraelectric) phase of NaH$_3$ (SeO$_3$)$_2$
Sodium Trihydrogen Selenite has been studied.

The space group is $P2_1/n$. Lattice parameters are: $a = 10.32$, $b = 4.83$, $c = 5.78$ and $\beta = 91.8^\circ$ as determined by Unterleitner (1964).

While the final refinement has not been carried out, the ordered set of hydrogen positions indicated in Fig. 3 was determined. The Sodium, Oxygen, and Selenium parameters were refined, starting with Unterleitner's positions.

The notable features of the structure shown in Fig. 3 are described as follows: The Sodium is octahedrally coordinated to six oxygens, 2 each at 2.38 Å, 2.43 Å and 2.48 Å. The Se-O distances are 1.75 Å, 1.70 Å, 1.74 Å for O1, O2, O3, respectively. Comments on the significance of these distances should probably await the final refinement of the structure. The selenite groups are linked through two types of hydrogen bonds (Fig. 3).

The O1 - H1 - O1 seems to be symmetrical, although a double minimum cannot be ruled out, with an O - O distance of 2.52 Å and O - H distance of 1.26 Å. The other, O2 - H2 - O3, hydrogen bond which is even shorter seems asymmetric. The O2 - O3 distance is 2.49 Å with O2 - H2 = 1.10 Å and H2 - O3 = 1.39 Å. The anisotropic motion should be analyzed before commenting further on the above results.

Cross Section Measurements. In connection with a neutron liquid scattering project by Dr. H. Ritter (See section on Staff), the scattering lengths for tin isotopes 116, 117, 118, 119, 120, 122, 124 have been measured. The results are $0.58 \pm .01$, $0.64 \pm .02$, $0.58 \pm .01$, $0.60 \pm .02$, $0.64 \pm .01$, $0.55 \pm .03$, $0.59 \pm .02 \times 10^{-12}$ cm. Scattering lengths are the data that describes the scattering power of an atomic or isotopic species for coherent processes.

New Equipment. An experimental dewar for taking diffraction data at liquid nitrogen or helium temperatures has been purchased. The dewar is now being tested.

The U.S.I. four circle neutron spectrometer has been automated and is now taking data under punched-card control.
Dr. Howard Ritter, professor of Chemistry at Miami University, Oxford, Ohio, returned to Oxford in August 1967 after a year at PRNC and a month at Argonne National Laboratory.

Dr. Ritter worked on neutron scattering by molten salts. He tried to develop the technique of separating parts of the radial distribution function by means of isotopic substitution. While at PRNC, he measured the coherent scattering cross sections of seven stable tin isotopes. He was going to measure the scattering from Molten Cu Cl$^{35}$, Cu Cl$^{37}$, Cu$^{65}$Cl, and Cu Cl at Argonne, where the higher reactor flux would provide more suitable conditions. Since Dr. Ritter's untimely death in November of this year, the project has been continued under the auspices of the Argonne metallurgy group.

The neutron spectrometer unit at PRNC Mayaguez.
Mr. Jaime A. Castellanos measures the length of a 100 megawatt Q-switched laser pulse.
SOLID STATE PHYSICS
STUDY OF RADIATION DAMAGE IN ORGANIC CRYSTALS

The primary interest in this project is to study the effects of radiation on organic crystals. It is felt that such studies on well defined crystalline structures can provide a firm foundation for a later study of more complex materials including those of direct biological interest. Anthracene was chosen as the initial material to be studied for the following reasons: (1) large and very pure anthracene crystals can be obtained; (2) a large amount of information is known about its electrical and optical properties; (3) radiation damage due to high doses of neutron and gamma irradiation in anthracene has been studied. Radiation damage in anthracene after gamma irradiation in the high dose range (larger than 10^5 R) was studied by measuring the degradation of its fluorescence. Radiation damage due to neutron irradiation was studied by measuring the changes in the photoconductivity properties.

EXPERIMENTAL TECHNIQUES

Experimental methods used in studying radiation damage include: (1) Space-charge limited current measurements; (2) delayed fluorescence measurements; (3) electron spin resonance measurements; (4) chemical analysis. A complete description of the detection of radiation by the space-charged limited current technique and by the delayed fluorescence techniques are given in PRNC-58 and PRNC-107, respectively.

ACHIEVEMENTS

1. Singlet-Singlet Annihilation Rate Constant in Anthracene. As described previously (PRNC-107), by measuring the degradation of fluorescence and delayed fluorescence caused by gamma radiation, valuable information concerning radiation damage is obtained. The luminescence of anthracene is blue and is due to excitation of the singlet states. By illuminating the crystal with red light, triplet excitons are produced. The triplet state energy level in anthracene lies more than half way between the ground state and the first excited singlet state. Two triplet excitons can annihilate each other, producing a ground state and an excited singlet. The fluorescence arising from this excited singlet is known as the
delayed fluorescence. By irradiating the anthracene crystals with gamma rays, triplet and singlet quenching centers are introduced. Since the diffusion length of the triplet exciton is about one hundred times larger than that of the singlet exciton, degradation of the delayed fluorescence will occur by irradiating the crystal with a lower gamma dose than that needed for observing degradation in the normal fluorescence. The equation, \( \gamma s/2 \gamma T/2 = 2.5 \) \((K_S/K_T)(\alpha T^N/\alpha s^N)\), that relates the radiation doses needed to reduce the intensities of the normal and delayed fluorescence to half their original values and the concentrations of the introduced singlet and triplet quenching centers, was derived. In this equation, \( \gamma s/2 \) and \( \gamma T/2 \) are the radiation doses in rads that are needed to reduce the intensity of the normal and delayed fluorescence, respectively, to half their original values. \( K_S \) and \( K_T \) are the monomolecular rate constants, and \( \alpha_s \) and \( \alpha_T \) are the bimolecular rate constants for the singlets and triplets, respectively. \( N_S \) and \( N_T \) are the concentrations of the singlet and triplet quenching centers induced in the crystal by one rad. The values of \( \gamma s/2, \gamma T/2 \) and \( K_T \) are obtained from the above experiments. The values of \( K_S \) and \( \alpha_T \) are taken from the literature. The value of the singlet-singlet annihilation rate constant \( \alpha_s \) found in the literature \(1,2,3\) varies from \( \alpha_s = 5 \times 10^{-11} \text{ cm}^3 \text{ sec}^{-1} \) to \( \alpha_s = 2.5 \times 10^{-7} \text{ cm}^3 \text{ sec}^{-1} \). We calculated \( \alpha_s \) by measuring the fluorescent quantum yield as a function of light intensity using very high intensity pulsed light sources and found \( \alpha_s = 10^{-10} \text{ cm}^3 \text{ sec}^{-1} \). Using that value in the above equation, it is found that the concentration of the singlet quenching centers introduced by one rad is three orders of magnitude larger than the concentration of the triplet quenching centers induced by one rad.

2. Phenanthrene. Extensive information is available on the optical properties of phenanthrene. However, the electrical properties and radiation damage have not been studied as thoroughly. The reason appears to be that optical properties and part of the

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Instrumentation used in conductivity measurements.
radiation damage can be studied utilizing thin vapor or solution grown crystals; while the study of electrical properties and low dose radiation damage requires the bulkier pure melt grown crystals which are more difficult to obtain. Attempts are being made to study low dose radiation damage in phenanthrene along the same lines as used in studying anthracene. No delayed fluorescence has been observed in phenanthrene by utilizing a high intensity xenon flashlight in an attempt to excite enough triplets directly from the ground singlets. It appears that the singlet-triplet absorption coefficient for phenanthrene is smaller than $10^{-4}$ cm$^{-1}$, which is the value for anthracene. Experiments are in progress utilizing the second harmonics of a 100 megawatt "Q" switched neodymium doped glass laser. Results of these experiments might lead to the calculation of parameters such as the singlet-triplet absorption coefficient, triplet-triplet annihilation rate constant and the diffusion coefficient for the triplet excitons.

3. Electron Spin Resonance Measurements. The quenching centers introduced by gamma radiation in anthracene are paramagnetic and their ESR spectra are studied. The ESR signal consists of apparently four unresolved lines. The magnitude of the signal increases linearly with the dose. The half band width of the signal is 60 gauss. The $g$ value calculated is approximately 2. There is no difference in the ESR spectrum by using the highly purified Eastman X-480 anthracene or by using the synthetic Eastman H-480 anthracene. A model was proposed in which a cross-linking between the anthracene molecules takes place. The validity of that model is now being checked by using deuterated anthracene.

4. Chemical Analysis. Attempts were made to analyze chemically the radiation induced quenching centers in anthracene by using the thin layer chromatography method. The following solutions were used: Benzene, chloroform, ethyl acetate, heptanol, isopropyl alcohol, acetone, carbon tetrachloride, nitryl acetate, ether, dichloroethylene, trichloroethylene and tetrachloroethylene. No separation could be seen. The maximum radiation dose to the crystal was $2 \times 10^9$ rad. By assuming that the linear extrapolation is valid up to such a high radiation dose, the maximum concentration of the quenching centers would have been $10^{-3}$ cm$^{-3}$.

LABORATORY SPACE

This project is housed in the Facundo Bueso Science Building at the Río Piedras Campus of the University of Puerto Rico because of the shortage of space in the PRNC Bio-Medical Building. When the project was initiated in January 1962, a room with 360 square feet of space was utilized. In February 1965, the project was moved into a room with approximately 900 square feet of floor space. In addition, two offices with a combined area of approximately 240 square feet were provided. The increase in space has made it possible to set up the equipment more advantageously and to make additions to the staff.
Analysis for $\text{H}_2$ obtained in radiolytic experiments.
HOT-ATOM CHEMISTRY

The program is principally concerned with the study of the mechanism of Szilard-Chalmers processes in organic compounds containing multiple bonds between the activated atom and carbon atoms of the organic radical. These organic radicals are either phenyl or cyclopentadienyl. The activated atoms include Si, P, S, Hg, Bi, Sn, Tl, and I as phenyl derivatives, and the cyclopentadienyl derivatives (metallocenes) of Co, Ni, Zn, Hf, Os, and Re.

The equipment available includes glove boxes, and a specially ventilated laboratory to handle radioactive gases. The analytical techniques used cover various forms of chromatography, including radio-gas chromatography and high-voltage electrophoresis.

WORK IN PROGRESS

Metallocenes - with Mrs. M.L. McClain. Previous studies on metallocenes (see following section) are being extended to dicyclopentadienyl osmium and dicyclopentadienyl rhenium hydride. These compounds were activated and processed by solvent extraction in a nitrogen atmosphere. The organic fraction was analyzed by sublimation and the inorganic fraction by paper electrophoresis.

Organic Silicon Compounds - with Miss J.E. Trabal. The neutron activation products formed from tetraphenylsilane, triphenylsilane, and diphenylsilane are being separated by rapid column chromatography and electrophoresis.

Recoil Labeling with Iodine and Bromine - with Miss H. López Alonso (graduate student). Liquid aromatic compounds such as benzene, nitrobenzene, aniline, and methyl benzoate, are being labeled with $^{128}$I and $^{80}$Br by recoil from dissolved iodoform, carbon tetraiodide, bromoform or carbon tetrabromide.

WORK COMPLETED

Szilard-Chalmers Reactions in Metallocenes - O.H. Wheeler and M.L. McClain. The radioactive products formed in the neutron activation of cobaltocene and nickelocene have been separated by solvent extraction in a nitrogen atmosphere. This rapid procedure permitted the study of $^{60}$mCo (half-life 10.5 min.). Nickelocene
showed 65% retention of activity, and 35% as nickelous ion. Cobaltocene gave 25% retention, 15% as cobalticinium ion and 5% as cobaltous iron. The activity in the cobalticinium ion fraction was greater for $^{60}\text{mCo}$. Thermal annealing increased the retention in both cases.

Dicyclopentadienylzirconium dichloride and dicyclopentadienyl hafnium dichloride were similarly activated and the products separated by solvent extraction. The retention was only 1.8 and 0.5%, respectively, in these compounds. This must be due to the low probability of reforming multiple bonds. The inorganic zirconium activity amounted to 64.2%, and inorganic hafnium 80.9%. The remainder of the activity appeared in a polymeric fraction. Thermal annealing did not increase the retention in these cases but led to a redistribution of the activity between the inorganic and polymeric fractions.

**Hot-Atom Synthesis of Phenylmercuric Compounds** - O.H. Wheeler and M.L. McClain. The products formed in the neutron activation of diphenylmercury and phenylmercuric chloride and acetate have been studied using chromatography on alumina. Diphenylmercury-203 and labeled phenylmercuric compounds were formed in low specific activity, and exchange was noted between inorganic $^{203}\text{Hg}$ in solution. The highest retention for diphenylmercury was obtained when the compound was activated in benzene.

**Hot-Atom Reactions in Aromatic Bismuth and Thallium Compounds**

O.H. Wheeler, J.E. Trabal, and M.L. McClain. The products formed in the activation of triphenylbismuth were separated by column chromatography on alumina. The triphenylbismuth fraction amounted to 27.5% of the total activity, with 35.0 and 23.5% appearing in the diphenylbismuth and phenylbismuth fractions. Activation in benzene resulted in 92.0% "retention." Diphenylbismuth chloride afforded 24.6% triphenylbismuth, little Ph$_2$Bi$^+$(2.4%), PhBi$_2^+$(16.4%) and a large (56.5%) inorganic Bi fraction.

Similar studies on diphenylthallium chloride showed an increase in retention with activation time as a result of radiolytic decomposition. Thermal annealing led to an increase in retention and an increase in the $^{204}\text{Tl}$ inorganic fraction, at the expense of the PhTl$_2^+$ activity.

**Radioactive Decay Correction Tables** - O.H. Wheeler, A.S. Kay and J.E. Trabal. A FORTRAN program was written to compute the fraction of activity remaining at intervals of time for a series of short half-life isotopes.
Above: Graduate assistants and students using counting equipment. Below: Dr. Luis Feliu (Chemistry Dept., UPR) and Misses Hilda Lopez and Carmen Lecumberry (graduate students) preparing radioiodine compounds in special facility.
STAFF

The group consists of Mrs. María Luisa McClin, M.S., Miss J. Elisín Trabal, B.S. (part time) and Miss Hilda López Alonso, B.S. Miss Carmen Lecumberry, instructor, Chemistry Department, University of Puerto Rico at Mayaguez, also participates in the program. Both Miss López Alonso and Miss Lecumberry are graduate students in chemistry. Mrs. Carmen L. González, M.S., resigned in May 1967, in order to accept an appointment as Chemist in Charge of the Analytical Laboratories, Puerto Rico Public Health Service in Mayaguez.

Dr. A. Gordus, University of Michigan; Dr. A.P. Wolf, Brookhaven National Laboratory; and Dr. H. Ache, Virginia Polytechnic Institute; visited the Center to consult on the project.

Dr. Owen H. Wheeler gave a seminar on the "Hot-Atom Chemistry of Aromatic Phosphorus Compounds" at the Chemistry Department, University of Puerto Rico at Río Piedras, on October 4, 1967.

Miss Elisín Trabal and Mrs. María Luisa McClin (research assistants) using dry boxes to process unstable radioactive compounds.
PEACEFUL NUCLEAR EXPLOSIVES

PRNC's studies in this area are financed by the U. S. Atomic Energy Commission's Division of Peaceful Nuclear Explosives (PNE).
At right: Mr. Samuel Rodriguez observes the operation of a column used in dynamics studies of leaching with sulphuric acid. Below: Dr. Dan Taylor lectures on Project Gasbuggy at a Plowshare Workshop session.
MINING WITH NUCLEAR EXPLOSIVES

Studies on this project were begun in May 1967. From July onward, they were financed by the Atomic Energy Commission's Division of Peaceful Nuclear Explosives (PNE). The project consists of a feasibility study of the use of an underground nuclear explosion to facilitate leaching a copper ore body. The effects are expected to be largely mechanical, resulting from the fracturing of the rock and the foundation of a rubble chimney, together with possible chemical effects due to the shock wave produced by the explosion.

WORK IN PROGRESS

Effect of Heat and Shock Wave on Chalcopyrite. With S. Rodríguez and Carmen Vincenty. Chalcopyrite type copper ore was obtained from the Puerto Rico Mines at Utuado, Puerto Rico, and its physical properties were determined: density, humidity, and screen analysis. Data is presented in Table 1. Based on screen analysis data, a statistical procedure was made following recommendations given by Rodean (Geophysics 4, 616 (1965)), and from Figure 1, one can see that the logarithmic size-weight distribution found in the rubble material of nuclear chimneys can also be applied to study the size-weight distribution of a pulverized ore.

Several analytical techniques for copper quantitative determination were put in operation: spectrophotometric determinations, electrolytic techniques and titration with sodium sulfate solutions. Statistical analysis of the time employed for analytical measurements indicated the convenience of using titration methods. Using Na₂S₂O₃ complexed with iron (Fe⁺⁺⁺) and ammonium bifluoride, the ore samples were separated from silica with hydrofluoric acid, followed by evaporation to dryness with concentrated sulfuric acid. Following this procedure, the content of copper in the Utuado ore was found to be between 3/4 and 1%. The precise data are contained in the scientific reports of this work.

Studies on the differential thermal analysis of chalcopyrite up to 850°C have been made. A Cahn electrobalance has been modified to permit thermogravimetric analysis in atmosphere of air or nitrogen. Statistical analyses of available results are being done; the structural changes corresponding to thermal transitions are being analyzed by X-ray. Procedures to study the leachability of the copper from the chalcopyrite by pumping the acid through an
aerated column of mineral, as well as a method for determining the free-acid content of the copper leach solutions, are being considered.

WORK COMPLETED

Leaching of Chalcopyrite Ore: O.H. Wheeler, F. Muñoz-Ribadeneira, and S. Rodriguez Morales. Preliminary studies on the leaching behavior of the Puerto Rico chalcopyrite ore with 0.5N H2SO4 have been initiated, using batch type operations when no air is present during the leach time. After 3 days, 13.2% of copper was leached; 26.7% after 8 days and 35.9% after 18 days. Exposure of the ore to gamma irradiation either in the dry state, or wetted with water, did not alter the leachability. After heating to 500°C, 60% of the available copper could be leached in 6 days. Heating to 650°C released 75% of the copper in a similar period.

OTHER ACTIVITIES

Puerto Rico Summer Workshop on Nuclear Civil Engineering. Dr. James A. Cheney and Dr. Wilson K. Talley from the University of California, Davis, California, attended a summer workshop on Plowshare technology during June-August 1967, at the invitation of PRNC Director, Dr. Henry J. Gomberg. Representatives from the departments of chemical civil, mechanical and nuclear engineering, physics, and staff members of PRNC also attended. At the end of the workshop, each participant presented a detailed analysis of a specific engineering or scientific use of nuclear explosives. The topics covered in the summer workshop are listed in Table 2.

AEC International Exhibits Program. During the Atoms in Action Exhibit in Quito, Ecuador, Eng. Muñoz-Ribadeneira offered a series of lectures at universities and other institutions, describing the current status of Plowshare, as well as applications of nuclear explosives in modern technology. Only two lectures at the University of Guayaquil were originally scheduled, but broad local press coverage prompted invitations to speak at other institutions. Economic assistance is now being sought to permit several young Ecuadorian engineers to take graduate studies at UPR and participate in Plowshare-related research projects at PRNC. See Table 3 for details on the lectures offered.
Figure 1 - Logarithmic Size-Weight Normal Distribution (Rodean) for a Pulverized Chalcopirite Type Ore, Utuado Mines, P.R.
The Ecuadorian Institute of Hydraulic Resources (INERHI) displayed strong interest in Flowshare, as related to water conservation. According to INERHI staff members, it appears that two possible applications of nuclear explosives for irrigation purposes presently exist. One is to dig a channel through a mountain range; another is to excavate a huge reservoir.

1. **The Channel** -- The Province of Manabí is one of Ecuador's most fertile regions, but sparse rainfall and few rivers hinder agricultural development. There are several rivers heading south-southwest for one-third of their length, but a series of small mountain chains diverts them in a north-northwest direction. INERHI has studied the possibility of tunnelling through these mountains to permit the water to reach the other side, but the time and money involved make it prohibitive for Ecuador to undertake such a project. The proposed cut is 8 kilometers long; the peak height is 280 meters.

2. **The Reservoir** -- INERHI believes that between the mountains of Chimborazo and Carahuayrazgo there is enough water to irrigate the Province of Tungurahua, where most of Ecuador's temperate zone fruits and vegetables could be planted. A huge reservoir is needed to contain rain water and melting snow on the mountainsides. INERHI states that a crater similar in dimension to Project SEDAN would hold an adequate water supply.

**STAFF**

The staff consists of Dr. John D. Weaver, Chairman of the Department of Geology, University of Puerto Rico at Mayagüez, on a part time basis, and Mr. Samuel Rodríguez-Morales, B.S., research assistant and graduate student in chemistry. The leaching studies are directed by Dr. O.H. Wheeler, Associate Director and Mr. Fausto Muñoz-Ribadeneira, M.S., Associate Scientist. Miss Carmen Vincenty, (B.S.), is research assistant in charge of computer calculations.
TABLE 1

Physical Characteristics of the Copper Ore,
Chalcopyrite Type Orte (Utuado)

a) Screen Analysis

<table>
<thead>
<tr>
<th>Screen Number</th>
<th>% Weight Retained</th>
<th>% Weight Passing</th>
<th>Screen Microns</th>
<th>Average Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.11</td>
<td>99.89</td>
<td>1190</td>
<td>1435</td>
</tr>
<tr>
<td>20</td>
<td>0.81</td>
<td>99.08</td>
<td>840</td>
<td>1015</td>
</tr>
<tr>
<td>28</td>
<td>3.16</td>
<td>95.92</td>
<td>590</td>
<td>715</td>
</tr>
<tr>
<td>35</td>
<td>4.50</td>
<td>90.52</td>
<td>420</td>
<td>505</td>
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<tr>
<td>48</td>
<td>7.85</td>
<td>82.67</td>
<td>297</td>
<td>359</td>
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<tr>
<td>65</td>
<td>11.41</td>
<td>71.26</td>
<td>210</td>
<td>254</td>
</tr>
<tr>
<td>100</td>
<td>14.70</td>
<td>56.56</td>
<td>149</td>
<td>229</td>
</tr>
<tr>
<td>150</td>
<td>13.18</td>
<td>43.38</td>
<td>105</td>
<td>127</td>
</tr>
<tr>
<td>200</td>
<td>13.70</td>
<td>29.68</td>
<td>74</td>
<td>89</td>
</tr>
<tr>
<td>270</td>
<td>5.20</td>
<td>24.48</td>
<td>53</td>
<td>64</td>
</tr>
<tr>
<td>-270</td>
<td>24.48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) Other properties:
- **Density** (by volume of water displaced) \(2.76 \pm 0.13 \text{ gr/cc}\)
- **Humidity** (dried at 110°C for 48 hours) \(0.54 \pm 0.02\%\)
### TABLE 2

**Topics Covered in Summer Workshop**

- **Mechanical Action of Nuclear Explosions**
  - Equations of hydrodynamics
  - Thermodynamics and statistical mechanics
  - Equation of state; Thomas-Fermi model
  - Rankine-Hugoniot relations
  - Reflection and interaction of shocks
  - Physical and mathematical instabilities

- **Engineering Principles Applicable to Nuclear Explosions**
  - Planning and organization of construction projects; CPM
  - Theory of elasticity
  - Waves in elastic media
  - Failure theories in mechanics
  - Soil mechanics; slope stability
  - Flow in porous media
  - Seepage forces; settlement of saturated soils
  - Structural dynamics; response spectra
  - Descriptive geology
  - Drilling of large diameter holes

- **Phenomenology of Nuclear Explosions**
  - Size, shape, cost and yield
  - Nuclear radiation and hazards
  - Radioactivity production
  - Fission and fusion
  - Distribution of radioactivity
  - Fallout and groundwater
  - Contained explosions: cavity, chimney formation
  - Crating explosions: crater formation
  - Measurement of parameters: instrumentation
  - Prediction of parameters: scaling laws and hydrocodes
  - Hazards due to groundshock and airblast
**Engineering Applications**

Earthmoving Applications:
- Canals and mountain cuts
- Harbors
- Water resource development

Contained Applications:
- Aggregate production
- Petroleum reservoir stimulation
- Underground storage
- Tar sands and oil shale
- Mining

**Scientific Applications**

- Neutron physics
- Nuclear structure
- Seismology
- Meteorology
- Chemistry
- Material science

Mr. Fausto J. Munoz-Ribadeneira working on problems related to temperature on the solubility of chalcopyrite type ores.
**TABLE 3**

Flowshare Lectures Offered During Atoms in Action in Ecuador

<table>
<thead>
<tr>
<th>Lecture Title</th>
<th>Place where they were offered</th>
<th>Attendance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of Nuclear Explosives and Nuclear Explosives</td>
<td>College of Engineering University of Guayaquil</td>
<td>49</td>
</tr>
<tr>
<td>Civil Engineering Applications of Nuclear Explosives</td>
<td>College of Engineering University of Guayaquil</td>
<td>39</td>
</tr>
<tr>
<td>Flowshare, its nature and its goals</td>
<td>Association of Former Students of &quot;La Salle,&quot; Quito</td>
<td>82</td>
</tr>
<tr>
<td>Flowshare, its nature and its goals</td>
<td>Professors and students, Univ. of Manabí, at the Exhibit's Conference Room, Quito</td>
<td>32</td>
</tr>
<tr>
<td>Industrial Applications of Nuclear Explosives</td>
<td>Professors and students, Univ. of Manabí at the Exhibit's Conference Room, Quito</td>
<td>28</td>
</tr>
<tr>
<td>Application of Nuclear Explosives in Water Conservation Programs</td>
<td>Institute of Hydraulic Resources, Quito</td>
<td>42</td>
</tr>
<tr>
<td>Flowshare its Nature and its Goals</td>
<td>College of Sciences, Catholic Univ., Quito</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>392</td>
</tr>
</tbody>
</table>
ISOTOPES DEVELOPMENT

PRNC's food irradiation program (08 Program) was started during the latter part of 1965 and is supported by the US AEC Division of Isotopes Development.
Technician C. Asencio determines the weight average molecular weight of pectins by viscometry.
RADIATION PRESERVATION OF TROPICAL FOODSTUFFS

Research has continued in our base program of determining the feasibility of preserving tropical fruits by radiation. Emphasis is placed on those factors of pre-irradiation condition, radiation dose, and post-irradiation treatment which produce maximal delay of ripening and extension of shelf life. Quantification by chemical assay of changes in food quality due to irradiation treatment and post-irradiation storage has permitted evaluation of the feasibility of radiopasteurization of important varieties of banana, plantain, and mango produced in the Caribbean area. Much program emphasis is now directed to evaluation of the economic feasibility of radiation treatment of such fruits. Study of the marketing potential for mangoes in the United States has been initiated and simulated shipping studies using air and ocean shipment have begun.

Bananas of the Montecristo variety were studied intensively during the period October, 1966 to March, 1967, since in earlier work ripening patterns were highly erratic. The factor of fruit age became recognized in the course of the later work, in that in 115-day old fruit after 20 days of storage, only 28% of fruit irradiated at 40 Kr were ripe, compared with 72% ripening in controls. On the other hand, in younger fruit (90-95 days) at this storage time there is little difference in extent of ripening between irradiated and control lots. The young fruit showed no significant shelf life extension, whereas in the more mature fruit a 5 to 6 day extension was observed. Fruits--at least of the Montecristo variety--younger than about 100 days or older than 130 days do not show marked shelf life extension within normal temperatures (65-70°F). That ripening may be delayed from the 21st to the 27th storage day offers considerable promise of irradiation treatment at 40 Kr levels. In the older fruit, there is no significant loss of vitamin C with doses to 40 Kr. While the younger fruit show some loss of ascorbic acid with irradiation after 0-3 days storage, at longer storage times (18-19 days) there is no real difference between controls and irradiated fruit, nor between the two lots of bananas. The data for changes in sugar content, total acidity, and starch tend to correlate with the observed state of ripening in all fruit. Research in the radiation treatment of the banana has not been continued, despite the favorable response of this fruit, due to the apparent lack of economic feasibility for such treatment.

The plantain or "cooking banana" is an important food source in the humid tropics and large quantities are shipped regularly
to the U.S. Mature plantains (Guayabero variety) have been irradiated at 10, 20, 25, 30, 35, 40, and 50 Kr. The irradiated and control fruits were then stored at 68°F and 80% RH. At intervals of 4-6 days, fruits were taken for biochemical tests; daily observations were made for ripening, spoilage, and blackening due to irradiation. It was found that 20-30 Kr doses retard ripening rather considerably. After 19 days storage at 68°F only 25% of irradiated fruit had ripened as compared with 100% for the controls. Delay in complete ripening, i.e. shelf extension, is about 9 days. This delay, coupled with the insignificant losses of vitamin content, indicates a very favorable response of plantains to radiation treatment. That ripening delay is induced at doses as low as 10 Kr is interesting. Such a difference in ripening response in the plantain as compared with the banana, even though both belong to the same genus, poses an interesting problem of respective ripening mechanisms and specific effect of ionizing radiation.

Mangoes of the Mayaguezano, or "Native", variety have been irradiated in the mature or almost-ripe stage at levels of 50, 75, 100, 150, and 200 Kr, stored at 68°F, checked daily for ripening, and analyzed at intervals for sugars, starch, acidity, ascorbic acid, and crude carotenoids. For this variety, we have confirmed previous results, which indicate a 6-8 day shelf-life extension from 75 Kr doses. The effect of a hot water dip (120°F for 7 min., then 7 min. cooling) followed by irradiation to 50, 75, or 100 Kr doses has been studied for the Redondo variety mango. Here complete control of fruit spoilage due to anthracnose infection was obtained, a benefit not found by irradiation alone. Fruits given both heat and irradiation retained a firmer texture than those irradiated only. An initial loss of ascorbic acid (about 15%) occurred due to the heat; no significant difference between controls and irradiated fruit, with or without heat, was noted after 8 and 16 days storage.

Simulated shipping experiments have been initiated on irradiated mangoes. Mayaguezano variety fruit were sent on a 1000 mile auto trip, extending over five days. Fruits given 0, 50, 75 or 100 Kr doses, plus non-irradiated controls, were packed sixty in a crate using shredded paper as packaging material between layers of fruit. Following the travel, the fruit were stored at 68°F and analyzed chemically at intervals. The vibration and exposure to temperatures up to 84°F during travel speeded ripening in all fruits, but the radiation doses caused a considerable re-
Drs. Graham and Deshpande and Miss Asencio chemically measure methoxyl content of mango pectins following microdistillation.

Iodometric titration for characterization of polysaccharides degraded by ionizing radiation is done by technician A. Alemany.

Mr. Cuevas presents student with $350 scholarship award in agriculture at SACA's 1967 Annual Meeting. Dr. Luse, 1967 Society president, is at right.
tardation of ripening (4-6 days later than controls) and elimina-
tion of anthracnose spotting. There was no significant loss of
vitamin content in the fruits. Data obtained from shipping mangoes
by air from San Juan to Miami and back to San Juan are now being
collected.

Students in Radiobiology course measure the effect of gamma radiation on plant height.
APPENDIX
PUERTO RICO NUCLEAR CENTER
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Hada L. Rodríguez de Colón, Res. Tech. in Clinical Applications of Radioisotopes.

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Sara Jean de Jesus, Med. Res. Technician II in Virology and Tissue Culture
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Fernando Vallecillo, Associate in Health Physics
Efigenio Rivera, Associate in Health Physics
Michael Gileadi, Health Physics Assistant II
Miriam H. Vega Soderstrom, Health Physics Assistant II

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Juan Silva Parra, Research Associate III
Marine Biology

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John H. Martin, Associate Scientist I, Ph.D., U. of Rhode Island (Biological Oceanography; Zooplankton)
Stephen H. Walsh, Technical Assistant to the Director (See also Office of the Director)

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Allan G. Gordon, Research Associate II
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Raul McClint Escalera, Research Associate I, M.S., U. of Puerto Rico (Physics)

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George Drewry, Associate Scientist I, Ph.D., U. of Texas (Zoology)
Carl F. Jordan, Associate Scientist I, Ph.D., Rutgers U. (Botany)
Barbara Bannister*, Research Associate I

Radiation Chemistry

Alec Grimison, Head (See also Physical Sciences Division)
George A. Simpson, Associate Scientist I (See also Physical Sciences Division)

Schistosoma mansoni Project

Jorge M. Chiriboga, Head (See also Office of the Director)
Genoveva M. de Umpierre, Research Associate I, B.S., U. of Puerto Rico at Mayaguez (Biology)
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Felix Liard Bertin, Research Assistant II

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Victoriano Quintana, Research Assistant I

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Hot-Atom Chemistry Project
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Josefa Elisa Trabal, Research Associate I (See also Nuclear Sciences Division)
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Donald S. Sasscer, Head (See also Nuclear Engineering Division)
Fausto Muñoz Ribadeneira, Research Associate III (See also "Atoms in Action" Exhibit Project)

Radiation Preservation of Tropical Foodstuffs
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Jose Cuevas Ruíz, Research Associate I (See also Agricultural Bio-Sciences Division)
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Guillermo Torres Carmona, Building and Grounds Maintenance Supervisor

Mayagüez:

Victor Lequerique, Scientific Glassblower
Alfredo Carmona Trutten, Electronic Technician Supervisor
Nelson Quiñones, Technical Supervisor

Ad Honorem

Waldemar Adam, Ph.D.
Chemistry

Conrado Asenjo, Ph.D.
Chemistry

Gustavo Candelas Reyes, Ph.D.
Biology

Julio I. Colón, Ph.D.
Microbiology

Graciela Casanova de Candelas, Ph.D.
Biology

Antonio Ortiz, M.D.
Pediatrics

Máximo Cerame Vivas, Ph.D.
Oceanography

Luis Otero Villaderbó, Ph.D.
Biochemistry

Edwin Roig, Ph.D.
Chemistry

Américo Pomales, Ph.D.
Bacteriology

Angel Rodríguez Olleros, M.D.
Gastroenterology

Nelson Biaggi, Ph.D.
Sanitary Science
Jesús M. Tharrats, Ph.D.
Physics

Alfredo Torruella, Ph.D.
Physics

Arthur Block, Ph.D.
Chemistry

Julián Roldán Regus, M.S.
Soil Chemistry

Arturo Roque Perez, M.S.
Genetics

Jose A. del Castillo, M.D.
Pharmacology

Mario Rosa, M.D.
Internal Medicine

Lawrence S. Ritchie, Ph.D.
Parasitology; Zoology

Víctor Dávila Cintrón, B.S.
Mechanical Engineering

Jose Noel Correa, M.D.
Radiotherapy
PAPERS PRESENTED


   Adam, W. - See also Grimison, A.


   Almodóvar, I. - See Kay, M. I.

   Blanco, M. S. - See Marcial, V. A.


   Bosch, A. - See also Lanaro, A. E.; and Marcial, V. A.

   Brar, S. S. - See Kline, J. R.

   Butler, J. M. - See Knight, W. B.

   Cardona, M. - See Vázquez, F.

   Cashman, R. J. - See Muir, J. A.

   Castillo, M. - See Lanaro, A. E.

   Castrillón, J. P. A. - See Freer Calderón, J.; and Szmant, H. H.


Chiriboga, J. - See also Colón, J. I.; Knight, W. B.; Liard, F.; Martínez Silva, R.; Murilo, C.; and Oquendo, I.


Cobas, A. - See also Weisz, S. Z.


Colón, J. I. - See also Knight, W. B.; Liard, F.; Martínez Silva, R.; Murilo, C.; and Oquendo, I.


De León, E. - See Marcial, V. A.


Deshpande, S. N. - See also Luse, R. A.


Forman, R. A. - See Vázquez, F.

Foss, J. E. - See Kline, J. R.


Gomberg, H. J. - See also Koo, F. K. S.

Gonzalo, J. A. - See Ortiz, N. R.


Graham, H. D. - See also Cuevas Ruiz, J; and Deshpande, S. N.


Grimison, A. - See also Daniels, M.

Irizarry, N. - See Phelps, D. K.

Irizarry, S. - See Lanaro, A. E.; and Rodríguez Olleros, A.


Jhaveri, J. - See also Grimison, A.


Kaplan, S. F. - See Kay, M. I.


Knight, W. B. - See also Liard, F.


Liard, F. - See also Knight, W. B.

López, V. - See Martínez Silva, R.


Luciano, D. - See Phelps, D. K.


Luse, R. A. - See also Cuevas Ruiz, J.; Deshpande, S. N.; and Graham, H. D.


Marcial Rojas, R. - See Lanaro, A. E.


Marcial, V. A. - See also Bosch, A.


Martínez Silva, R. - See also Colón, J. I.; Knight, W. B.; Liard, F.; Murilo, C.; and Oquendo, I.


Mata, A. - See also Szmant, H. H.


Muir, J. A. - See also Cobas, A.


Pantoja, R. - See Wheeler, O. H.


Pellegrino, J. - See Knight, W. B.; Liard, F.; and Murilo, J.


Quintana, V. - See Walker, D. W.

Rigau, J. J. - See Szmant, H. H.

Ritchie, L. S. - See Knight, W. B.


Rivera, C. - See Colón, J. I.

Rodríguez, G. - See Grimison, A.


Román de Vega, V. - See Ting, R. Y.

Santiago, R. J. - See Phelps, D. K.

Santos, M. - See Wheeler, O. H.

Simpson, G. A. - See Grimison, A.


Szmant, H. H. - See also Freer Calderón, J.


Toro, M. - See Colón, J. I.

Trabal, J. E. - See Wheeler, O. H.


Valencia, A. - See Bosch, A.


Weisz, S. Z. - See also Cobas, A.


PUBLICATIONS


   Alexander, A. G. - See Maretzki, A.

   Almodóvar, I. - See Kay, M. I.


   Bosch, A. - See also Caldwell, W. L. (U. S. Army Trop. Res. Med. Lab.); Correa, J. N.


Cromer, D. T. - See also Kay, M. I.


Gileadi, A. E. - See also Sánchez del Río, A.

Gonzalo, J. A. - See Okada, K.

Graham, H. D. - See Luse, R. A.


Grimison, A. - See also Adam, W.; and Daniels, M.


Kaplan, S. F. - See Kay, M. I.

Kay, A. S. - See Wheeler, O. H.


Kay, M. I. - See also Cromer, D. T.


Kleinberg, R. - See also Kay, M. I.


Larson, A. C. - See Cromer, D. T.


Lowman, F. G. - See also Miró, M.


McClin, M. L. - See Wheeler, O. H.


Marcial, V. A. - See also Bosch, A.; and Correa, J. N.


Montalvo, R. - See Wheeler, O. H.


Oliver de Padovani, I. - See Miró, M.


Ramos, E. - See Miró, M.

Rigau, J. J. - See Szmant, H. H.

Ritter, H. L. - See Kay, M. I.

Rivera, J. M. - See Okada, K.

Rodríguez, G. - See Adam, W.


Román de Vega, V. - See Miró, M.


Simpson, G. A. - See Grimison, A.


Szmant, H. H. - See also Castrillón, J. P. A.


Trabal, J. E. - See Wheeler, O. H.

Valencia, A. - See Bosch, A.

Vázquez, S. - See Szmant, H. H.

Vidal de Alemañy, A. - See Walker, D. W.


Weisz, S. Z. - See Cobas, A.


**WEEKLY SEMINARS, RIO PIEDRAS**

Drs. W. L. Russell and Liane Russell, Oak Ridge National Laboratory, Recent Developments in Radiation Induced Genetic Changes, January 4.

Dr. George Simpson, Recent Advances in the Matrix Isolation of Intermediates from Gamma Irradiated Heterocyclic Compounds, January 13.

Dr. Mortimer Kay, Crystal Structures of Transition Metal Formates, January 20.

Dr. Martin Pope, Radiation and Solid State Laboratory, New York University, Electric Currents in Organic Crystals, January 27.

Dr. Owen H. Wheeler, Radiolysis of Proteins, February 3.

Dr. Howard L. Andrews, Clinical Applications of Whole Body Counters, February 13.

Dr. Ezra Khedouri, Inter-American University, San Juan Campus, On the Mechanism of Action of Glutamine synthetase, February 24.

Dr. Jean P. Changeux, Pasteur Institute, Paris, Allosteric Interactions and Their Relevance to Membrane Problems, February 28.

Dr. Adon Gordus, University of Michigan, Ann Arbor, Chemical Effects of Nuclear Transformation, March 3.

Dr. Efrain Toro Goyco, UPR School of Medicine, Substrate Specificity and Possible Mechanism of Action of Pinguinain, the Proteolytic Enzyme of the Maya Fruit, March 10.

Professor Malcolm Dole, Technological Institute, Northwestern University, The Use of Ultraviolet Spectroscopy in the Study of the Effects of Radiation on Polyethylene, March 16.

Dr. Peter Paraskevoudakis, Total Absorption X-Ray Calorimeter, March 17.
Dr. William A. Mosher, University of Delaware and American Chemical Society, 1, 3-Rearrangements, March 21.

Dr. Alfred P. Wolf, Brookhaven National Laboratory, Non-Synthetic Methods of Labeling, April 7.

Dr. Michael Byer, University of Puerto Rico, Microdistribution Analysis by Correlation Between Species Abundance, April 14.

Dr. Evelina Ortiz, University of Puerto Rico, Hormones and Mammalian Sex Differentiation, April 21.

Dr. Shrinivas H. Kamath, Myeloperoxidase, April 28.

Dr. Glen A. Russel, Iowa State University, Addition Reactions of the Methylsulfinyl Carbamion, May 5.

Dr. Heriberto Batiz, UPR Agricultural Experiment Station, Experimental and Theoretical Measurements of Geminal Spin-Spin Coupling Constants, May 12.

Dr. Mortimer Mendelshon, University of Pennsylvania, Tumor Models and Cancer Therapy, May 17.

Dr. James Muir, Preparation and Properties of a New Semiconductor, May 19.

Dr. Luis R. Otero Villaderbó, UPR School of Medicine, Structural and Functional Aspects of Mitochondria, May 26.

Dr. Aldo Lanaro, Tratamiento de Afecciones Tiroideas con Iodo-131 (Treatment of Thyroid Disorders With Iodine-131), June 2.

Dr. Thomas R. Tosteson, University of Puerto Rico, Biological Effects of Melanocyte Stimulating Hormones, June 9.

Dr. George Drewry, Hybridization and Behavioral Analysis as Taxonomic Tools, June 16.

Dr. John H. Martin, Diurnal Variations in Zooplankton Abundance, Species Composition, and Elemental Composition, June 23.

Mrs. Rosa Tirado, Exchange Reactions in Inorganic Coordination Complexes, June 3.

Dr. William H. Hamill, Radiation Laboratory, University of Notre Dame, Fundamental Ionic Process Observed by Mass Spectroscopy; Ionic Processes in Glasses Relevant to Radiation Chemistry; Ion Dynamics in Viscous Liquids: Rate of Charge Recombination, Charge Separation, Evidence for Ion Pair Correlations; Chemical Consequences of Ionic Processes in Organic Liquids: Charge Transfer, Electron Capture, Proton Transfer, Recombination; and Unsolved Problems and New Approaches With Low Energy Electron Impact and Thin Films; July 10, 11, 13, 14.


Dr. Carl F. Jordan, Recovery of Tropical Rain Forest After Gamma Irradiation, July 28.

Dr. Wilson K. Talley, University of California, Davis, Phenomenology of Underground Explosions, August 4.

Dr. James A. Cheney, University of California, Davis, Critical Path Method for Scheduling, August 11.

Dr. Ramiro Martínez Silva, Radiation Effects on *Trypanosoma cruzi*, August 18.

Dr. Eduardo Touya, "Centro de Medicina Nuclear," Uruguay, Gamagrafía de Cerebro con 99mTc (Brain Gammagraphy with 99mTc), September 1.

Dr. H. Harry Szmant, Dethionylation, September 8.

Dr. Luis Amorós, University of Puerto Rico, Synthesis and Reactions of Dibenzo-quinazolinium Salts, September 22.

Dr. Art Bloch, University of Puerto Rico, Radial Intensity Distributions of Infrared Super-radiant Sources (All about lasers!), September 29.

Dr. Owen H. Wheeler, Hot-Atom Chemistry of Aromatic Phosphorus Compounds, October 4.

Dr. Jerry Kline, Resonance Times of Fallout Radionuclides in a Tropical Rain Forest, October 6.

Dr. William Caldwell, Vanderbilt University School of Medicine, Hyperbaric Oxygen in Radiotherapy, October 13.

Dr. José Pellegrino, University of Minas Gerais, Brazil, Science Against Schistosomiasis, October 20.

Dr. Anthony H. W. Nias, Christie Hospital and the Holt Radium Institute, Effect of Continuous Irradiation on Cell Cultures, October 27.
Dr. Jesús Tharrats, University of Puerto Rico, Monte Carlo Methods, November 3.
Dr. Antonio Bosch, Results of Cancer Radiotherapy, November 10.
Dr. Robert A. Luse, Status of Radiation Preservation of Food Project at PRNC, November 17.
Dr. Angel L. Rodríguez Olleros, UPR School of Medicine, Experimental Cirrhosis, November 24.
Dr. José A. de Jesús, UPR School of Medicine, Alcohol in the Central Nervous System, December 1.
Dr. H. Harry Szmant, Advances in the Study of the Wolff-Kishner Reaction Mechanism, December 15.

WEEKLY SEMINARS, MAYAGUEZ

Dr. Julio A. Gonzalo, Radiation Effects on Ferroelectric Crystals, January 30.
Dr. Frank G. Lowman, The Application of the Specific Activity Approach to Environmental Assessment of Hazards from Radioisotopes, February 13.
Dr. Adon A. Gordus, University of Michigan, Ann Arbor, Neutron Activation Analysis in Archaeology, February 27; Chemical Effects of Nuclear Transformations, March 1.
Dr. Mortimer Kay, Crystal Structures of Transition Metal Formates, March 6.
Rev. Dr. Ignacio Cantarell, Research on Color Centers in Alkali Halides, March 13.
Dr. José A. Ferrer Monge, Aberraciones Cromosómicas Inducidas por Radiación (Chromosome Aberrations Induced by Radiation), March 20.
Mr. Antonio Macías, Indiana University, Quantum Mechanical Calculations in the H³⁻ Molecular Ion, March 21.
Dr. Alfred P. Wolf, Brookhaven National Laboratory, Non-Synthetic Methods of Labeling, April 4; Reactions of Energetic Carbon Atoms, April 5.

Dr. Donald J. Swift, Late Quartenary Glaciation and Sea Level Rise in the Bay of Fundy, Canada, April 10.

Mr. Antonio Rivera Cordero, Investigation of Puerto Rico Nuclear Center Reactor Beam Tube Explosion, April 18.

Professor Kenneth G. Soderstrom, University of Puerto Rico, Free Convection Heat Transfer to Air Through Narrow Vertical Channels, April 24.

Dr. Wilson K. Talley, University of California, Peaceful Uses of Nuclear Explosives in Outer Space, April 28.

Dr. Don T. Cromer, Los Alamos Scientific Laboratory, The Crystal Structure of K₃Cu(CN)₄ and the Determination of its Absolute Configuration, May 1.

Dr. Walter Snyder, Oak Ridge National Laboratory, Tritium Retention in Humans, May 3.

Dr. George C. Summerfield, University of Michigan, Neutron Scattering and the Dynamics of High Polymers, May 16.

Mr. Frank Kabot, Perkins Elmer Corporation, Program on Gas Chromatography, May 21.

Dr. Dan Taylor, University of Puerto Rico, Computer Control of Chemical Plants, May 29.

Dr. William H. Hamill, University of Notre Dame, Chemical Consequences of Ionizing Processes in Organic Liquids, July 12.

Dr. David Fisher, University of Miami, Potassium-Argon Dating of Oceans and Meteorites, October 10.

Dr. Kurt Bostrom, University of Miami, Exhalation from the Mantle Into Pelagic Sediments, October 11.

Dr. Knud Pedersen, Measurement of Shut-down Margin, October 16.

Dr. Florencio Vázquez, Band Structure Studies in Semiconductors Mg₂Sn, Mg₂Ge, and Mg₂Si, Through Electroreflectance Measurements, November 13.

Dr. Fred H. Pollak, Brown University, Piezoelectro Reflectance in Semiconductors, November 24.

Dr. José A. Ferrer Monge, Efectos de Radiación en Cromosomas (Effects of Radiation on Chromosomes), December 1.

Dr. Rupert A. Lee, Radiation Chemistry of Gases, December 11.
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**Total Non-U.S. Citizens**: 9 20 27 21 21 36 35 13 32 37 251
**Total U.S. Citizens**: 50 52 71 74 101 161 176 198 141 199 1223

**TOTAL STUDENTS**: 59 72 98 95 122 197 211 211 173 236 1474

* A student is counted once each Fiscal Year he is in training.  

July 1, 1967
### Programs and Courses

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### Rio Piedras Total

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### Mayaguez Totals

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### Rio Piedras

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### Mayaguez

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### UPR Totals

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### Grand Totals

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<td>Students</td>
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The United States Atomic Energy Commission

Presented to
THE PUERTO RICO NUCLEAR CENTER
in Recognition of
Ten Years of Progress

The Puerto Rico Nuclear Center through quality of research, training and leadership has attained eminence in furtherance of the peaceful uses of nuclear energy throughout the Western Hemisphere. The accomplishments of the first decade are but a preamble to the challenges in the decades ahead.

S. F. Soper
Manager, Oak Ridge Operations
United States Atomic Energy Commission

10th Anniversary plaque presented to Puerto Rico Nuclear Center by the U. S. Atomic Energy Commission. See Introduction and chapter on Office of the Director for more details.