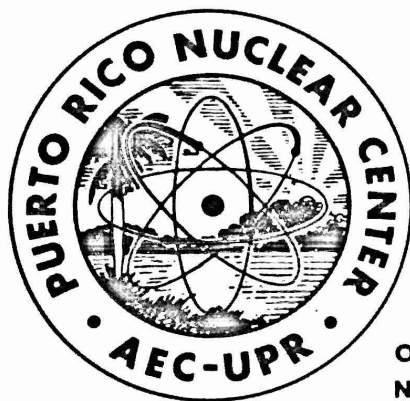


PRNC - 131

PUERTO RICO NUCLEAR CENTER

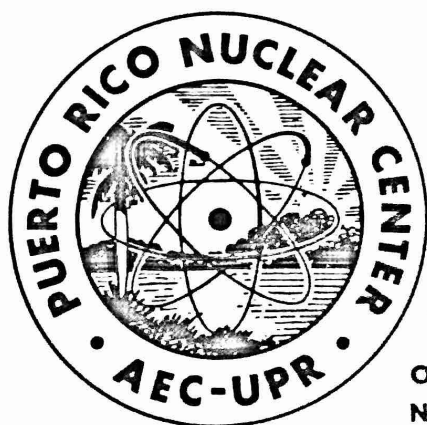
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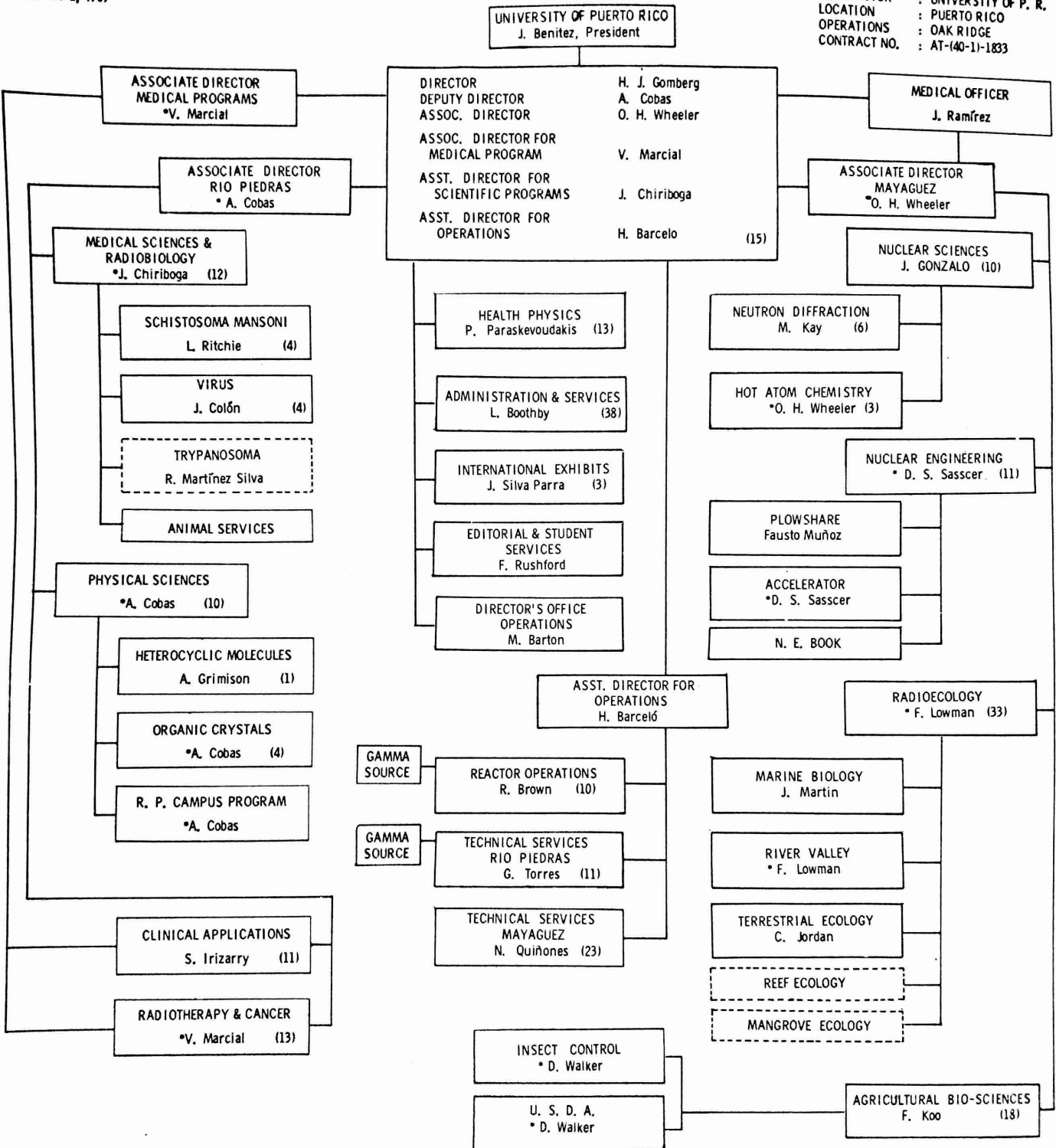
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PUERTO RICO NUCLEAR CENTER
 ORGANIZATION CHART
 JANUARY 1, 1969

TOTAL PERSONNEL: 257
 CONTRACTOR : UNIVERSITY OF P. R.
 LOCATION : PUERTO RICO
 OPERATIONS : OAK RIDGE
 CONTRACT NO. : AT-(40-1)-1833



APPROVED FOR AEC

[Signature]
 AREA MANAGER
 PUERTO RICO AREA OFFICE

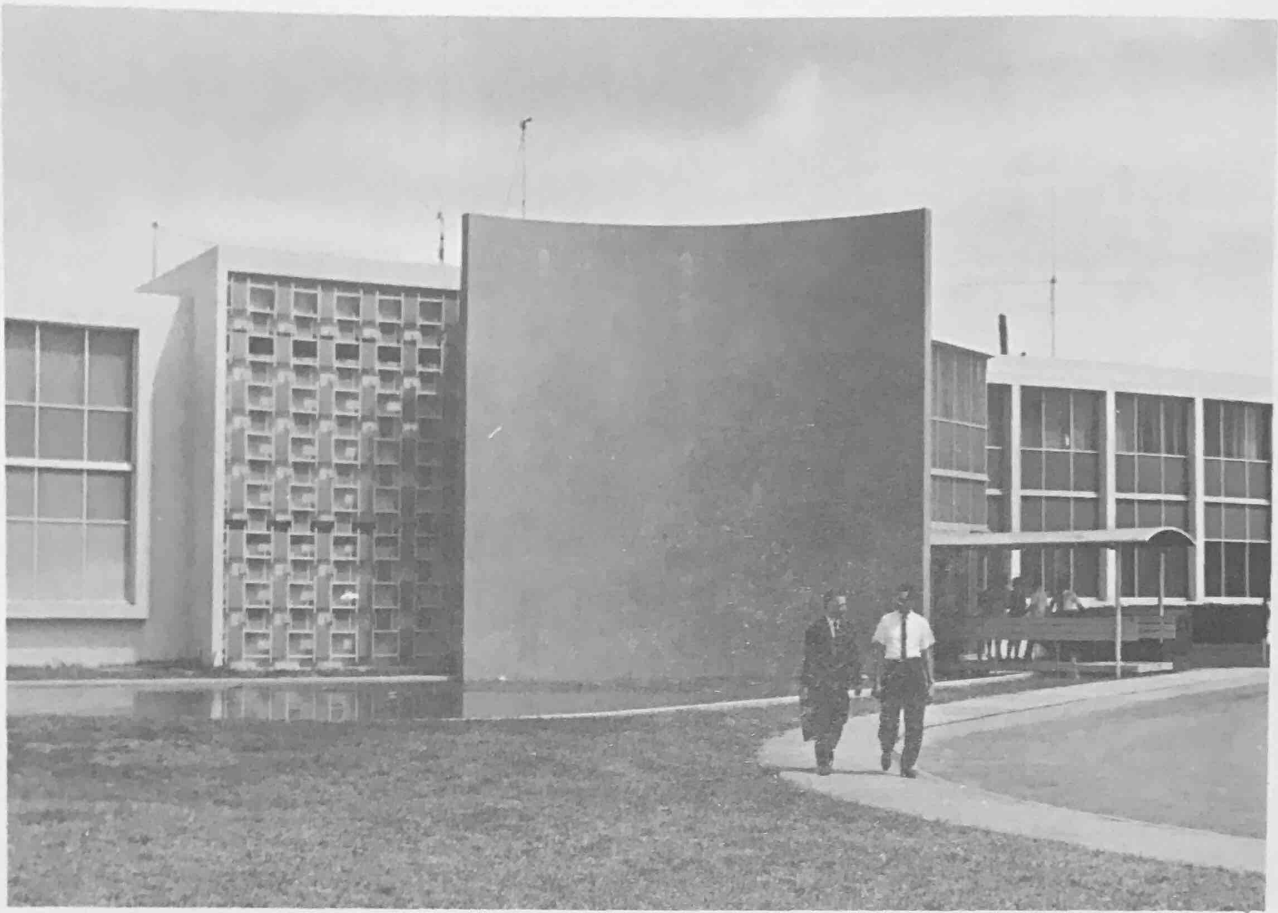
* Multiple Function
 ---- Planned Projects

APPROVED FOR CONTRACTOR

[Signature]
 DIRECTOR
 PUERTO RICO NUCLEAR CENTER

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PRNC's main installations in Río Piedras (top) and Mayaguez (bottom).

INTRODUCTION

The Puerto Rico Nuclear Center, founded in 1957, is operated under contract for the U. S. Atomic Energy Commission by the University of Puerto Rico, whose student body of 34,000 (which has doubled in each of the past three decades) makes it the island's largest university.

The Nuclear Center engages in training and research in the peaceful use of nuclear energy, with special emphasis upon the needs of Puerto Rico and Latin America. The idea for a nuclear center on this Caribbean island stemmed from President Dwight D. Eisenhower's historic "Atoms for Peace" address before the United Nations General Assembly in 1953.

Since 1957, the Nuclear Center has grown rapidly. Its first year staff of 43 has multiplied to nearly 300, including 80 scientists. PRNC's student enrollment last year was 236, four times the amount during its first year. About one-sixth of its 1,474 alumni are foreign nationals, from 18 Latin American republics as well as other nations.

The Center is small compared to major nuclear labs on the U. S. mainland, but its modern facilities are excellent.

One of PRNC's two main facilities is at the University's Mayaguez campus on the west coast. There, it has three reactors (one pool-type research reactor and two training reactors), a subcritical assembly, a 14 MeV neutron generator, neutron spectrometers, a laboratory for work with high- and low-level radioactivity, a large gamma facility, a chemistry laboratory, and separate buildings for plant sciences, nuclear engineering and marine biology. The marine biology program has a 100-ton oceanographic research vessel, fully equipped with laboratory.

The other main facility is at the new Medical Center in Río Piedras, on the outskirts of San Juan. The Bio-Medical building in Río Piedras is equipped for research in several fields. Irradiation facilities include a cobalt-60 teletherapy unit, a 300 KVP X-ray therapy unit, and a cobalt-60 irradiator. An animal house next door is stocked with colonies of mice and snails for experimental use. A solid state physics laboratory is located at the University's College of Natural Sciences in Río Piedras; terrestrial ecology laboratories are located in the Luquillo National Forest.

The Nuclear Center's academic program is closely integrated with the Master degree programs of the UPR in the physical and life sciences, agriculture and engineering. Students enroll at the University and receive academic credit through the corresponding University department. Their professors are scientists who have joint appointments at both PRNC and the University. Inroads are also being made at the doctoral level. New doctoral programs are now offered in biochemistry, microbiology and physiology; proposals for programs in physical sciences and chemistry are under study.

PRNC also gives non-credit training courses. It provides facilities for

graduate research and offers courses in the nuclear field, with students receiving credit from the university they are attending.

The Center's bilingual policy--most formal lectures are in Spanish--has encouraged enrollment by Puerto Rican students and Latin Americans. Spanish-speaking scientists come from abroad to teach, or to take advanced level courses. For example, PRNC's Physical Sciences Division--in addition to supporting the University's M.S. degree programs in chemistry, physics and biology--provides four-week basic courses in radioisotope techniques. Last year these courses were taken by--among others--a Uruguayan doctor, a Dominican engineer, and a Peruvian biochemist.

The Nuclear Center has also become known in Latin America by participating since 1965 in the US AEC's "Atoms in Action" exhibits, held twice yearly in a different South or Central American republic. The exhibits provide data on the peaceful uses of atomic energy for scientists, teachers and the general public. PRNC's scientists lecture, work on graduate thesis projects with local students, and cooperate with institutions in the country being visited. During 1967 in Ecuador, for example, research dealt with radiation preservation of agricultural products, including the banana, Ecuador's biggest money crop.

By far PRNC's biggest "growth area" in its first decade has been research, much of it aimed at solving problems germane to Puerto Rico and/or Latin America.

PRNC's marine biologists are playing a key role in determining how feasible it might be to dig a new sea-level Isthmus of Panama Canal with nuclear explosives. Its research ships spent seven months in the waters off Panama and Colombia, collecting tons of samples of water, sediment, phytoplankton, fish, crustaceans, etc. The results will be used to evaluate possible hazards caused by incorporating radionuclides into food webs leading to humans.

PRNC's terrestrial ecology specialists have radiated a small section of the Rain Forest in eastern Puerto Rico. Preliminary and follow-up studies show how radiation affects the total environment (plants, animals, insects, soil, water, mineral cycling, etc.).

The main thrust of PRNC's research in medicine and radiobiology studies how radiation affects the relationship between the host and parasite in various parasitic diseases which cripple millions of persons, particularly on the South American and African continents. Studies are being made of Schistosomiasis (also known as Bilharzia), of Trypanosomiasis (also called Chagas' Disease), and of coxsackie virus. These parasites and viruses are being observed in mice, in snails, in human cells, and other host environments.

An insect called the sugarcane borer (Diatraea saccharalis) causes losses in Puerto Rico alone of \$2 to \$3 million a year. It is also a serious pest in the U. S. and Central and South America. A PRNC project begun in 1963 hopes to eradicate the sugarcane borer by breeding adults which have been sterilized by radiation and releasing them to mate with pests in the cane fields. The Center plans to release sterile insects on a 1,000 acre tract of cane land on the nearby island of Vieques to test the success of this method.

Food irradiation is another of PRNC's interests. Many areas of South and Central America produce abundant fruits and vegetables, but poor roads slow delivery to distant markets and cause severe spillage losses. The technique of extending the shelf-life of tropical fruits by radiation, without damaging flavor or nutritional value, is now being studied. Varieties of mango, banana, and plantain (a large cooking banana which is a food staple in many areas of the West Indies and Latin America) are now being irradiated.

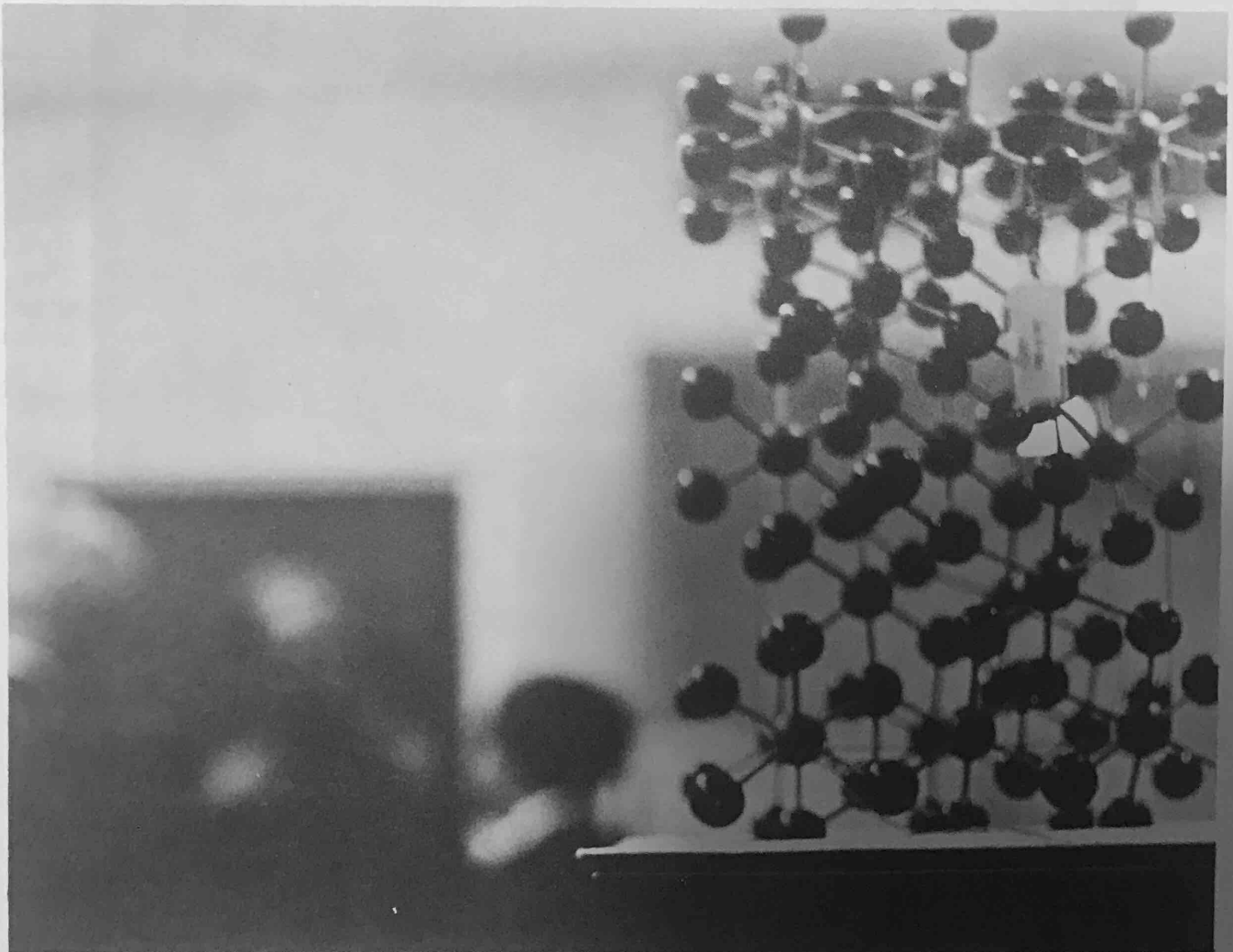
The Center has also been studying whether underground atomic blasts can be used in mining to leach copper ore from deep in the earth. If feasible, this technique reduces time and labor costs, and eliminates the pollution problems found in opencut mining.

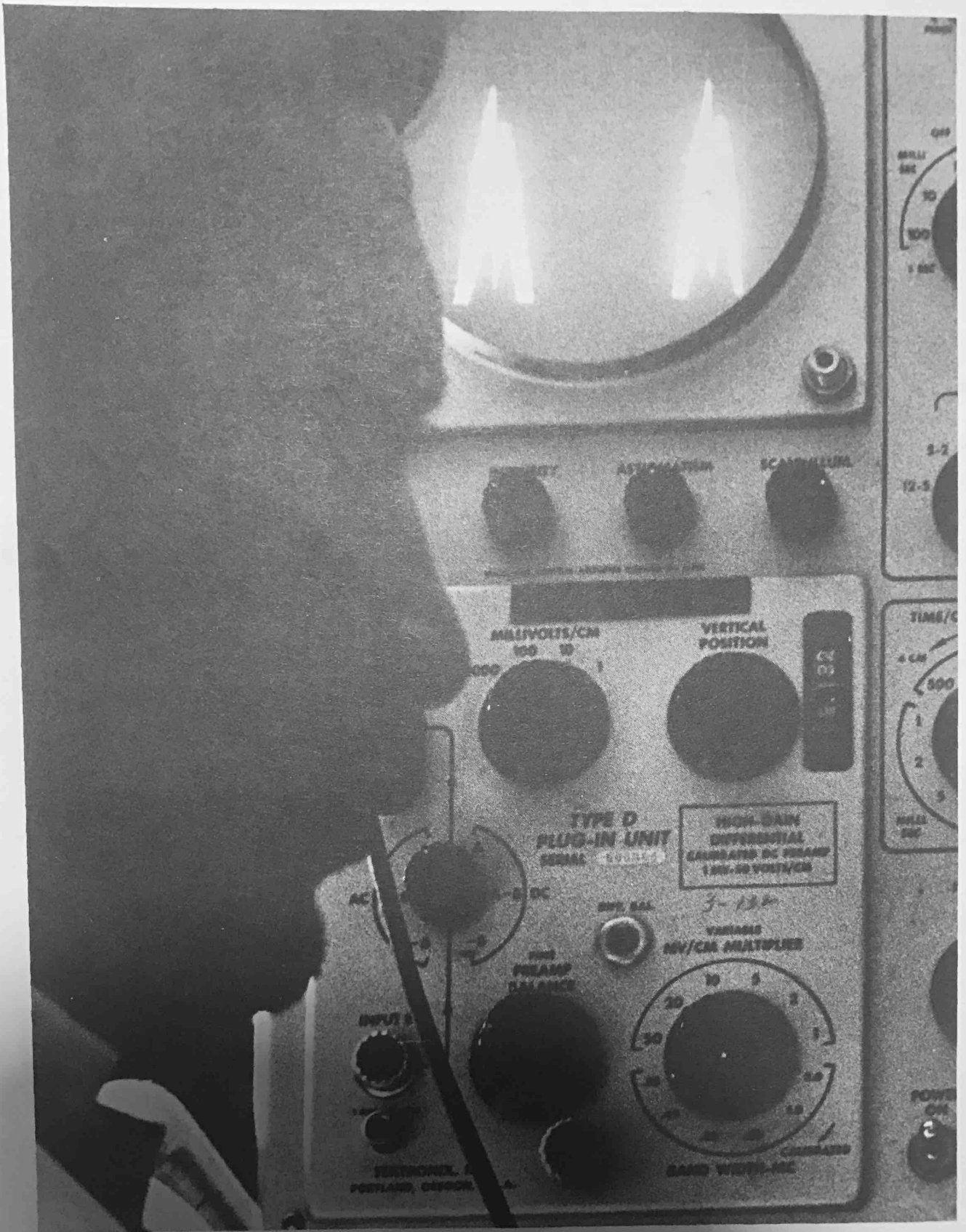
In the area of "pure" research, PRNC has programs in solid state physics, neutron diffraction, hot-atom chemistry, and radiation chemistry.

The Puerto Rico Nuclear Center has been productive in its first decade. Its alumni work in laboratories and hospitals throughout the hemisphere. Its scientific staff produced 52 research papers last year, over half the amount produced in the six-year period from 1957 through 1963.

The Center is expanding its activity in Latin America through a "sister laboratory" program with Colombia's Institute of Nuclear Affairs. This program could result in a number of far-reaching studies, including one of the vast Llanos area in eastern Colombia, where despite adequate rainfall, agricultural production is puzzlingly low. One tentative PRNC project is to study how the huge Llanos sector can someday be converted into a rich food-producing region, to help alleviate the inevitable crisis in years to come when world population multiplies over present levels.

PRNC is expanding its facilities at the Medical Center in Río Piedras. Construction of an entire new wing, costing over \$1 million, began this year, with completion scheduled for mid-1970. A new \$350,000 pulsing reactor will soon be installed at the Mayaguez facility.





Ivan Nazario observes the signal coming from a radiofrequency oscillator through a KDP crystal.

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.

The main facility incorporated this year has been a "Jeolco" Electron Paramagnetic Resonance Spectrometer. The use of this facility by people within the Division from different groups (Radiation Chemistry, Solid State) will encourage cooperative research.

EDUCATIONAL ACTIVITIES

Graduate Courses

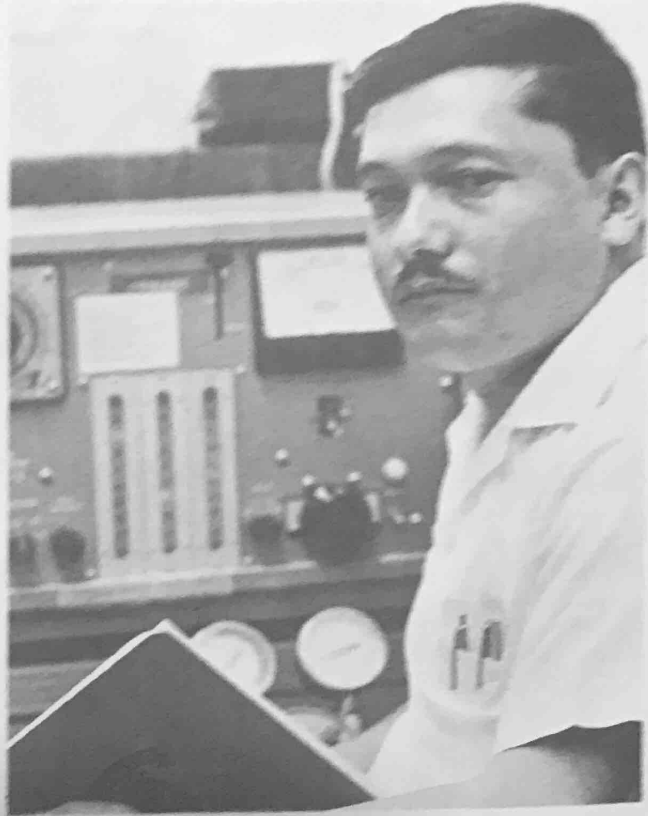
During 1968 six graduate courses were taught by PRNC personnel, with academic credit given by the UPR:

<u>Course</u>	<u>Professor</u>	<u>Enrollment</u>
Nuclear Chemistry	Dr. Owen H. Wheeler	10
Int. to Solid State Physics	Dr. Julio A. Gonzalo	4
Radiation Chemistry	Dr. Rupert A. Lee	3
Int. to Quantum Theory	Dr. Baltasar Cruz	4
Graduate Seminar	Dr. Baltasar Cruz	4
Solid State Electronics	Dr. Florencio Vázquez	8

Thesis Research

The following students from Puerto Rico, Colombia, El Salvador and the Phillipines completed thesis research under Nuclear Science Division staff supervision:

<u>Thesis Title</u>	<u>Student</u>	<u>Advisor</u>
A physico chemical study of the Chapman rearrangement.	Fernanda Román	Dr. Rosado
Effects of gamma irradiation on some components of essential oils.	Elba Díaz	Dr. O. H. Wheeler
Radiolysis of peptides.	Dolores Julián	Dr. O. H. Wheeler
Neutron activation of aromatic iodine compounds.	Carmen Lecumberry	Dr. O. H. Wheeler



- (1) Dr. Julio Gonzalo González, center, at the Nuclear Center's Mayaguez grounds, chats with two graduate students in Physics: Antonio Mock Rodríguez, of Panama, and Jenaro Coronel, Paraguay
- (2) Mr. Mock observes the oscilloscope
- (3) Another graduate student, Laureano Hijo Rojas, of Colombia, is working towards his M.S. in Physics

Dielectric properties of alkaline trihydrogen selenites.	Luis C. Niño	Dr. J. A. Gonzalo
HCl Radiolysis in a Nuclear Reactor.	Luis Rivera Oyola	Dr. R. A. Lee
W and G(H ₂) values for CH ₃ F and CHF ₃ .	José Mario Saca	Dr. R. A. Lee
F center formation at 78°K in RbBr during exposure to monochromatic X-ray energies around the bromine and rubidium K edges.	Fernando Díaz	Dr. B. Cruz
Electroreflectance in silicon and germanium crystals.	Julio Marrero	Dr. F. Vázquez

The following students from Puerto Rico, Colombia, Chile and Cuba are doing thesis research under Nuclear Science Division staff supervision:

<u>Thesis Title</u>	<u>Student</u>	<u>Advisor</u>
Synthesis of Thiosteroids.	Wilfredo Rodríguez	Dr. O. H. Wheeler
Recoil labeling of aromatic compounds with halogen.	Hilda López	Dr. O. H. Wheeler
Radiation damage in KDP and ADP single crystals.	Iván Nazario	Dr. J. A. Gonzalo
Synthesis of pyrimides labeled with radioiodine.	Ileana Casanova	Dr. O. H. Wheeler
Incorporation of radioisotopes in pharmacological compounds.	Cándida R. de Jesús	Dr. L. Feliú
Copolymerization of vinyl compounds with crotonic acid induced by gamma radiation.	Raquel Rodríguez	Dr. R. A. Lee
Mechanism radioprotection of peptides.	Gabriel Infante	Dr. O. H. Wheeler
Synthesis of Radiopharmaceuticals labeled with short half-life isotopes.	Carmen Cecilia Motta	Dr. O. H. Wheeler
Radiolysis of aqueous organic sulphur compounds	Manuel Lagunas	Dr. R. A. Lee
F center formation in potassium chloride at 78°K during exposure to monochromatic X-ray energies around the chlorine K edge.	Laureano Niño	Dr. B. Cruz
Electroreflectance on (γ -) irradiated silicon and germanium crystals.	Francisco Hernández	Dr. F. Vázquez

Specific heat anomaly in ferro-electric transitions (TGS and isomorphous compounds).

A. Mock

Dr. J. A. Gonzalo

RESEARCH COMPLETED

Radiolysis of Gaseous Hydrogen Bromide: The Effects of Pressure, Bromine, and an Applied Electric Field - R. A. Lee (PRNC) and D. A. Armstrong (University of Calgary, Alta., Canada). An ion pair yield of 4.7 for the decomposition of gaseous hydrogen bromide by ^{60}Co gamma rays has been obtained using an ionization chamber method. A $G(\text{H}_2)$ value of 9.7 can be calculated from the W value for hydrogen bromide. Studies with added bromine and sulfur hexafluoride show that about 30 per cent of the hydrogen yield can be inhibited at scavenger concentration of about 6 mole percent. The remainder of the hydrogen yield is not easily suppressed, indicating the presence of two hydrogen forming species. An electric field applied during radiolysis causes no change in the hydrogen yield in the ion-recombination region, implying that ion neutralization processes are unimportant in the formation of hydrogen.

HCl Radiolysis in a Nuclear Reactor - R. A. Lee and Luis Rivera Oyola (M.S. Nuclear Engineering, September 1968). Gaseous hydrogen chloride has been irradiated in quartz cells in a light water cooled reactor operating at 1 megawatt power. Irradiations were carried out in the pressure range 60-140 cm Hg of hydrogen chloride for times of 15 and 25 minutes. The method of dosimetry used was to compare the radiolysis of hydrogen chloride with that of nitrous oxide, irradiating one immediately after the other for the same time at approximately the same gas pressures. The Bragg-Gray theory of cavity ionization was used and corrections for the difference in stopping power of the two gases were made. A $G(\text{H}_2)$ value of 8.1 ± 0.4 has been determined. In the PRNC reactor, at a point where the neutron flux is 5×10^{10} neutrons per $\text{cm}^2\text{sec.}$, the gammas are the dominant source of energy deposition."

W and $G(\text{H}_2)$ values for CH_3F and CHF_3 - R. A. Lee and Mario Saca (M.S. Physics, October 1968). The gases methyl fluoride (CH_3F) and fluoroform (CHF_3) have both been irradiated with ^{60}Co gammas. The cell used is in the form of a parallel plate ionization chamber having its parallel faces coated with graphite. This cell with its electrical complements has been used to determine W (energy required to form an ion pair) and the ion pair yields from the decomposition of gases. The results obtained are not in complete agreement with the Bragg-Gray theory of cavity ionization chambers which predicts a constancy of the saturation ionization current per unit pressure as the pressure is varied. The irradiations were carried out in the pressure range 20-60 cm Hg at $25 \pm 2^\circ\text{C}$. By a comparison method W values of 28.3 eV and 27.6 eV were obtained for methyl fluoride and fluoroform respectively. $G(\text{H}_2)$ values of 4.0 and 1.25 were determined from the measured W values and ion pair yields for the two gases CH_3F and CHF_3 , respectively. Hydrogen formation may be due to the fact that the C-H bonds in these compounds are slightly weaker than the C-F bonds.

Thermal Hysteresis in Both Phase Transitions of $\text{NaH}_3(\text{SeO}_3)_2$ - J. A. Gonzalo and L. C. Niño (M.S. Chemistry, April 1968) Dielectric constant and hysteresis loops measurements in $\text{NaH}_3(\text{SeO}_3)_2$ show the existence of thermal hysteresis accompanying both phase transitions which appear at -79° and -180°C . The most reliable values obtained from ϵ vs. T curves give estimates of $\Delta T = 3.2^\circ\text{C}$ and $\Delta T = 20.1^\circ\text{C}$, respectively. Assuming that a double potential well along the hydrogen bonds is responsible for the dipole ordering, it is possible to correlate the thermal hysteresis with the discontinuous disappearance of spontaneous polarization and the height of the potential barrier. Estimated

energies for the potential barriers in both transitions yield values which are reasonable for a short hydrogen bond.

Set of Experimental Critical Exponents for Ferroelectric Triglycine Sulfate - J. A. Gonzalo. A series of curves of P vs E at various temperatures close to the transition temperature of triglycine sulfate have been used for determining the behavior of P , $(\partial P/\partial E)_{T=T_C}$, and $(\partial P/\partial T)_{E=0}$ with respect to field and temperature in the critical region. Log-log plots of the polarization and its derivatives versus E and T allow the direct experimental determination of six critical exponents. All of these experimental values are consistent with predictions of the mean-field theory.

Electroreflectance Measurements on Mg_2Si , Mg_2Ge , and Mg_2Sn -

F. Vázquez, Richard A. Forman and Manuel Cardona (PRNC and Brown University). The room-temperature electroreflectance spectra of the II-VI compounds Mg_2Si , Mg_2Ge , and Mg_2Sn are reported in the energy region 1.5-4.5 eV. All measurements were performed using the electrolyte technique with a nonaqueous electrolyte. These materials crystallize with the antiferroite crystal structure and are small-band-gap semiconductors. In all of the materials, a large number of sharp peaks were observed; the spectra are interpreted in terms of the reflectivity spectra and existing energy-band calculations. Special mention should be made of a doublet observed (1.64-1.84 eV) in the spectrum of Mg_2Ge . This doublet appears to be due to the spin-orbit splitting of the Γ_{15} valence band.

F center formation at 78°K in RbBr during exposure to monochromatic X-ray energies around the bromine and rubidium K edges - Baltasar Cruz and Fernando Díaz. Measurements of the relative efficiency of formation of F centers in RbBr during exposure to monochromatic X-ray photons of energy either below, between, or above the bromine and rubidium K absorption edges were completed during the summer of 1968. This work verified similar research on KBr and verified calculations on the fluorescence during irradiation of alkali halide crystals.

RESEARCH IN PROGRESS

Improved statistical theory for ferroelectric TGS - J. A. Gonzalo. The inclusion of temperature dependent tunneling effects within the H-bonds, along the line recently suggested by Blinc and Svetina for KDP leads to an improvement of the agreement between the theoretical and experimental results for the spontaneous polarization and specific heat in the whole temperature interval up to the Curie point. This tunneling effect correction is formally similar to the "bicadratic exchange correction" used in the theory of magnetic transitions.

The Antiferroelectric Phase Transition in $CsH_3(SeO_3)_2$ - J. A. Gonzalo and L. C. Niño. Careful measurements of temperature dependence of the dielectric constant perpendicular to the (100) plane have been performed on single crystal samples of $CsH_3(SeO_3)_2$. The transition takes place at -123°C. The temperature interval between measurements was 0.25°C. Our results indicate no discontinuity in the dielectric constant at the peak temperature, strongly suggesting a 2nd. order transition.

Gamma induced copolymerization of crotonic acid and vinyl compounds - R. A. Lee and Raquel Rodriguez. The copolymerization of crotonic acid with (a) vinyl acetate and (b) methyl acrylate is being carried out using ^{60}Co gammas. Since crotonic acid itself does not polymerize, it is hoped that the study of these two compounds would provide a comparison of the effect of a carbon to carbon double bond position relative to a carbonyl group in the

formation of copolymers. Thermal copolymerization using benzoyl peroxide as a catalyst has been shown to occur via a free radical mechanism. In the case of radiation induced reactions it is known that both ions and radicals are formed initially. By introducing various scavengers and studying their effects, the mechanism, whether radical or ionic in these cases, would be determined.

The γ -radiolysis of aqueous solutions of organic sulfur compounds -

R. A. Lee and Manuel Lagunas. Organic sulfur compounds have been shown to be extremely good protecting agents against radiation. Thiourea in particular is exceptional. The reason for this is unknown; it is hoped that a complete study of the radiolysis of this compound in aqueous solution along with its methyl analogues (tetramethyl thiourea) would shed some light on the subject. Aqueous solutions of these 2 compounds will be irradiated both in presence and absence of air. $G(H_2)$ will be determined and the effect of pH will be studied. By using ^{14}C labelled compounds a product analysis will be attempted to help elucidate the reaction mechanism.

Radiolysis of CHF_3 and CH_3F in the presence of scavengers - R. A. Lee.

Preliminary work on these two gases has already been carried out. W and $G(H_2)$ values were obtained. It is believed that H_2 formation is due to the reaction of the H atom with either of these two compounds, since the C-H bonds are weaker than the C-F bonds in either of these compounds. Scavenger effects (both electron and H atom scavengers) on the $G(H_2)$ yield will be undertaken, along with product analyses to determine the mechanism of the radiolytic decomposition.

Electroreflectance from Semiconductor Crystals - F. Vázquez. Electroreflectance method is being used for γ -radiation damage on germanium and silicon crystals. It is also used to continue studying the band structure properties of these two semiconductors.

F center formation in potassium chloride at $78^\circ K$ during exposure to monochromatic X-ray energies around the chlorine K edge - Baltasar Cruz and Laureano Niño. Equipment has been designed and built to irradiate KCl under vacuum during exposure to monochromatic x-ray photons of energy, either below or above the chlorine K absorption energy (2.82 eV). We are now trying to develop means to obtain relative measurements of radiation beams in the energy range from 2 to 4 keV.

Equipment has been assembled and tested to investigate the energy of the fluorescence during irradiation of alkali halides as a function of sample temperature, purity, and incident photon energy. The thermal luminescence of alkali halides will also be studied. Basic information is sought on the reactions involving vacancy and interstitial defects in alkali halides, and the effect of these reactions on present theories on the mechanism of the production of defects. Through the study of the thermal luminescence of crystals it is hoped to improve present radiation dosimeters or develop new ones, particularly for photon energies from 5 or 6 eV through 4 or 5 keV.

STAFF

Mr. Luis Rivera Oyola left PRNC to begin graduate studies toward a Ph.D. at Pennsylvania State University.

Mr. José Mario Saca, after completion of his M.S. in Physics, has again joined the Physics Department, University of El Salvador.

Two "ad honorem" appointments have been extended to the following members of the Faculty of Arts & Sciences, U.P.R., Mayaguez. Dr. Luis Feliú, (Chemistry Department) will conduct work on labeling of organic compounds. Dr. Werner Fialla (Physics Department) will work on the design of a high intensity monochromator for reactor thermal neutrons.

Dr. A. Macías, from the Chemistry Department, who held an "ad honorem" appointment with our division, left P.R. to occupy a senior research position with the Quantum Chemistry group of the University of Ottawa, Canada.

The following graduate students from the U.P.R. and Institute of Modern Sciences, Mayaguez have joined our research staff as research assistants:

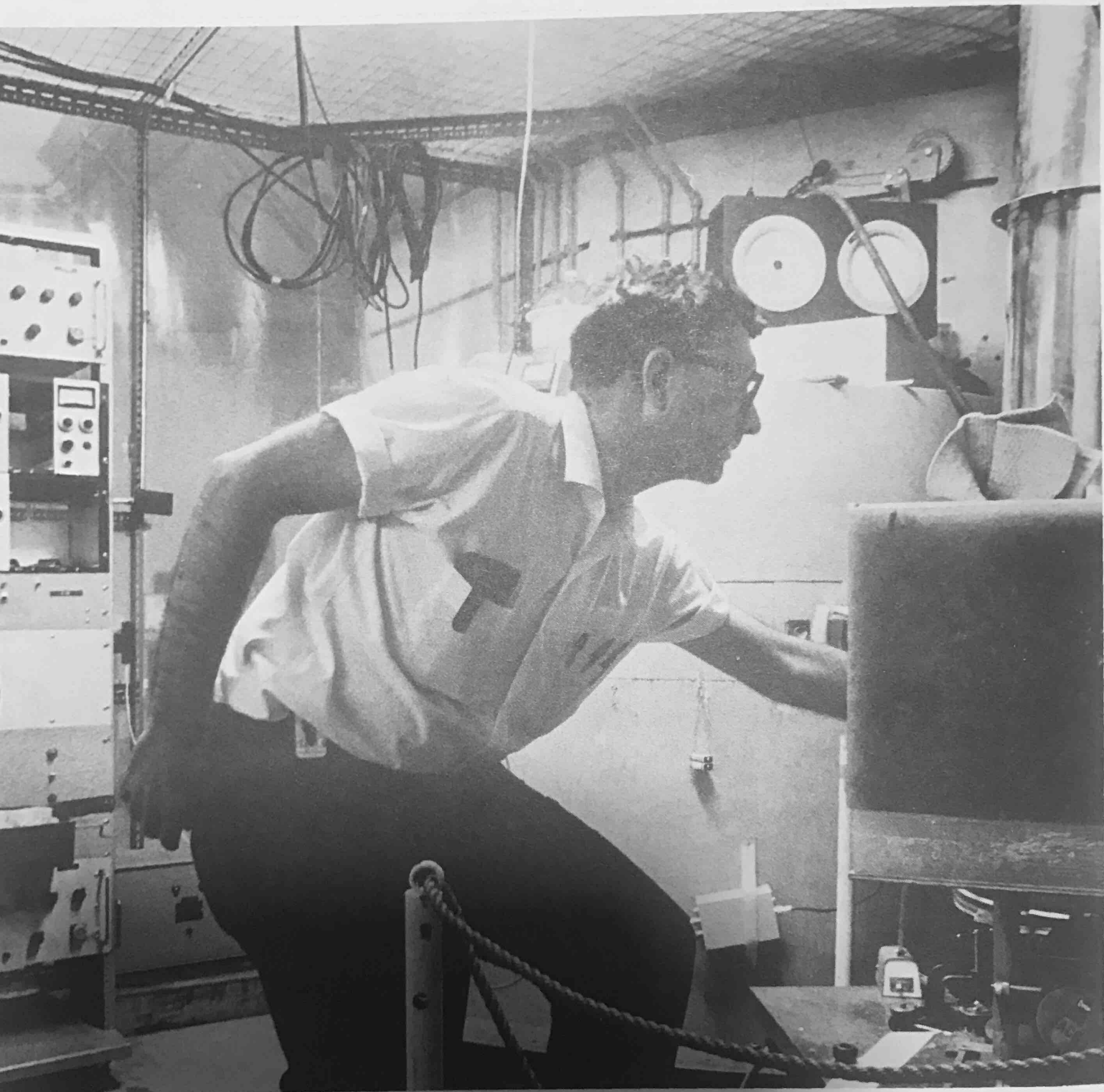
Raquel Rodríguez	(Chemistry)	Puerto Rico
Faustino Rivera	(Elec. Engineering)	" "
Iván Nazario	(Physics)	" "
Manuel Lagunas	(Chemistry)	Chile
Nelson Peña	(Chemistry)	Dominican Republic

Supported by I.A.E.A. of Vienna is also:

Carmen Cecilia Motta	Colombia
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Ivan Nazario does temperature dependent capacitance measurements using the following equipment: R.F. oscillator, capacitance bridge, potentiometer, and oscilloscope



Dr. Kay adjusts neutron spectrometer with liquid helium Dewar for maintaining crystals at very low temperature while measuring their neutron scattering

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

Several problems have been completed in the past year. An attempt will be made to briefly describe the results and give some idea of the importance of the investigation.

I. Phenanthrene. Damask and Arndt have reported that phenanthrene 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, or perhaps to hydrogen atom motion, since no change in x-ray pattern was noted on heating phenanthrene through its transition.

We have not yet done any work on the high temperature phase. The room temperature phase, however, has been analyzed.

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, Y. Okaya, then at IBM laboratories, took new x-ray data to obtain more accurate carbon position. Both x-ray and neutron data confirm the earlier results that the molecule is non-planar. Figure 1 gives the bond distances averaged over x-ray and neutron results; Figure 2 gives the angles; Figure 3 the distances of each atom from the average plane of the six central carbon atoms. Figure 1 shows the 2.04 \AA H (4) - H (5) distance. This distance, determined from neutron diffraction data, is well under the 2.4 \AA Van der Waals distance. Because these two hydrogen atoms are so close, the molecule is forced to bend to try to relieve the repulsive stresses.

It may be seen from Figure 3 that the top ring is bent down and the bottom ring up with respect to the central ring, although the nature of the twisting seems to be somewhat different in the two rings. Each six membered ring is close to planar.

We note that the carbon atoms determined from x-ray data are an average of 0.006 Å closer to the center of mass of the molecule than are the carbon positions determined from neutron data. Such systematic differences between data sets may be due to systematic experimental errors, but may also be consistent with differences between the position of the nucleus of the atom, determined by neutron diffraction data, and the average position of the electron density of an atom, determined from x-ray data.

Bond distances have been corrected for rigid body libration of the carbon atoms. The rigid body motions were determined by the Schomaker-Trueblood (1968) Acta Cryst. B24 63 program. The carbon atoms fit a rigid body far better than did the hydrogens. The derived rigid body motion was subtracted at the hydrogen positions. The remainders of 25 of 30 diagonal tensor terms were positive, showing that the hydrogen vibrates on the phenanthrene carbon ring. The average uncorrected C-H distance was 1.076 Å after rigid body correction 1.082 Å.

The C(12) - C(13) distance is 1.46 Å as opposed to 1.43 Å derived from molecular orbital calculations. Coulson and Haigh (1963) Tetrahedron 19, 527 calculate that there should be an increase of about .02 Å due to the deformations. They did not, however, expect the out of plane bending, proving either crystal forces, or higher order potential terms are important or the H-H potential is harder than assumed. No clear-cut effects of over crowding show up in the H(4) or H(5) thermal parameters. Figure 2 shows that the angles in the "ring" formed by atoms C(4), H(4), H(5), C(5), C(13), C(12) are somewhat larger than normal, probably due to the H-H repulsion.

II. Thermal Vibrations of the Sulfate Group in Sodium Alum, NaAlSO₄·12H₂O.

The alum structures were reported in PRNC-94. On page 9 of that report, we reported that three of the sulfate oxygens seemed to undergo a translational motion down the three-fold axis which is correlated with a rotation about the three-fold axis to avoid a near hydrogen neighbor. That is, the O₁-S 3(O₂) (sulfate) group is on a three-fold rotation axis which passes through O₁ and S. The three O₂ atoms are at the apices of an equilateral triangle. The spiral or translation-rotation motion described above consists of a rotation about S-O₁ combined with a translation parallel to the axis.

PRNC-115 describes in detail the method of including such motion in the least squares refinement of structural parameters by means of a numerical integration of the scattering function and its derivatives. The usual method of treating small torsional oscillations is to assume linear harmonic motion and, from the amplitudes of such motion, try to guess the rotations that give rise to them. PRNC-115 on the other hand, describes a numerical method of directly putting the non-linear terms into the scattering expression which should be extendable to a wide variety of problems.

At the conclusion of the above analysis it was discovered that on top of the spiral motion described above, there was torsional or rocking motion. The numerical integration method could have been extended to include such a motion, however, by using some approximations. Kay and Behrendt (1963) Acta Cryst. 16 157 derived a function that describes torsional oscillations. This expression was used together with numerical derivatives in the least squares analysis of the x-ray data.

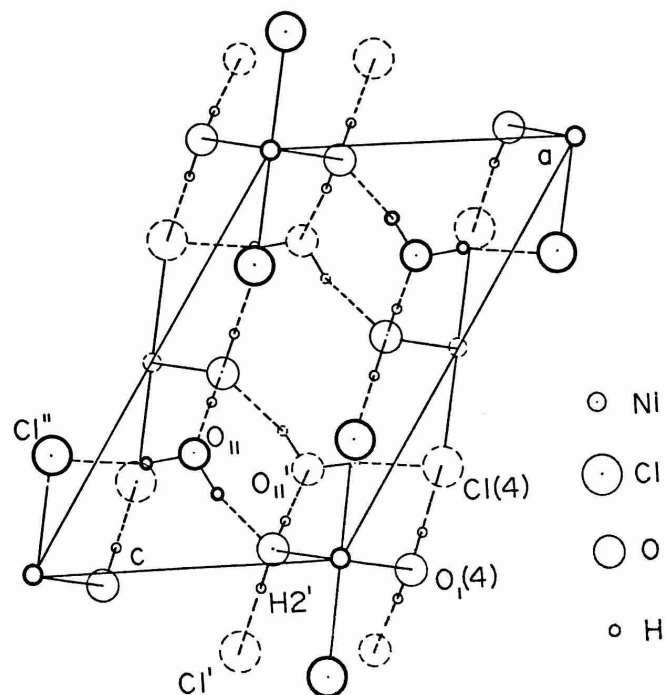
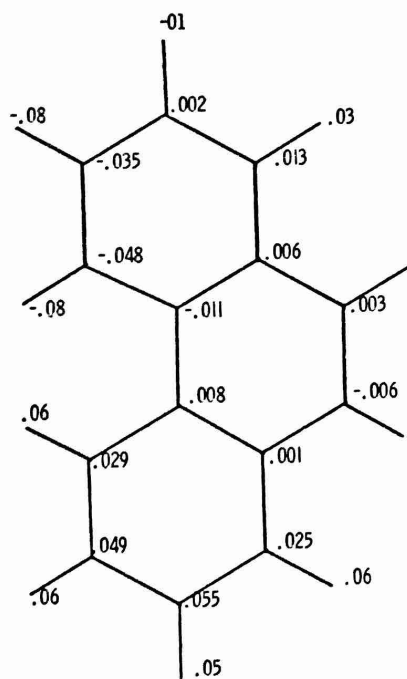
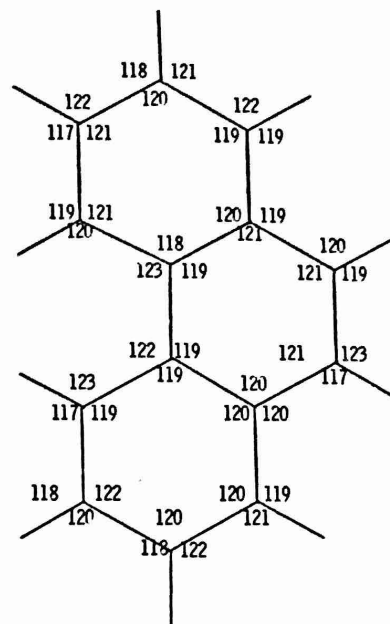
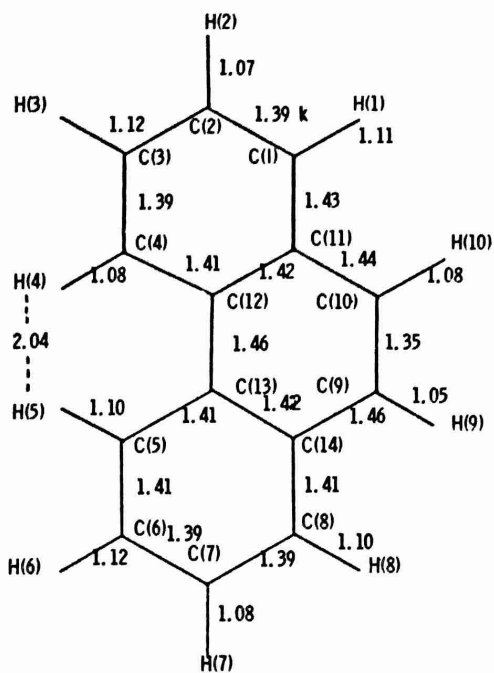


Fig. 1. Phenanthrene: Numbering system used to name atoms and bond distances averaged over x-ray and neutron results

Fig. 2. Phenanthrene: Bond angles

Fig. 3. Distances from least squares plane of central ring in Angstrom units

Fig. 4. A representation of the crystal structure of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ viewed along the b axis. Heavy lined and dashed circles represent atoms in the mirror planes $Y = 0$, and $Y = 1/2$ respectively. Thin lined circles represent atoms at $0 < Y < 1/2$. Hydrogen bonds are represented by dashed lines

The final results state that the sulfate group undergoes a spiral oscillation with a root mean square amplitude of 0.169 radians at a slope of 59° from the three-fold axis. The torsional oscillation is .278 radians.

One artefact of using an incorrect linear vibrational function is that bond distances are artificially shortened and must be corrected. The functions used in the current work give correct distances (to the validity of the vibrational model) directly. The S-O₁ and S-O₂ distance increased from 1.461 Å and 1.459 Å to 1.479 Å and 1.495 Å, respectively, in the present work.

III. A Relationship between Incoherent Neutron Scattering and Nuclear Magnetism. In NMR and nuclear spin scattering experiments, an external perturbation is applied to a nuclear spin system, causing nuclear spin transitions in the system. In the NMR experiment these transitions are caused by the interaction of the nuclear spins with an applied radiofrequency field, while in the latter they are caused by the interaction with the neutron spins. We have derived the relation between the functions describing both types of experiments.

Using the established connection between generalized susceptibilities and fluctuations of dynamical variables, we find for a system composed of identical particles, the relationship between the spin dependent, or so-called incoherent scattering law and the susceptibility arising from the nuclear magnetism to be,

$$\begin{aligned} \text{where,} \quad S_s(\underline{K}, \omega) &= 2\hbar v \mu^{-2} (e^{\hbar\omega\beta} - 1)^{-1} \chi'(\underline{K}, \omega), \\ \text{and,} \quad \chi'(\underline{K}, \omega) &= \text{Im}\{\text{Tr} \underline{\chi}(\underline{K}, \omega)\}, \\ S_s(\underline{K}, \omega) &= \sum_{\ell, \ell'} \int_{-\infty}^{\infty} dt e^{i\omega t} \langle e^{i\underline{K} \cdot \underline{r}_\ell} \underline{J}_\ell \cdot \underline{J}_{\ell'}(t) e^{-i\underline{K} \cdot \underline{r}_{\ell'}(t)} \rangle_T. \end{aligned}$$

Thus, "incoherent" neutron scattering and experiments on nuclear magnetism measure essentially the same function over different ranges of the same function over different ranges of the variables \underline{K} and ω .

IV. The Crystal Structure of NiCl₂·6H₂O at Room Temperature and at 4.2°K by Neutron Diffraction. In recent nuclear magnetic resonance experiments in the magnetic fields at the proton sites in antiferromagnetic NiCl₂·6H₂O have been measured.¹ Subsequent attempts to calculate these local fields on the basis of a simple dipole model, and thereby explain the results of the NMR experiment, were unsuccessful. Since these calculations required the use of several assumptions about the magnetic and crystallographic properties of the material, it was decided to experimentally determine the magnetic structure and spin direction in the antiferromagnetic state, as well as the crystal structure in the paramagnetic and antiferromagnetic states by means of neutron diffraction experiments before attempting another extensive calculation of the local fields. The magnetic properties have already been discussed in a previous report,² while this work is concerned with crystallographic properties at room temperature and at 4.2°K.

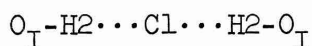
The heavy atom structure at room temperature has been determined by Mizuno using x-rays.³ The unit cell is monoclinic, with space group C2/m, and contains two formula units per unit cell. Cell dimensions determined by x-rays are a = 10.23, b = 7.05, c = 6.57 Å, with $\beta = 122^\circ 10'$. The nickel ions are

situated on $2/m$ inversion centers of the ab faces. They are all octahedrally coordinated to four oxygen and two chlorine atoms. The oxygens form a slightly distorted square, with the nickel at the center, while the chlorines are located on the two normals to the oxygen plane. The remaining two water molecules of the formula unit are located in the mirror plane and are relatively free, but do take part in the hydrogen bonding scheme.

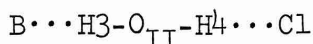
Unit cell parameters were determined by neutrons and found to be $a = 10.24$, $b = 7.04$, $c = 6.58$ Å, and $\beta = 122^\circ 14'$, at room temperature, and $a = 10.20$, $c = 6.50$ Å, and $\beta = 122^\circ 32'$ at 4.2°K .

Isotropic least-squares refinement of the intensity data gave the positional and isotropic thermal parameters given in Table I. The positions of the atoms measured at 4.2°K are in good agreement with the room temperature results, indicating that the crystallographic structure of the antiferromagnetic state at 4.2°K is the same as in the paramagnetic state at room temperature. A projection along the b axis is given in Fig. 4, wherein most of the atomic symbols are defined, and the hydrogen bonding scheme is indicated. Some bond lengths and angles calculated from the two sets of parameters are given in Table II. Values calculated from the x-ray determination are also given.

The crystal structure is determined by hydrogen bridges of the type



which link the $\text{NiCl}_2 \cdot 4\text{H}_2\text{O}$ octahedra into a face centered two dimensional network parallel to the ab plane, and bridges of the type



which bond together the neighboring two dimensional networks. Hydrogen atoms from each O_I water molecule enter into two hydrogen bridges which are essentially "parallel", while those from each O_{II} water molecule enter into bonds which are essentially "collinear" in their effect on the crystal structure. Thus, there are only half as many structural bridges along the (001) as along other directions, and this results in the perfect cleavage parallel to the ab plane.

In the hydrogen bonding system, $H3$ has a bifurcated type bond with O_I and its mirror image $O_I(2)$, but it is interesting to note that $H3$ appears to be hydrogen bonded to the point B , the midpoint between O_I and $O_I(2)$. The distances for $H3 \cdots B$, and $O_{II}-H3 \cdots B$ of 1.77 and 2.70 Å, are essentially the same as the corresponding distances of 1.80 and 2.72 Å, associated with $O_I-H1 \cdots O_{II}'$. Curiously enough, if the hydrogen bond is essentially electrostatic, having effective point charge q on each oxygen atom, the O_I and $O_I(2)$ may be replaced by an imaginary charge of $1.5q$ at the point B . This would seem to imply that the bifurcated bond is not necessarily a weak one. The apparent exceptional length of the $O_{II}-H4$ bond is due to overlap in projection between Cl and $H4$ atoms in adjacent mirror planes. Other bonds and angles are in agreement with previously reported results.

Proton-proton vectors for $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in the paramagnetic state have been measured by El Saffar, using the NMR method.⁴ Since this salt is isomorphous to $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, with respect to the heavy atoms, it was of interest to compare his results to those calculated from the hydrogen parameters presented above. Therefore, p-p vectors determined from the NMR and neutron diffraction experiments were compared and agreement was found to be quite good. Thus, within experimental error, it was found that these salts are probably also isomorphous with respect to the hydrogen atoms.

TABLE II

Interatomic distances and bond angles in $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
at room temperature, and at 4.2°K

Ni-Cl	2.359 (7) ^a 2.366 (4)	2.375 ^b	O _I -O _I (2)	2.86 (1) 2.936	2.92 ^b
Ni-O _I	2.05 (1) 2.091	2.094	Cl-O _I	3.10 (1) 3.133	3.146
Ni-O _{II}	3.90 (1) 3.853 (5)	3.932	Cl-O _{II} '	3.73 (1) 3.706	3.731
O _I -O _I (4)	2.93 (1) 2.979 (5)	3.006	Cl-Cl(4)	4.06 (1)	4.053
O _{II} -H ₃	.96 (4) .95 (1)		O _I -H ₁	.94 (2) .97	
O _{II} -H ⁴	1.07 (3) .97 (1)		O _I -H ₂ '	.94 (2) .95	
H ₃ -H ⁴	1.63 (4) 1.54 (2)		H ₁ -H ₂ '	1.48 (2) 1.55	
O _{II} -B	2.70 (1) 2.639 (5)	2.703	O _I -O _{II} '	2.74 (1) 2.67	2.715
O _{II} -Cl''	3.17 (2) 3.109 (7)	3.126	O _I -Cl'	3.21 (1) 3.14	3.176
B-H ₃	1.77 (2) 1.700 (8)		O _{II} '-H ₁	1.80 (2) 1.71	
Cl''-H ⁴	2.11 (3) 2.16 (1)		Cl'-H ₂ '	2.30 (2) 2.22	
O _I -H ₃	2.27 (2)		O _I -O _{II}	3.06 (1)	3.072
H ₃ -O _{II} -H ⁴	107° (2) 107 (1)		H ₁ -O _I -H ₂	104° (2) 108	
O _{II} -H ₃ -B	165 (1) 168 (1)		O _I -H ₁ -O _{II} '	174 (2) 172	
O _{II} -H ⁴ -Cl''	168 (1) 166 (1)		O _I -H ₂ '-Cl'	164 (2) 161	
B-O _{II} -Cl''	124.7 (4) 124 (1)	124.4	O _{II} '-O _I -Cl'	89.5 (4) 91	90.7

^a Upper number in each number set refers to room temperature, lower number to 4.2°K bond or angle

^b Bond lengths and angles in these columns were calculated from x-ray data

- ¹ R.D. Spence, P. Middents, Z.M. El Saffar, and R. Kleinberg, J. Appl. Phys. Suppl. 35, 854 (1964)
- ² R. Kleinberg, J. Appl. Phys. 38, 1453 (1967)
- ³ J. Mizuno, J. Phys. Soc. Japan 16, 1574 (1961)
- ⁴ Z. M. El Saffar, J. Phys. Soc. Japan 17, 1334 (1962)

TABLE I

Comparison of parameters in NiCl₂·6H₂O determined from: anisotropic least-squares refinement of room temperature x-ray data; isotropic refinement of room temperature neutron data; and isotropic refinement of 4.2°K neutron data

	x	y	z	B
Ni	0	0	0	1.8 (2) .7 (1)
Cl	.2708 (2) ^a .2691 (8) .2720 (5)	0	.1674 (3) .167 .1700 (7)	1.5 (1) .8 (1)
O _I	.0307 (4) .0296 (7) .0320 (3)	.2072 (7) .203 (2) .210 ^c	.2503 (6) .244 (1) .2525 (5)	2.7 (2) 1.0 (1)
O _{II}	.2825 (7) .281 (1) .2823 (6)	0	.7023 (11) .696 (2) .700 (1)	2.3 (2) .9 (1)
H ₁	.100 (1) ^b .107 (1)	.299 (3) .308	.269 (2) .280 (1)	3.5 (3) 2.1 (1)
H ₂	.444 (1) .441 (1)	.232 (4) .228	.225 (2) .227 (1)	3.4 (3) 2.3 (1)
H ₃	.181 (4) .184 (1)	0 0	.549 (5) .549 (2)	5.9 (7) 2.0 (2)
H ₄	.260 (2) .261 (2)	0 0	.839 (4) .828 (2)	1.2 (4) 1.7 (2)

^a In each heavy atom set of numbers the upper, middle, and lower number subsets correspond to positions determined by: x-rays at room temperature; neutrons at room temperature; and neutrons at 4.2°K, respectively

^b In each hydrogen atom set of numbers, the upper and lower subsets correspond to positions determined by neutrons at room temperature and 4.2°K, respectively

^c At 4.2°K, the y atomic parameters for the water molecule in the general position were selected to give reasonable molecular and hydrogen bonds

HOT ATOM CHEMISTRY

The studies being carried out in the field of hot-atom chemistry involve the investigation of the products formed when an atom covalently bound to carbon undergoes nuclear recoil. The recoiling nuclei have included the transition metals and heavy metals, as well as non-metallic atoms. The carbon compounds employed have been phenyl derivatives, metallocenes and metal carbonyls. The purpose of these studies is to determine the mechanism of high energy reactions in organic compounds through a study of the products formed under different activation conditions. The possibility of directly preparing labeled compounds and of obtaining radioisotopes of high specific activity by recoil methods is also being investigated.

The equipment available for handling unstable compounds includes vacuum systems and glove boxes. The experimental techniques used for separating the radioactive products include various methods of chromatography and electrophoresis.

WORK IN PROGRESS

Metallocenes - Solid solutions of nickelocene in cyclopentadienylthallium and in ferrocene, or solutions in indene, afforded an increased activity in the radioactive organic Ni^{III} fraction. There was no increase, however, in the organic Ni^{II} activity, indicating that recombination results in the formation of organic Ni^{III} products.

Metal Carbonyls - The retention of 2.56 hour ⁶⁵Ni in nickel carbonyl is being studied by rapid vacuum distillation of the products, as a means of obtaining ⁶⁵Ni in high specific activity.

Carbon-14 - The recoil labeled products from glycine are being separated by chromatography on ion exchange columns, and on Sephadex and silica gel.

WORK COMPLETED

Metallocenes - Bicyclopentadienylosmium (osmocene) on activation afforded 80% of the activity in the organic fraction of which about 10% was "retention." The inorganic Os activity was largely Os⁺⁴. Bicyclopentadienylrhenium hydride gave only 4% activity in the organic fraction and 4% "retention." The inorganic activity consisted of Re⁺³, Re⁺⁴ and Re⁺⁷.

Molybdenum-99 - ⁹⁹Mo has been prepared as a source of ^{99m}Tc by activating K₄Mo(CN)₈·2H₂O. The unchanged salt was precipitated with ethanol from an aqueous solution, leaving the ⁹⁹Mo activity in solution.

Silanes - The ³¹Si activities formed in the activation of tetraphenylsilane, triphenylsilane and diphenylsilane were separated by chromatography on alumina from benzene, after washing out the silicic acid activity with sodium

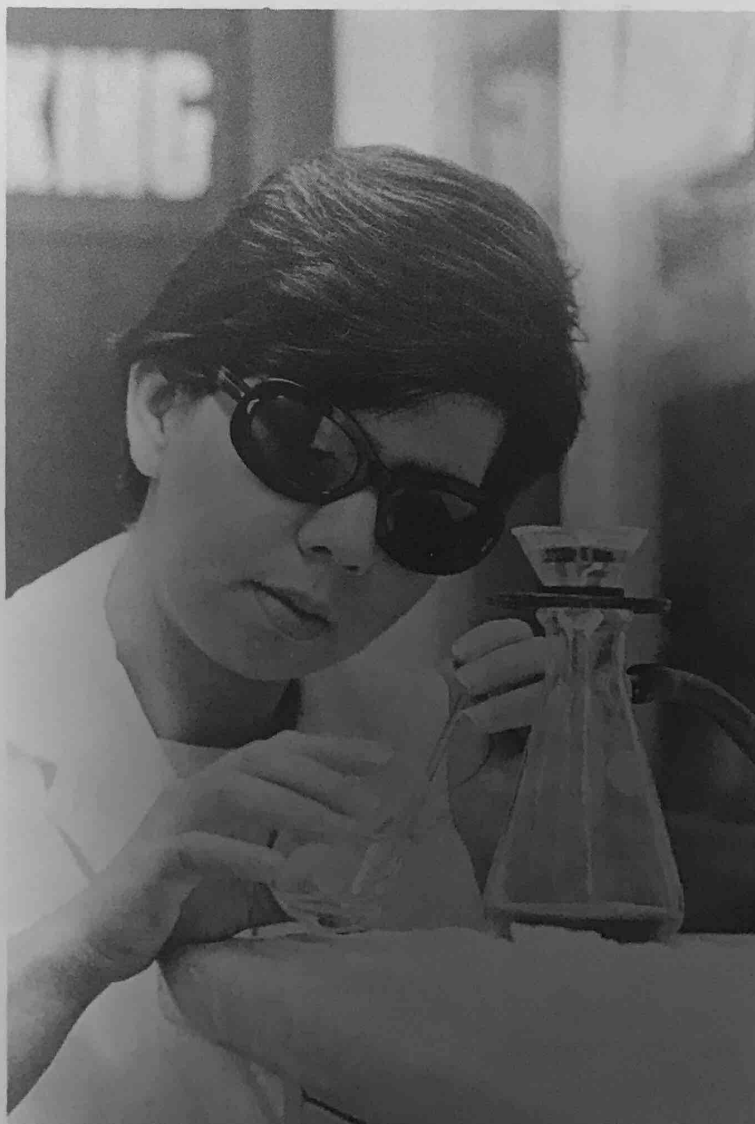
hydroxide. The principal activity formed from tetraphenylsilane was triphenylsilane, whereas triphenylsilane gave equal amounts of labeled tetraphenyl and triphenyl-silane. Diphenylsilane showed activities in all three fractions. The retention (9%) of activity in tetraphenylsilane increased (to 17%) when the compound was activated in benzene solution, but decreased (to 7.5%) when hexafluorocyclobutane was added to the benzene. This indicates that the tetraphenylsilane was partially formed through the reaction of radicals, which were scavenged by hexafluorocyclobutane.

Selenium and Tellurium - Diphenyl selenide, selenoxide and selenone were activated and the ^{81m}Se activities separated by paper chromatography and electrophoresis. The inorganic activity from diphenyl selenide was exclusively selenite, whereas the selenoxide gave largely selenate and the selenone formed both labelled selenite and selenate. The "retention" in diphenyl selenide, selenoxide and selenone was 2.0, 12.9 and 7.1%, respectively. Diphenyl tellurium gave 11.8% "retention."

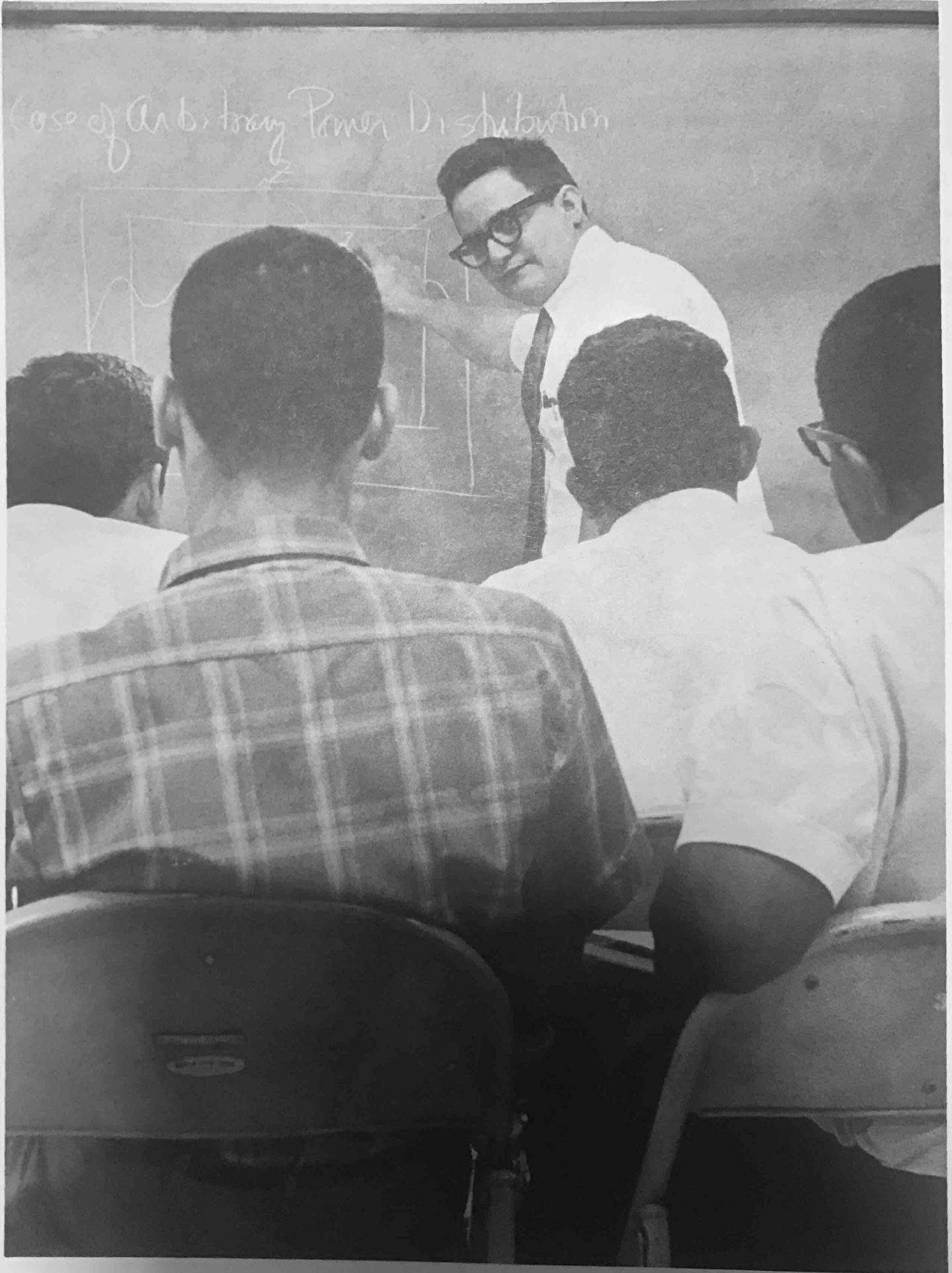
STAFF

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 completed the requirements of an M.S. degree in August, 1968 and is now teaching in the Chemistry Department, U.P.R., Mayaguez.

Dr. D. A. Wiles of the Department of Chemistry, Carlton University, Ottawa, visited the laboratories to consult on the project.



Miss Angela Vallejos from the Universidad Nacional de Asunción, Paraguay, is engaged in Hot Atom Chemistry studies at PRNC Mayaguez. She is working on activation analysis of native Paraguayan woods.



Dr. Heriberto Plaza teaches Nuclear Engineering to UPR students at PRNC's Mayaguez facility.

NUCLEAR ENGINEERING

The Nuclear Engineering Division teaches graduate courses at UPR, Mayaguez and conducts research in nuclear engineering. The staff also directs thesis research of nuclear engineering students from UPR and from other universities in the United States and Latin America. In addition, the Division offers short courses for scientists, engineers, and technicians, and for staff members engaged in individual research.

EDUCATIONAL ACTIVITIES

Master of Science Degree Program

UPR at Mayaguez, 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 UPR Department of Nuclear Engineering is composed largely of staff members of the PRNC Nuclear Engineering Division; the director of the UPR department is head of the PRNC division as well. The Division also provides the classrooms, offices, laboratories, equipment, and administrative personnel necessary for the education and training of UPR nuclear engineering students. The Masters degree in Nuclear Engineering requires 30 hours of graduate work and the satisfactory completion of a thesis. A bachelor's degree in engineering is a prerequisite.

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 L-77 low power reactor, and the PRNC one-megawatt reactor. The student is encouraged to use both an analog and a digital computer and to present a seminar on his research to the PRNC staff. Students are guided 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 system; 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, and gamma ray spectrometry.

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. Consist 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 Wavemechanics 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 Wavemechanics II. Two lecture discussions per week. A continuation of the same topics covered in the "Wavemechanics I" course, including perturbation and control rod theory and the effect of an isotropic scattering.

Special Problems. One to three periods per week each semester related to the investigation of special problems in Nuclear Engineering.

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.

New Courses

Peaceful Use of Nuclear Explosives. For graduate or advanced undergraduate civil engineers and nuclear engineers. Three lectures per week in the basic engineering concepts of nuclear explosives, nuclear physics as applied to health and safety, structural mechanics and the application of nuclear explosives to large-scale engineering projects.

During the Spring of 1968 a special problems course, Nuclear Civil Engineering, was offered jointly by the Civil and the Nuclear Engineering Departments. The course was taught by Dr. Luis Mora Faria of the Civil Engineering Department and Dr. Knud Pedersen of the Nuclear Engineering Department.

Although practical, peaceful applications of nuclear explosives have not yet been achieved, a large amount of research is being conducted in this field, and the results indicate that an introduction of this knowledge to civil and nuclear engineering students is advantageous.

Non-Degree Program

In cooperation with the Reactor Division, a special five month course in Reactor Physics was offered for PRNC reactor operators in order to review and upgrade their knowledge in this area.

The academic aspects of the program were presented by the Division of Nuclear Engineering. The applied aspects were obtained by on-the-job training in the Reactor Division.

STUDENTS

During 1968, one student from Mexico, one from Nationalist China, and ten students from Puerto Rico participated in the M.S. Degree Program and a total of 18 students (five from foreign countries) in fields other than Nuclear Engineering took semester length courses taught by the staff of the Division of Nuclear Engineering. One graduate student of the Physics Department conducted her research in the Nuclear Engineering Division under the supervision of a PRNC staff member.

The 12 students in the Masters degree program are listed by name, country of origin, and sponsor in Table 1.

Six students had papers accepted for presentation at the Seventh Annual Student Conference of the American Nuclear Society at the University of Florida (April 4-7):

1. Gilberto Vélez Delgado, "Measurement of Temperature Distribution in the Water Poll of PRNC Reactor"
2. Cho Fu lee, "Depletion Calculations on Several Reactor Models."
3. Fernando López Carrasco, "Determination of the Transfer Function and Certain Neutron Kinetics Parameters of the L-77 Reactor."
4. Fernando Pérez Bracetti, "Use of a Gamma Ray Threshold Detector in Reactor Control."
5. Antonio Rivera Cordero, "Investigation of Gas Produced by the Nuclear Irradiation of Barytes Concrete Containing a Boron Additive."
6. Luis Rivera Oyola, "Radiolysis of Hydrogen Chloride in a Nuclear Reactor."

Mr. Lee and Mr. López Carrasco were unable to attend the conference and present their papers since both had finished their research and returned to their home countries before April, 1968.

Six students obtained the M.S. degree in Nuclear Engineering from UPR, Mayaguez during 1968. The list of students and their date of graduation is found in Table 2.

The progress of students active in the Nuclear Engineering degree programs is shown in Table 3.

Mr. Lee, since returning to China, has been working in the Design and Planning Section of the Taiwan Power Company.

Mr. López is now in a reactor supervisory position with the "Comisión Nacional de Energía Nuclear" of Mexico.

TABLE 1

Students enrolled in Master of Science Degree Program

<u>Name</u>	<u>Country of Origin</u>	<u>Sponsor</u>
Cho-fu Lee	Nationalist China	IAEA
Fernando López Carrasco	Mexico	CNEN
Fernando Pérez Bracetti	Puerto Rico	Self
Antonio Rivera Cordero	Puerto Rico	AEC
Luis Rivera Oyola	Puerto Rico	AEC
Rafael L. Ufret Acevedo	Puerto Rico	AEC
Gilberto Vélez Delgado	Puerto Rico	PRWRA
Francisco Rodríguez Perazza	Puerto Rico	UPR
Rafael Alcalá Quesada	Puerto Rico	AEC
Braulio Mejía Avilés	Puerto Rico	AEC
José Castro Montalvo	Puerto Rico	AEC
Antonio Castro Rosario	Puerto Rico	Self

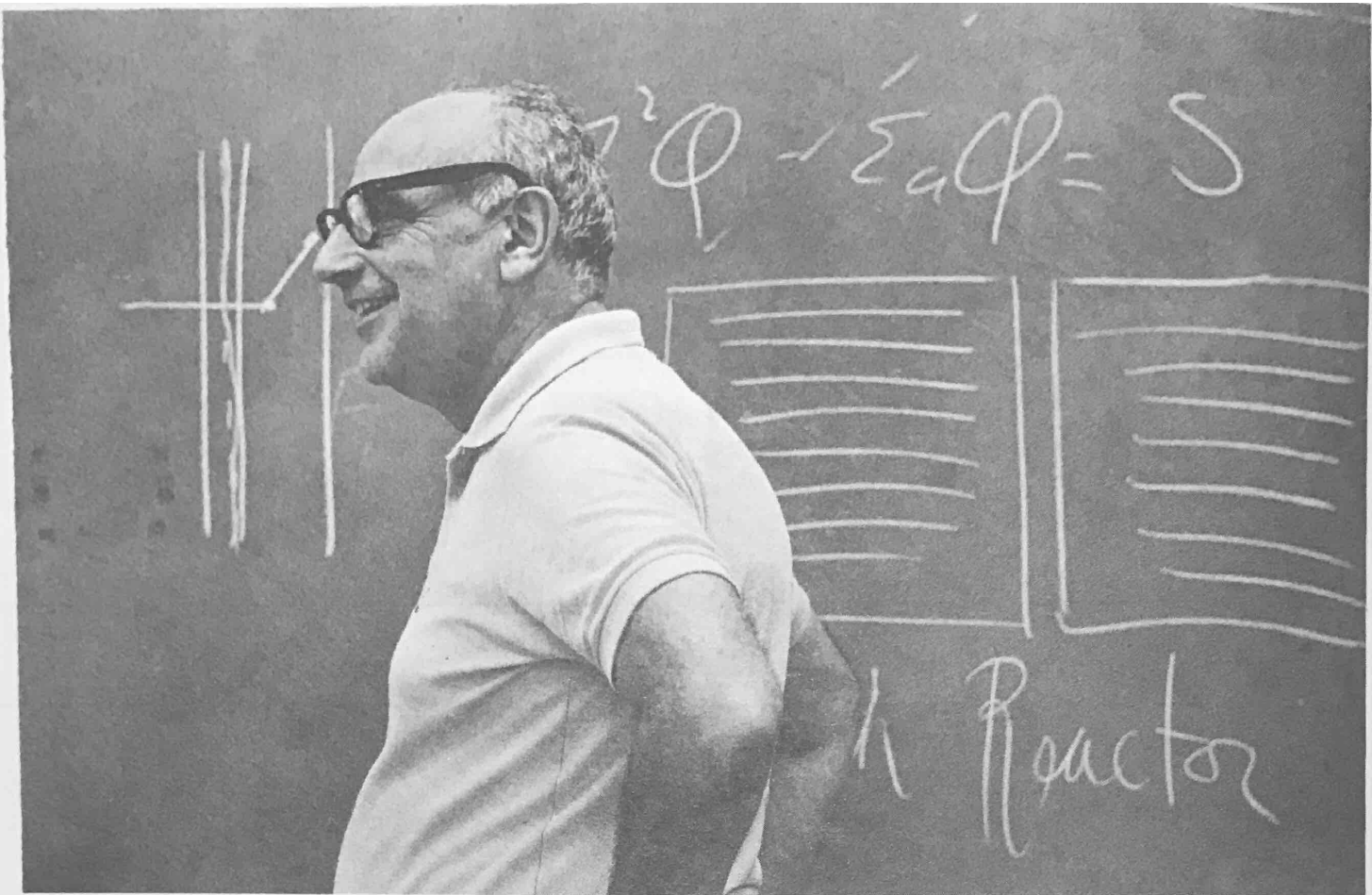
TABLE 3

Progress of Nuclear Engineering Students in 1968

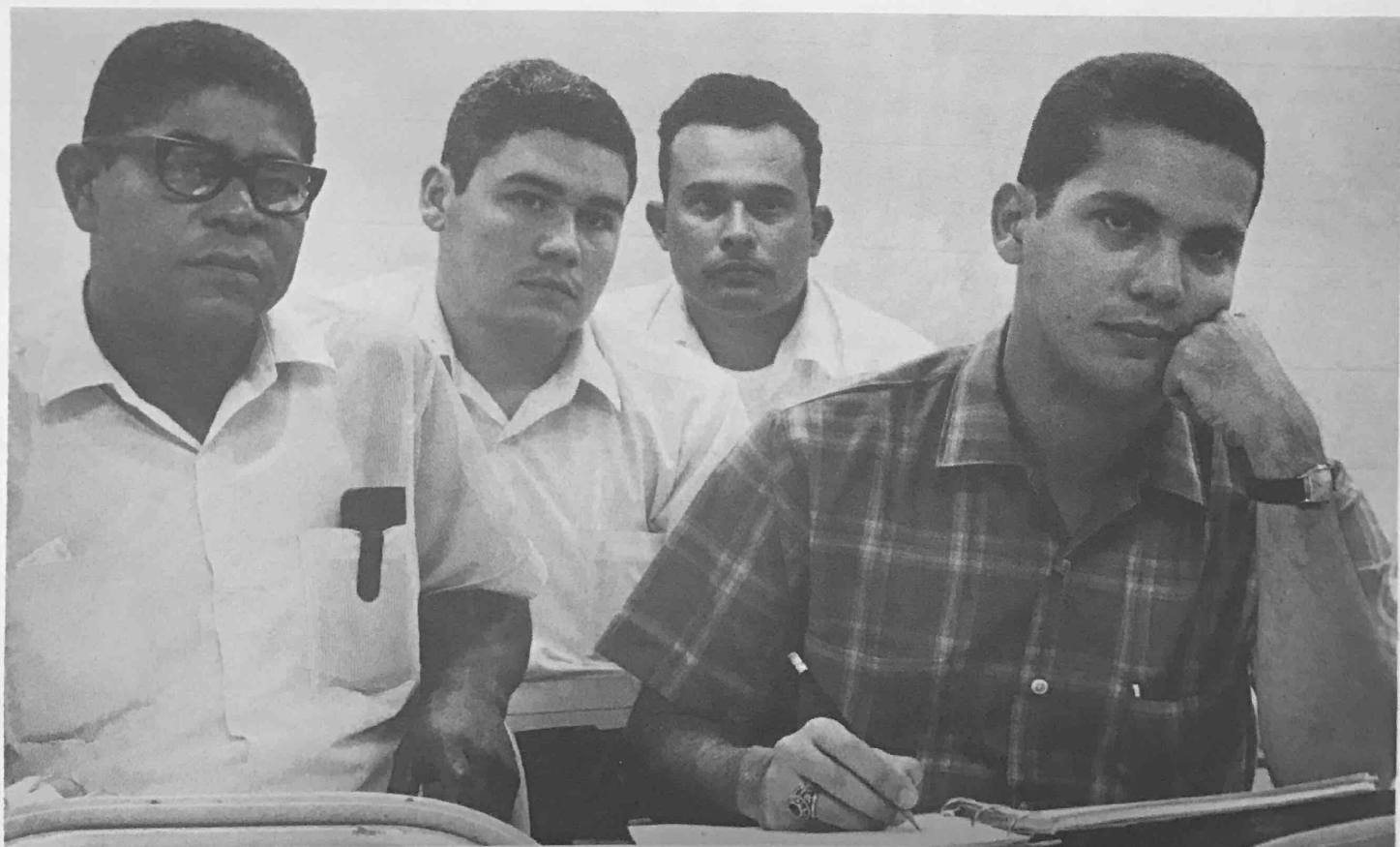
Students who received the M.S. degree in Nuclear Engineering during 1968	6
Students who have completed all course work for degree (presently working on theses)	1
Students engaged in course work for M.S. degree in Nuclear Engineering	5

TABLE 2
Master of Science Degrees in Nuclear Engineering 1968

Student	Thesis	Research Director	Graduation Date
Lee, Cho-fu	"A Study of Computational Methods Used to Determine Fuel Depletion in Nuclear Reactors"	Dr. Gileadi, Aviva E.	May, 1968
López Carrasco, Fernando	"Determination of the Transfer Function and Certain Kinetics Parameters of the L-77 Reactor"	Dr. Gileadi, Aviva E.	May, 1968
Pérez Bracetti, Fernando	"Use of a Gamma Ray Threshold Detector in Reactor Control"	Dr. Ortiz, Eddie	May, 1968
Rivera Cordero, Antonio	"Investigation of Gas Produced by the Nuclear Irradiation of Barytes Concrete Containing a Boron Additive"	Dr. Sasscer, Donald S.	Dec. 1968
Rivera Oyola, Luis	"Radiolysis of Hydrogen Chloride in a Nuclear Reactor"	Dr. Lee, Rupert	Sept. 1968
Vélez Delgado, Gilberto	"Measurements of Temperature Distribution in the Water Pool of Puerto Rico Nuclear Center Reactor"	Mr. Barceló, Héctor	May, 1968



- (1) Dr. Jack Chernick of Brookhaven National Laboratory joined the Nuclear Engineering Division for seven months starting in Nov. 1968 as a visiting scientist, during which time he gave lectures to students and seminars to PRNC staff
- (2) a group of UPR Nuclear Engineering students at PRNC's Mayaguez facility



RESEARCH PROJECTS

The research projects of the division, in process or completed during last year are as follows:

1. Determination of Reactor Transfer Functions and Certain Neutron Kinetic Parameters (A. E. Gileadi, F. López Carrasco). The value of β/λ was determined from the transfer function of the L-77 reactor by the use of a pile oscillator. Project completed in May 1968.

2. Population Models for Suppression of Sugarcane Borer (D. Walker, K. Pedersen). Two population models for the suppression of the sugarcane borer Diatraea Saccharalis Fab. (Crambidae, Lepidoptera) were investigated to determine if the release of irradiated adults would manifest sterility in the F₁ and subsequent generations. Project completed in December 1968.

3. Determination of Certain Neutron Kinetic Parameters by Means of Stochastic Methods (A. E. Gileadi). The applicability of stochastic methods including Rossi- α , variance to mean auto correlation to determine reactor transfer functions and certain neutron kinetic parameters, is studied, using a fast response time analyzer. The obtained data are processed on the IBM 360/40 computer. In progress.

4. Fuel Burn Up Studies (A. E. Gileadi, Cho-fu Lee). A computer code was written and used to determine the burn up in a water moderated reactor fueled with a mixture of U²³⁵/U²³⁸. To date this code has been used for two reactor models. Completed in May 1968.

5. Gas Evolution of Borated Concrete in a Neutron Environment (D. S. Sasscer, A. Rivera Cordero). The rate of gas produced as a function of the boron content in heavy concrete is determined by placing a sample of concrete in the pool of the PRNC reactor and monitoring the amount of gas produced as a function of nvt. In process.

6. Activation Analysis in Water Pollution Studies (K. B. Pedersen). Determination of aluminum content by activation analysis has been used to measure the pollution of Mayaguez Bay. In process.

7. Calculation of Time and Space Dependent Neutron Densities Following a Point Burst in an Infinite Medium (A.E. Gileadi, M. Rodríguez Perazza). The time and space dependent contaminant concentration due to a three dimensional block-shaped instantaneous source diffusing within an infinite medium is being computed. In process.

8. Initial Testing Program of the PRNC-TRIGA Reactor (A. E. Gileadi). The PRNC-TRIGA reactor will be the first reactor of its type in operation. A testing program is, therefore, being designed to determine the pertinent characteristics of the reactor. In process.

9. Measurement of Fluorescent Radiation in Various Substances Induced by Radioisotope Gamma Ray Sources (E. Ortiz, K. Pagan de Ramirez). Gamma rays from a ⁵⁷Co source fall on a radiator, exciting its characteristic X-ray spectrum. The X-rays are detected by a proportional chamber and the electric pulses from the chamber are analyzed by a Multichannel Analyzer. In process.

10. Vertical Biological Transport in the Ocean (A. E. Gileadi). A mathematical model of the vertical biological transport in the ocean is being devised to aid the work of the Marine Biology Division of PRNC. In process.

11. Applied Nuclear Power Engineering for Practicing Engineers (K. Pedersen). Studies are being conducted with Dr. A. E. Gileadi and Dr. D. S. Sasscer for material to be used in a book to be published by the firm of Barnes and Noble to aid the engineering understanding of nuclear power plants. In process.

12. Escape Peaks From a Proportional Chamber (E. Ortíz). When the energy E_r of the incident radiation is larger than the activation energy, $E(K)$, of the gas in a chamber, a spurious line appears in the spectrum. A study of the spurious lines is being made. In progress.

13. Effects of the Temperature and Time of Heating on the Leaching of a Copper Chalcopyrite Type Ore in Sulfuric Acid Solutions (F. J. Muñoz). A copper ore identified by X-ray diffraction techniques as chalcopyrite ($CuFeS_2$) was heated to different temperatures for various times. The heated samples showed an increased leachability of copper from samples heated up to $350^\circ C$ as well as sharp reduction in its solubility from ore samples heated at higher temperatures. Completed September 1968.

14. Energy Deposition in Shock Wave in Media (Fausto J. Muñoz-Ribadeneira). The aim is to calculate the energy deposited by the shock wave in media and derive the relationships of this energy, with the possible induced vibration of atoms in the crystal of chalcopyrite or on its molecular rearrangement, or with the activation energy required for the desulfurization of this copper ore. In progress.

15. Technological Studies on the Leaching of Chalcopyrite (F. J. Muñoz). Studies have been initiated as to its possible technological importance, using different concentrations of sulfuric acid, sulfuric acid plus oxidants, and other leaching agents, to the long-term increasing solubility of chalcopyrite when it has been heated up to $350^\circ C$. In progress.

16. Development of Prediction Equations for Cratering from Models (K. B. Pedersen). By using distorted model theory in conjunction with properly designed models a method is proposed whereby it is possible to predict many properties of craters whose variables fall outside the ranges of direct test experience. Completed October 1968.

17. Computation of Time and Space Dependent Contaminant Concentration Due to a Three Dimensional Block Shaped Instantaneous Source Diffusing within an Infinite Medium (open see) by A. E. Gileadi. Prepared at the request of Drs. F. Lowman and S. Barnes for the American Institute of Biological Sciences held in Columbus, Ohio. September 4, 1968.

STAFF ACTIVITIES

Dr. Jack Chernick, Head of the Theoretical Reactor Physics Division of Brookhaven National Laboratory, joined the division as a Visiting Scientist in November. Dr. Chernick is advising the division in its research projects, giving lectures to nuclear engineering students and seminars to PRNC staff. In addition, he is using his stay with PRNC to complete a book on reactor physics. He will be at PRNC until May 1969.

Dr. Aviva E. Gileadi was a Guest Research Collaborator with the Nuclear Engineering Division of the Brookhaven National Laboratory from June 10 to July 20, 1968.

Dr. Knud B. Pedersen attended a short course (May 7-10, 1968) to familiarize nuclear engineering educators with the facilities of ORNL.

Dr. Eddie Ortiz attended The Small Accelerators for Teaching and Research Conference (April 8-11, 1968) at Oak Ridge, Tennessee, held to acquaint participants with educational uses of a small accelerator (less than 0.5 Mev).

Dr. Ortiz was a participant at the Institute in Small Accelerators (July 16-Aug 23, 1968) at Oak Ridge, Tennessee. The purpose was to acquaint the participants with educational and research uses of various types of accelerators in the energy range from 150 Kev - 5 Mev.

Dr. Ortiz, Dr. Gileadi and Dr. Sasscer attended the Ninth Annual AUA-ANL, Nuclear Engineering Education Conference held at the Argonne National Laboratory (March 25-26)

Dr. Pedersen and Dr. Sasscer attended the joint meeting of the ANS-CNS held in Toronto, Canada in June.

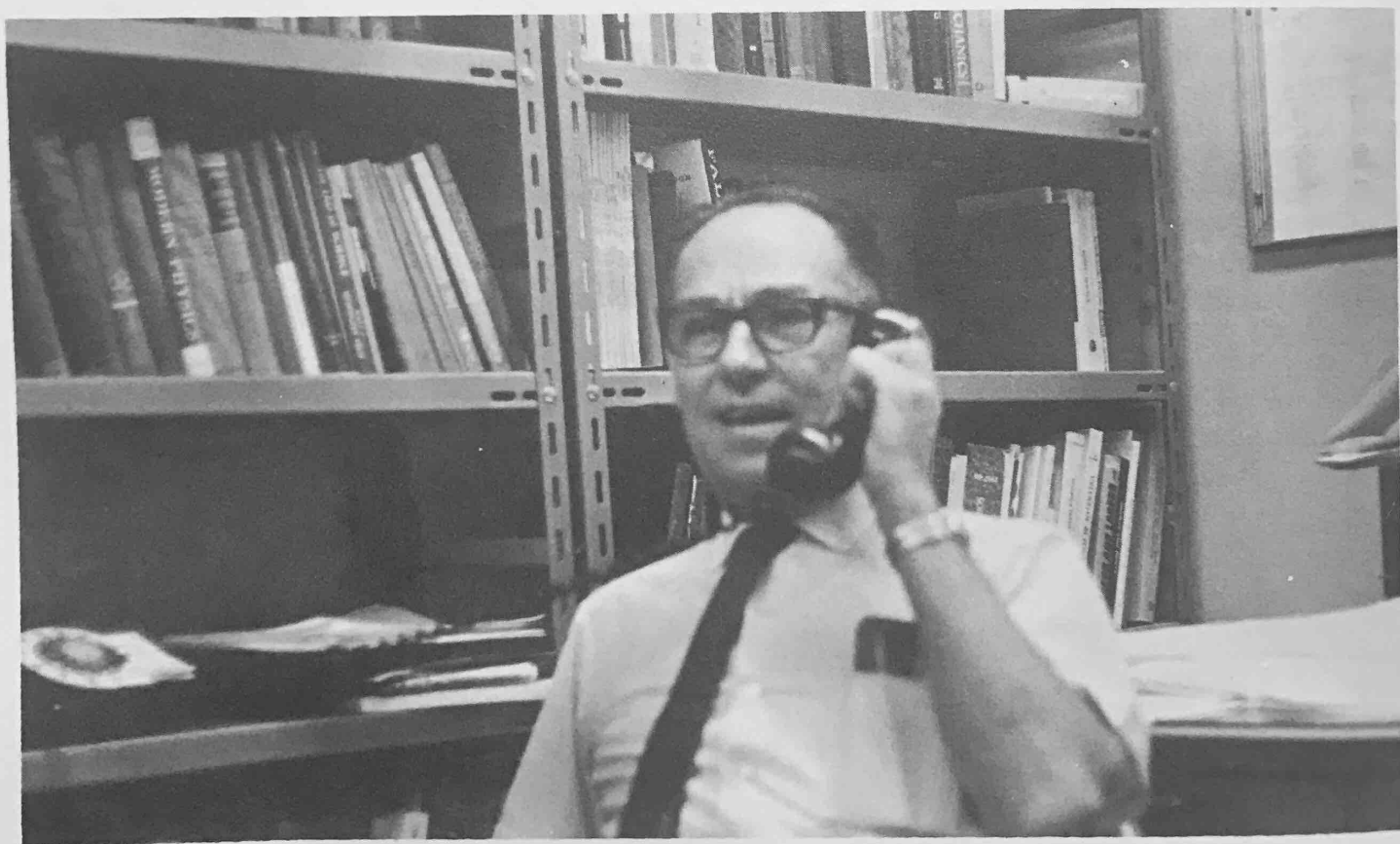
Dr. Gileadi attended the American Nuclear Society Winter Meeting and conference on the Constructive Uses of Atomic Energy held in Washington, D. C. (Nov. 11-15, 1968).

Dr. Sasscer attended the Conference on Abundant Nuclear Energy held at Gatlinburg, Tennessee from (August 26-29, 1968).

ADDITIONAL INFORMATION

During the year the Division has been becoming engaged in a new, major area of teaching and research, called "plowshare", or the peaceful uses of nuclear devices. The research has been conducted by F. J. Muñoz Ribadeneira and K. B. Pedersen and the teaching by Dr. Pedersen.

Three publications (See appendix) have resulted from this research. The research projects pertain to : (a) in situ mining by nuclear devices, specifically, the leachability of chalcopyrite (CuFeS_2) in sulfuric acid solutions and, (b) applications of distorted model theory to predicting cratering dimensions.



- (1) Dr. Amador Cobas, deputy director of the Nuclear Center, also heads the Physical Sciences Division
- (2) Dr. Manfred Eberhardt works at an IBM card punch in preparation for molecular orbital calculations

PHYSICAL SCIENCES

The long range objective of the Physical Sciences 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 radioisotopes, and requires heavy participation of the scientific personnel in the academic activities of the natural sciences departments of the University of Puerto Rico, Rio Piedras campus. The latter cooperative effort is encouraged through joint appointments. The Division also participates in the AEC "Atoms in Action" exhibits.

EDUCATIONAL ACTIVITIES

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

a) The Radioisotopes Techniques Course was offered four times during 1968. The distribution of the fourteen trainees by geographical origin (Table I) shows seven from Puerto Rico, one from Spain, two from U.S.A., one from China, two from Colombia, and one from the Dominican Republic.

b) A course in Radiation Chemistry (PRNC 505, two credits) for M.S. students in Health Physics was taught by Dr. Alec Grimison and Dr. Manfred Eberhardt, with the participation of four students.

c) University courses:

1. Advanced Physical Chemistry (Chem. 464, three credits). A one-semester graduate course, taught twice by Dr. Alec Grimison, with a total of thirty students.
2. Photochemistry and Radiation Chemistry (Chem. 660, three credits) A one semester graduate course, taught by Dr. George A. Simpson, with three students.
3. Undergraduate Inorganic Chemistry (Chem. 311, three credits). A one semester course, taught by Dr. Mariel Muir, with thirty students.
4. Advanced Inorganic Chemistry (Chem. 521). The second semester of a two-semester graduate course, taught by Dr. Mariel Muir, with ten students.
5. Graduate Research (Chem. 599 or Phys. 501, one to six credits). Graduate students supervised by PRNC personnel and their geographical origins are shown in Table II.

6. Undergraduate Research Training. Three senior science students took advantage of PRNC's research training opportunities during 1968; José Marrero and César Cordero with Drs. A. Cobas and S. Z. Weisz; Janice Petrovich with Dr. J. P. A. Castrillón.

PARTICIPATION IN THE "ATOMS IN ACTION" EXHIBITS

Dr. Alec Grimison participated in the "Atoms in Action" exhibit in Caracas, Venezuela (April 27-May 5, 1968). He presented two lectures in Caracas: "Radiation Chemistry of Organic Glasses" at the Instituto Venezolano de Investigaciones Científicas (IVIC), and "Uses of Radioisotopes in Scientific Research" at the Central University, Caracas. A small project was initiated on the thermoluminescence of gamma-irradiated uracil and cytosine, coupled with electron spin resonance measurements in conjunction with Dr. Bemski, of the Physics Department, IVIC.

Dr. José Castrillón participated in the "Atoms in Action" exhibit in Córdoba, Argentina (October 26 - November 2, 1968). He presented three lectures to the Institute of Chemical Sciences, University of Córdoba on "The Use of Radioisotopes in Organic Chemistry", and two seminars for the Biological Chemistry Group at the University of Córdoba on "Liquid Scintillation Counting". Research projects were initiated on the tritium labelling of sphingosine and dihydrosphingosine, using the catalytic technique, and on the use of tracers in the study of the oxidation of aldehydes with n-bromosuccinamide.

VISITING LECTURERS

During 1968 the following visiting lecturers presented conferences of particular interest to the division staff:

February 23, Dr. G. J. Dienes, Head Solid State Physics Group, Brookhaven National Laboratory. "The Properties of Small Substitutional Impurities in the Alkali Halides."

April 10, Dr. Allan Maccoll; Reader in Physical Chemistry, University College London, London, England. "Mass Spectroscopy".

May 28, 29: Dr. Martin Pope, Solid State Physics and Radiation Laboratory, Dept. of Physics, New York University, "Autoionization in Anthracene," and "Photoconductivity as a Spectroscopic Adjunct."

June 12, 13, Dr. Marvin Silver, Organic Crystals Solid State Physics Project, Dept. of Physics, University of North Carolina. "Study of Quasi Free Electrons in Liquid Helium by Electron Injection," and "Determination of Electronic States in Anthracene from a Study of the Temperature Dependence of the Recombination Coefficient."

July 11, Dr. Gabriel Chuchani, Head Chemistry Dept., Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas. "The Effects of Amine Groups in Aromatic Electrophilic Substitution."

July 18, Dr. Michael Barfield, Chemistry Dept., University of Arizona. "Theory of Nuclear Spin-Spin Coupling."

August 8, Dr. D. A. Armstrong, Chemistry Dept., University of Calgary, Canada. "Radiolysis of Aqueous Solutions of Sulfur Compounds."

November 6, Dr. David Skelly, General Electric Research Laboratory.
"A Novel Photographic Process: Photoplastic Recording."

STAFF CHANGES

Dr. Harry Szmant, Division Head, who had been on leave of absence in the Dominican Republic, resigned from PRNC in August 1968. Dr. Szmant has accepted a position as Chairman of the Chemistry Department, University of Detroit. Two of the graduate students working with Dr. Szmant, A. Birke (Chile) and A. Mata (Costa Rica) will shortly join him at the University of Detroit. The remainder have transferred to work under the supervision of Dr. Castrillón.

Mr. Gerardo Molina, Research Associate, resigned from the Division in September, 1968, and has entered the graduate school, Duke University, to study for the Ph.D. degree in Chemistry.

Miss Dolores Julian has joined the Division staff as Research Assistant. She completed the research for her M.S. thesis in Radiation Chemistry under the direction of Dr. Owen Wheeler, PRNC, Mayaguez. Miss Julian will soon present this thesis for the M.S. degree.

SCIENTIFIC MEETINGS AND COURSES OUTSIDE PUERTO RICO

March 17-20, 1968: American Physical Society Meeting, University of California, Berkeley. Attended by A. Cobas and S. Z. Weisz.

March 31-April 5, 1968: American Chemical Society Meeting, San Francisco. The following paper was presented: "Steric Effects in the Radiolysis of Cis- and Trans- 1,2-dimethylcyclohexane," by Manfred Eberhardt.

April 6-9, 1968: American Chemical Society Short Course, San Francisco. Dr. Manfred Eberhardt received special training in Molecular Orbital Theory.

April 22-25, 1968: Second Inter-American Conference on Radiochemistry, Mexico City. The following paper was presented: "Radiation Effects in Organic Glasses," by Alec Grimison.

May 4, 1968: Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas. Dr. Alec Grimison delivered a lecture entitled "Radiation Chemistry of Organic Glasses."

May 4, 1968: Central University, Caracas. Dr. Alec Grimison delivered a lecture entitled "Uses of Radioisotopes in Scientific Research."

June 28, 1968: Hebrew University, Jerusalem, Israel. Dr. S. Z. Weisz delivered a lecture entitled "Radiation Damage in Organic Crystals."

July 9-12, 1968: 4th Molecular Crystal Symposium, Enschede, Netherlands. The following paper was presented: "Radiation Induced Paramagnetic Centers in Anthracene and Deuterated Anthracene" by S. Z. Weisz, J. Castellanos, A. Cobas, and G. A. Simpson.

August 20-23, 1968: Loyola University, Chicago, Illinois. Dr. George A. Simpson Attended an International Conference on Molecular Luminescence.

September 26, 1968: University of Montreal, Montreal. Dr. Alec Grimison delivered a lecture entitled "Electron Attachment to Pyridine and the Diazines."

September 27, 1968: McGill University, Montreal. Dr. Alec Grimison delivered a lecture entitled "Electron Attachment to Pyridine and the Diazines: Experimental and Theoretical Considerations."

October 2-23, 1968: Buck Hill Falls, Penn. Dr. S. Z. Weisz attended a Conference on Electrical Insulation and Dielectric Phenomena.

RESEARCH

Division research 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 effect of high-energy deposition in chemical systems. Some of the projects emphasize the initial, or primary, products of radiation; 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.

a) Stereochemical Effects in the Gamma Radiolysis of Cis- and Trans-1,2-dimethylcyclohexane (M. K. Eberhardt). The gamma radiolysis of cis and trans-1,2-dimethylcyclohexane in the liquid phase was studied from 2×10^{20} ev/ml to 16×10^{20} ev/ml at a dose-rate of 8.3×10^{18} ev/ml hr. using a ^{60}Co source. The main products are H_2 , 1,2-, 2,3-, 3,4-, and 4,5- dimethylcyclohexene, octene-2, dimeric products, and isomerization. The trans compound shows a greater decrease in $G(\text{olefin})$ with increasing dose than the cis isomer. Iodine ($2 \times 10^{-2}\text{M}$) reduces $G(\text{H}_2)$ in the trans-1,2-dimethylcyclohexane from 3.54 to 2.36 (H_2) = 1.8, but in the cis isomer the $G(\text{H}_2)$ is only reduced from 3.70 to 3.05 (H_2) = 0.65. Our results suggest that the difference of unscavengeable hydrogen between the two isomers $3.05 - 2.36 = 0.69$ is due to molecular hydrogen elimination in the cis-1,2-dimethylcyclohexane, and that tertiary axial hydrogens lead preferentially to the formation of scavengeable hydrogen atoms. A similar behavior was observed with cis and trans-decalin.

b) Radiation-Induced Addition of Thiophenols to Indene (H. H. Szmant and M. K. Eberhardt). The gamma-radiation induced addition of p-substituted thiophenols to indene in cyclohexane follows first order kinetics with respect to both the thiol and the olefin. In the case of p-thiocresol the bimolecular rate constant is $1.2 \times 10^{-4}\text{M}^{-1}\text{sec}^{-1}$ at 25° . The rates give a linear Hammett plot when sigma plus values are employed and the large, positive rho value of 2.29 is obtained. The G-values for the disappearance of p-thiocresol from an equimolar (0.5M) mixture with indene is strongly solvent dependent: 720 in carbon tetrachloride, 512 in cyclohexane, 219 in benzene and 131 in 1,2-dimethoxyethane. In a competitive reaction of p-thiocresol with 1-octene and indene the latter olefin is more reactive. These and other observations suggest a mechanism that involves the olefin and a thiol residue, and a rate-determining step favored by electron-withdrawal in the sulfur-containing moiety. Graduate student trainee: Irma Y. Zea Ponce.

c) Tritium Recoil Labelling (J. P. A. Castrillón). The analysis of samples of lithium phenylacetate following neutron irradiation is well advanced. An alternative method of analysis via the nitration of the labelled acid is being studied.

Work has begun on the steric aspects of recoil labelling, and for this purpose p-phenylbutyric acid is being resolved into its enantiomers. Graduate student trainee: Agnes Costa.

d) Matrix Isolation Studies of the Gamma-Radiolysis of Heterocyclic Molecules (A. Grimison and 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 the O6 Program. Graduate student trainees: Myrta Trujillo Sánchez, Francisco Bernasconi, José Revuelta.

e) Radiation Damage in Organic Crystals (A. Cobas, S. Z. Weisz, G. A. Simpson). This project receives support from the AEC Physical Sciences Division. Radiation damage in well-defined crystalline organic materials is studied by conductivity and spectroscopic measurements. The work is described fully elsewhere in this Annual Report under the O5 Program. Graduate student trainees: Jaime Castellanos and Eva Arzola.

Radioisotope Studies. These projects include the use of incorporated radioactive tracer atoms, as a diagnostic aid to the study of reaction mechanisms, as well as studies of counting techniques.

a) Oxidation of Diarylethanes (J. P. A. Castrillón). The individual steps in the synthesis of 1,1 diodosiphenylethane have been examined in an effort to improve the yields. The synthesis of the labelled compound was later performed successfully. Oxidations of inactive samples have been carried out in preparation for the tracer experiments. Graduate student trainee: J. Colón.

b) The Influence of Chemical Structure on Quenching in Liquid Scintillation Counting (J. P. A. Castrillón). To measure more accurately the quenching caused by the displacement of the C^{14} β -spectrum toward lower energies, a variable discriminator was calibrated using several internal conversion electron emitters. Several compounds of chosen structure have been synthesized: p,p-diacetyldiamino benzophenone, p,p'-diaminobenzophenone, p-monoiodo benzophenone, p,p'-diiodo benzophenone. P,p'-diiodo diphenyl sulfoxide is currently being synthesized. No clear relationship between quenching constant and chemical structure has yet been established. However, the quenching seems to be additive in the sense that the quenching of p,p-dihalogen compounds is double that of corresponding p-monohalogen compounds. Work using terphenyl as a scintillator has been initiated. Graduate student trainee: Elsa Gómez.

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

a) Molecular Orbital Calculation on Lactam-Lactim Tautomers (M. K. Eberhardt). Huckel MO calculations have been made on a series of lactam-lactim tautomers. These results can be related to the ability of these compounds to undergo an aminomethylation reaction (Mannich reaction).

b) Molecular Orbital Calculations on Aminophenols and Amino thiophenols (M. K. Eberhardt and A. Grimison, in collaboration with Dr. Gabriel Chuchani, IVIC, Caracas, Venezuela). Huckel MO calculations have been made on o-, m-, and p-aminophenol, and o-, m-, and p-aminothiophenol to explain the differences in electrophilic substitution with triphenylmethyl carbonium ion.

c) Thioxanthone and Related Compounds (J. P. A. Castrillón). To improve the method of preparation and isolation of thioxanthone sulfoxide, its ability to act as a ligand in metallic complexes was investigated. Thioxanthone sulfoxide forms stable, crystalline compounds with the group IIB metals. This led to the unexpected discovery that thioxanthone itself yields less stable complexes with these same metals. The structure of these two new families of

compounds are being studied. Graduate student trainee: Sonia Vázquez.

d) Nuclear Spin-Spin Coupling Constants (A. Grimison and W. Adam). Calculations have been made of the nuclear spin-spin coupling constants in various small molecules. Only the dominant Fermi contact term was computed, using the complete perturbation expression, as well as various approximations. The best available self-consistent field wavefunctions were used. Graduate student trainee: Philip Sprangle.

e) Calculations of C^{13} Chemical Shifts (A. Grimison and W. Adam). The carbon-13 chemical shifts of the 6-membered ring heterocyclics pyridine, pyrazine, pyrimidine, pyridazine, s-triazine, s-tetrazine, and their cations and dications, and of the 5-membered ring heterocyclics pyrrole, imidazole, pyrazole, and their anions and cations have been calculated from various approximate perturbation theory expressions. The diamagnetic contribution to the screening tensor was calculated exactly. The paramagnetic contribution was calculated using (i) individual transition energies in the full perturbation theory expression (ii) the Karplus-Pople approximation (iii) the Karplus-Das approximation, and (iv) from π -electron densities. Valence electron wavefunctions computed by the extended Huckel theory (EHT) were used, in non-orthogonal and in orthogonal form. The detailed perturbation expansion fails badly; this failure can be linked to the limited basis set used in EHT calculations. The neglect of orbitals on vicinal atoms, or the use of actual geometries does not improve the results significantly. Good agreement with experiment is obtained by the judicious use of the average excitation energy approximation and orthogonal wavefunctions. A normalized E parameter calculated from the EHT energy values reproduces (a) the displacement of the chemical shift to higher field in the isoelectronic series $C_7H_7^+$, $C_6H_6^-$, $C_5H_5^-$, (b) the displacement of the chemical shift to lower field on successive nitrogen substitution, and (c) the displacement of the chemical shift to higher field on protonation of the nitrogen lone pairs. The use of the π -electron approximation does not permit the latter correlation to be made with experimental trends. Graduate student trainee: Gladys Rodríguez.

f) Hetaryne Intermediates (A. Grimison and W. Adam, in collaboration with R. Hoffmann, Cornell University). The electronic structures of all of the possible 1,2-, 1,3-, and 1,4- didehydroaromatic intermediates derived from pyridine and the diazines (hetaryne intermediates) have been calculated using the extended Huckel theory (EHT). The didehydropyridine is the most stable, and the 2,6-didehydropyridine the least stable. Great relative stability is also predicted for 4,5-didehydropyrazine and 4,6-didehydropyrimidine. The complex computational trends in the hetaryne stabilities can be rationalized very well by simple molecular orbital considerations of the orbital interactions among non-bonding radical lobes and lone pair orbitals. A dominant effect is shown to be a nitrogen lone pair destabilization of nearby radical lobes. The calculated stability sequences and total electron distributions provide an excellent correlation of the available experimental data on relative stability and orientation effects in the hetaryne intermediates.

TABLE 1

Participants in Basic Course in Radioisotopes Techniques, 1968

<u>Name</u>	<u>Country</u>	<u>Field of Interest</u>	<u>Financial Sponsor</u>
1. Martínez-S., R., M.D.	Spain	Microbiology	PRNC
2. Jordan, C.F., Ph.D.	United States	Ecology	PRNC
3. Ramos-S., M.A.	Puerto Rico (USA)	Hematology	Self
4. Ramírez-S., G., M.D.	Puerto Rico (USA)	Surgery	UPR
5. Pothier, L.J.	United States	Biochemistry	Self
6. Rivera-H., F.	Puerto Rico (USA)	Odontology	Self
7. Liu, Lii-Chyuan, Ph.D.	China	Biology	Self
8. del Río-R., N.	Puerto Rico (USA)	Microbiology	Self
9. Rodríguez-A., D.C.	Dominican Rep.	Nuclear Medicine	Self
10. Osorio-G., R.G.	Colombia	Pharmacology	WHO
11. Nassar-R., J.A., M.D.	Puerto Rico (USA)	Radiology	UPR
12. Colón-L., J.A.	Puerto Rico (USA)	Biology	PRNC
13. Soto-G., C.H., M.D.	Puerto Rico (USA)	Radiology	P. R. Medical Center
14. Rivera, P.J.	Puerto Rico (USA)	Biology	P. R. Health Department
15. Motta, C.C.	Colombia	Pharmacology	IAEA

TABLE II

Thesis Research Supervised by Division Personnel During 1968

<u>Student</u>	<u>Country of Origin</u>	<u>Supervisor</u>
1. Arzola, Eva	Puerto Rico	S. Z. Weisz, A. Cobas
2. Bernasconi, Francisco	Chile	A. Grimison, G.A. Simpson
3. Birke, Arnoldo	Chile	H. H. Szmant
4. Cancio, Edith	Puerto Rico	J. P. Castrillón
5. Castellanos, Jaime*	Colombia	S. Z. Weisz, A. Cobas
6. Colón, Jaime	Puerto Rico	J. P. Castrillón
7. Costa, Agnes	Puerto Rico	J. P. Castrillón
8. Fernández, Olga	Cuba	M. Muir
9. Gómez, Elsa	Venezuela	J. P. Castrillón
10. Mata, Alfonso	Costa Rica	H. H. Szmant
11. Rechani, Pio	Puerto Rico	M. Muir
12. Riquelme, Ida	Cuba	H. H. Szmant
13. Rodríguez, Gladys*	Puerto Rico	A. Grimison
14. Revuelta, José	Cuba	A. Grimison, G.A. Simpson
15. Trujillo, Myrtha	Cuba	A. Grimison, G.A. Simpson
16. Sprangle, Philip	United States	A. Grimison
17. Zea Ponce, Irma*	Guatemala	H. H. Szmant, M. Eberhardt

*M.S. Degree awarded during 1968.



Mirtha Trujillo Sánchez, a Cuban chemistry graduate student, examines the flash photolysis apparatus

RADIATION CHEMISTRY PROJECT: MATRIX ISOLATION STUDIES OF PRODUCTS OF GAMMA-RADIOLYSIS OF HETEROCYCLIC MOLECULES.

The project aims at trapping and subsequently characterizing the species formed by γ -radiolysis of heterocyclic molecules which are of possible biological importance. Emphasis is placed on direct observation of labile intermediates formed following absorption of high-energy radiation. This is made possible by using the matrix isolation technique, in which the molecule is irradiated in some form of rigid matrix, usually at low temperatures. Under appropriate conditions, radicals and radical ions can be stabilized by using this method 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 used in conjunction with the experimentally measured properties to help identify unknown intermediates.

A description of the current research topics follows:

1. Absorption Spectra of Radiolytic Intermediates at 77°K. The previous Annual Report mentioned the observation of electron attachment to pyridine and the diazines by γ -radiolysis in a 2-methyltetrahydrofuran (MTHF) glass at 77°K. This has now been confirmed for pyridine, pyrazine, pyridazine, and pyrimidine by studies of bleaching effects, solute concentration effects, and addition of electron scavengers. The spectra of the radical anions obtained in this way are in excellent agreement with the assignments of other workers. Hush and co-workers (H and H) (University of Bristol) have reported the radical anion spectra produced by chemical reduction with metallic sodium in methyltetrahydrofuran at room temperature. Kimmel and Strauss (K and S) (University of California) produced the same species by electrolytic reduction in liquid ammonia. The results are shown in Tables 1-4, together with the theoretical predictions (see later). This work has been presented for publication.

2. Thermoluminescence following Radiolysis at 77°K. Thermoluminescence following γ -radiolysis of biologically significant compounds has been detected by several investigators. Some qualitative observations are summarized in Table 5. After irradiating the listed compounds at 77°K to a dose of approximately 3×10^5 rads, luminescence can be observed visually on warming in the dark. For adenine, cytosine, DNA, guanine, thymine, and uracil, these observations confirm earlier work. While 2, 4, 5 triphenylimidazole gave a yellow emission, all other luminescences were blue. Triphenylimidazole is known to have yellow phosphorescence bands. The previous Annual Report mentioned a blue (450 nm) thermoluminescence of DNA in irradiated 3-methylpentane glasses. The blue phosphorescence bands of unirradiated DNA and the pyrimidine bases are well known.

A rationale of the observed luminescence bands (which is confirmed by our more detailed studies) is that the observed thermoluminescence is due to re-

combination luminescence of the trapped intermediates, which corresponds to the normal phosphorescence of the unirradiated molecule.

3. Thermoluminescence and ESR Signals following Radiolysis at Room Temperature. In conjunction with Dr. Bemski (Instituto Venezolano de Investigaciones Científicas) comparative studies were made of the ESR signal and thermoluminescence following room-temperature radiolysis of biologically significant compounds. Figure 1 shows the ESR spectrum obtained from irradiated cytosine powder. The radical associated with this unsymmetrical and unresolved spectrum has a g-value of approximately 2.02, and a half-width of approximately 16 gauss. The absence of detail is characteristic of powdered samples. The g-value and the width suggest a neutral radical, with the unpaired electron localized near a carbon atom joined to two hydrogen atoms. A similar spectrum is obtained from irradiated uracil. The presence of these radicals is sensitive to heat treatment following irradiation. The efficiency of production of radical centers is of the order of 10^{-4} radicals per 100 eV absorbed.

Following irradiation of thymine, cytosine, adenine, purine, guanine, or uracil to doses up to 6×10^4 rads at room temperature, a thermoluminescence is produced with a yield approximately one-ten thousandth that from a similarly treated sample of lithium fluoride, and is linear with dose in the range specified. For cytosine and uracil the maximum thermoluminescence is obtained from 63° to 155°C, which suggests a trap depth of about 1 eV.

If it is assumed that the trapped species giving rise to the thermoluminescence is associated with the free radical center observed by ESR, then the luminescent process can be attributed to chemiluminescence resulting from radical diffusion and annihilation in the crystalline matrix.

4. Photoionization in Rigid Glasses at 77°K. Several mechanisms of photoionization have been suggested thus far. The most significant of these for biological systems appears to be the low energy, or biphotonic, process which involves absorption of light by the triplet state of the molecule. However, little direct evidence has been presented for the occurrence of biphotonic photoionization in heterocyclic molecules.

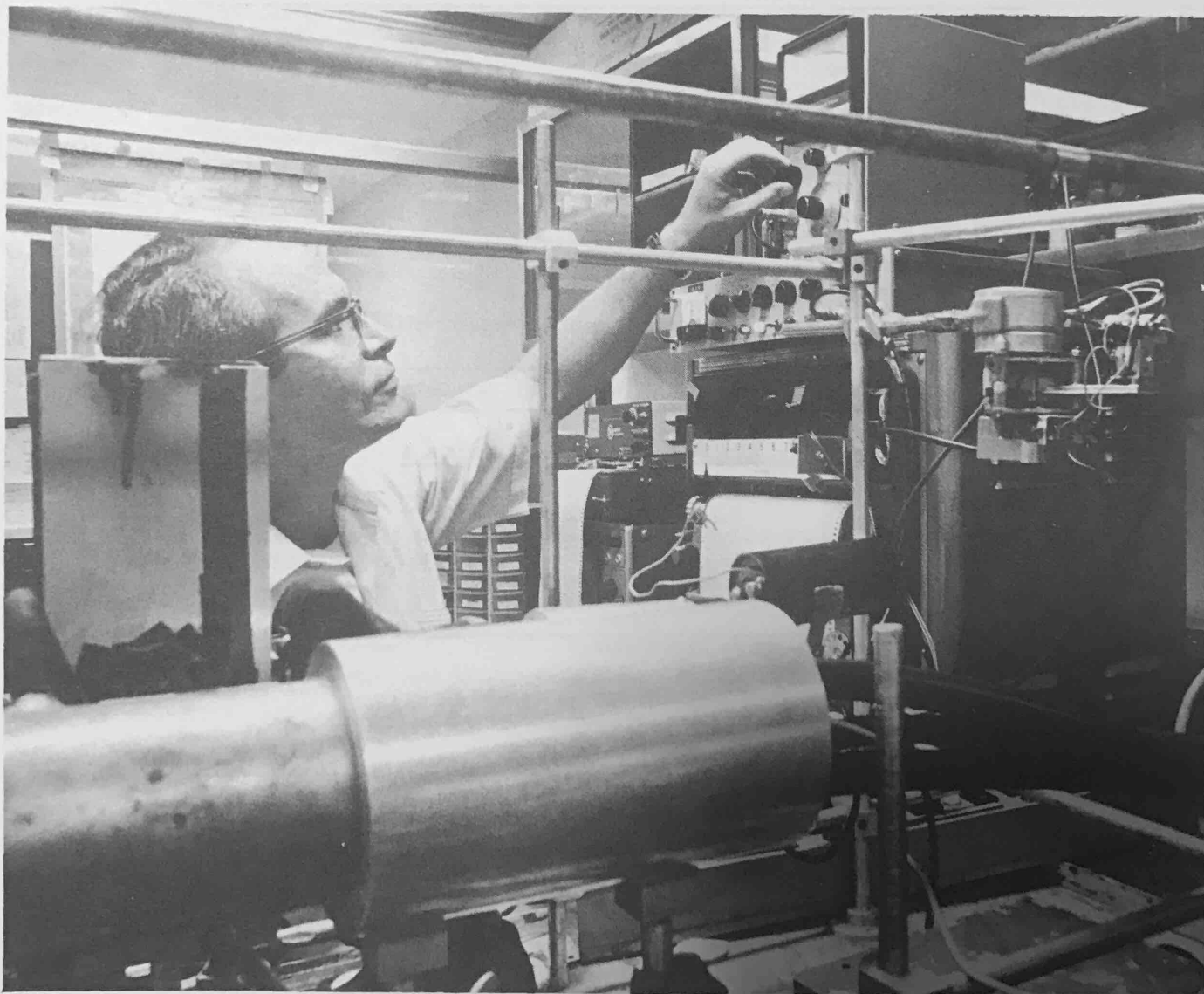
A test for photoionization is the occurrence of recombination luminescence subsequent to photolysis. This may be observed as thermoluminescence, optically stimulated luminescence, or prolonged isothermal luminescence. Table 6 reports on observed prolonged isothermal luminescence for a variety of aromatic amines and heterocyclic compounds in glasses at 77°K, after brief exposure to an intense UV source. This isothermal luminescence may last as long as 3 hours. The table lists the compounds in order of efficiency of production of prolonged luminescence, together with the normal phosphorescence life-times. There is a definite correlation between efficiency and triplet lifetime, which would be predicted by the biphotonic mechanism. Moreover, in the cases where we have obtained the photolysis intensity dependence of the luminescence, a squared dependence is obtained, as required by the biphotonic mechanism.

5. Self-Consistent-Field Calculations on Heterocyclic Radicals and Radical Ions. Pariser-Parr-Pople calculations have been carried out on the following neutral molecules and their radical anions and radical cations: pyrrole, furan, thiophene, pyridine, pyrimidine, pyrazine, and pyridazine. These calculations included limited Configuration Interaction, and used the computer program described in the previous Annual Report.

The agreement obtained with the well-characterized absorption spectra of the neutral molecules was very good. In harmony with recent theories, the use of a very limited configuration interaction worsens the agreement with experiment.

The agreement of the calculated transition energies for the radical anions is in good agreement with the assigned experimental spectra, and with other theoretical results (Tables 1-4). A much poorer agreement is obtained for the radical cations. These are pi-electron calculations, with no account taken of sigma-pi interactions. For the radical anions, the additional electron enters a pi-orbital, and sigma-pi interactions should not be of major importance. However, in the radical cations the electron may have been removed from a sigma- or from a pi-orbital. The results suggest that sigma-pi interaction terms are extremely important for the cations, and that these may be sigma-, rather than pi-radicals.

6. Valence-Bond Calculations on Heterocyclic Systems. These calculations use cyclopentadiene as a preliminary model, and aim at the electronic structure of simple heterocyclic molecules and radicals, using a non-empirical valence bond method. Much of this year's work was devoted to modifying a program originally written by Dr. Palmieri (University of Bologna). This uses as input the atomic integrals whose calculation was described in the previous Annual Report. All possible determinantal basis functions of a given multiplicity are generated; the integrals among these are evaluated using Lowdin's density matrix formalism for non-orthogonal Slater determinants.



Dr. George A. Simpson operates the low temperature photolysis and recombination luminescence detection apparatus

TABLE I
Transition Energies (eV) For The Pyridine Anion

Experimental Values			Calculated Values		
<u>H & H</u>	<u>K & S</u>	<u>This work</u>	<u>H & H</u>	<u>K & S</u>	<u>This work</u>
		1.			0.26 (.000)
		2.5	2.66 (.067)		2.25 (.231)
3.7 (.078)	3.46 (.071)	3.65		3.07 (.07)	4.29 (.195)
	4.75 (.25)		5.05 (.007)		4.65 (.200)
5.08 (.120)			5.23 (.830)	5.26 (.007)	5.40 (.173)

Oscillator strength values in parenthesis.

TABLE 2
Transition Energies (eV) For The Pyrazine Anion

Experimental Values			Calculated Values		
<u>H & H</u>	<u>K & S</u>	<u>This work</u>	<u>H & H</u>	<u>K & S</u>	<u>This work</u>
		2.45			0.49 (.000)
3.4 (.078)	3.58 (.091)	3.65	2.90 (.066)		2.28 (.188)
				3.15 (.053)	4.70 (.253)
4.98 (.215)	5.21 (.25)		5.16 (.215)		5.54 (.238)
				5.22 (.113)	

Oscillator strength values in parenthesis.

TABLE 3
Transition Energies (eV) For The Pyrimidine Anion

Experimental Values			Calculated Values		
<u>H & H</u>	<u>K & S</u>	<u>This work</u>	<u>H & H</u>	<u>K & S</u>	<u>This work</u>
		3.1			.29 (.003)
3.75 (.077)	3.72 (.065)	3.9	2.86 (.081)		2.53 (.193)
4.97 (1.94)	5.11 (.25)		3.01 (.169)	3.01 (.169)	4.04 (.216)
			5.09 (.10)	5.36 (.15)	4.73 (.181)
			5.47 (.060)		4.94 (.169)

Oscillator strength values in parenthesis.

TABLE 4
Transition Energies (eV) For The Pyridazine Anion

Experimental Values			Calculated Values		
<u>H & H</u>	<u>K & S</u>	<u>This work</u>	<u>H & H</u>	<u>K & S</u>	<u>This work</u>
		1.75			0.27 (.000)
3.52 (.052)	3.64 (.065)	2.3	2.73 (.051)		2.13 (.168)
		3.5		3.20 (.132)	4.54 (.185)
			4.59 (.030)		4.93 (.235)
5.13 (.182)			5.24 (.062)	5.63 (.125)	5.27 (.210)

Oscillator strength values in parenthesis.

TABLE 5

Compounds Irradiated at 77°K Giving Thermoluminescence

Compound	Observed Intensity
Acridine	Strong
Adenine	Strong
Anthracene	Weak
Ascorbic acid	Weak
Benzimidazole	Strong
Benzotriazole	Weak
5-Bromoindole	Strong
5-Bromouracil	Strong
Cytosine	Strong
DNA	Weak
Eosin-Y	None
Fluorescein	None
Guanine	Weak
Hematoporphorin	Weak
L. histidine	Weak
Indole	Weak
Imidazole	Weak
Indazole	Weak
5-methylcytosine	Weak
1,10-phenanthroline	Weak
Purine	Strong
Pyrazole	Weak
Pyranine B	None
Pyronine Y	Strong
Sodium chloride	Weak
Sucrose	Weak
Tetraphenyl pyrrole	Weak
Thionine	Weak
Triphenyl amine	Strong
2,4,5-triphenyl imidazole (TPI)	Weak
Thymine	Weak
Uracil	Weak
Xanthine	Weak

(All compounds had blue emission, except TPI which appeared blue. dose approx. 3×10^7 rads).

TABLE 6

Compounds Undergoing Photoionization At 77°K In MTHF
In Order Of Decreasing Efficiency

Compound	Lifetime (Seconds)
Aniline	5.1
Indole	6.4
Triphenylamine	5.1
Indazole	3.6
Tetraphenylpyrrole	1.5
Purine	1.7

Produced one second exposures to a 1000 watt high pressure mercury lamp.



Dr. Shmvel Zvi Weisz, of Israel, observes the interaction rate constant between trapped electrons and triplet excitons in anthracene crystals

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 because: (1) large, very pure anthracene crystals can be obtained; (2) much 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 rad) 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.

ACHIEVEMENTS

1. Electron Spin Resonance (ESR) Measurements in Anthracene $C_{14}H_{10}$ and Deuterated Anthracene $C_{14}D_{10}$. Several types of damage centers are introduced by irradiating anthracene with gamma rays. These defects are detected by different methods such as: delayed fluorescence, fluorescence, optical absorption, space charge limited currents (A description of detecting radiation by these methods is given in PRNC-58 and PRNC 107.) and electron spin resonance. Crystals irradiated with doses up to 10^9 rad were dissolved and analyzed by thin layer chromatography but no traces of impurities could be found. In spite of the extensive study of the radiation damage in anthracene until now, only the nature of the paramagnetic centers could be revealed. It became possible to identify these centers by studying the ESR spectra of gamma irradiated anthracene and deuterated anthracene. Doses up to 7×10^8 rad were measured. The spectra of the gamma irradiated anthracene was found to be that expected from three equivalent spin $1/2$ nuclei coupling with an unpaired electron. The intensity ratios and hyperfine splittings are approximately the same as for the methyl radical. Blum et. al.⁽¹⁾ suggested that the spectra results from the hyperfine interaction of 3 equivalent protons on a linear segment of a dimeric anthracene radical. It was anticipated that if the proposed cross-linking between the anthracene molecules is indeed produced, then the spectra of the paramagnetic centers produced on irradiation of deuterated anthracene will be considerably narrower. They would be less than $1/3$ of the methyl radical and have a 7 line structure with intensity distribution of 1:3:6:7:6:3:1. The spectra obtained in gamma irradiated deuterated anthracene was very close to the predicted one. A small excess in the width of the spectra is thought to be due to the partially deuterated anthracene $C_{14}D_9H$ which is nominally present in the sample. Since the $C_{14}D_9H$ has only one hydrogen, at low concentrations all the nearest neighbors in the crystal lattice would be fully deuterated and the resultant radical could have no more than one hydrogen. Hence, the spectrum

1. Blum, et. al. Mol. Cryst. 3, 269 (1967).



Dr. Jacob Levinson, of Israel, detecting radiation damage in anthracene crystals by using non-linear optical methods with a cathode ray oscilloscope and a 100 mw Q switch laser

obtained is that of a mixture of deuterated and partially deuterated radicals. Studies of ESR spectra produced by irradiation of selectively deuterated anthracene should provide even greater insight into the nature of the gamma ray induced paramagnetic centers in anthracene.

2. Annealing of the Triplet Quenching Radiation Damage in Anthracene.

As described in PRNC-58 and PRNC-107, gamma irradiation induces triplet quenching centers in anthracene. Due to the long diffusion length of the triplet excitons in pure crystals, concentrations of damage centers as low as 10^{10} N/cm⁻³ can be detected. From transient delayed fluorescence measurements it was found that these damage centers shorten the monomolecular lifetime of the triplet excitons. These centers are stable at room temperature for more than one year. Annealing the crystal at 120°C, the change in the monomolecular lifetime is restored by approximately 30%. The ability to anneal these centers at relatively low temperature suggests that these defects are physical ones. Measurements now in progress of the lifetime of the triplets in anthracene grown from irradiated powder can reveal more about the nature of these centers.

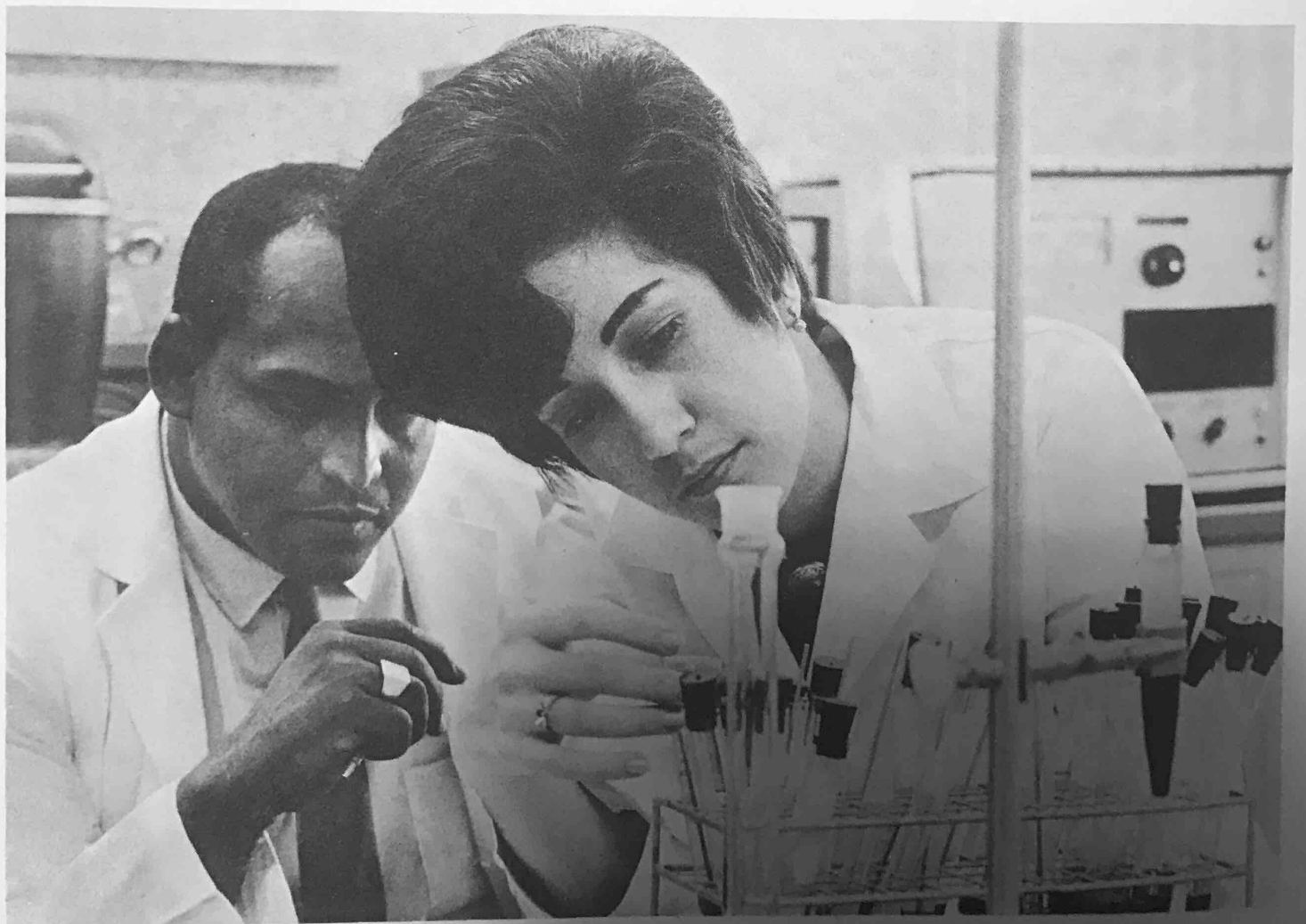
3. Photoenhanced Space Charge Limited Currents. Several authors reported recently on observation of interactions of triplet excitons with trapped and free electrons, respectively. Our calculations indicate that radiation damage measurements on the photoenhanced electron space charge limited currents in anthracene can distinguish between the two processes. The measurements are made on solution grown crystals. Sodium amalgam is used as the electron injecting electrode. It is found that electron traps are induced in anthracene by gamma rays. For radiation doses higher than 10^4 rads the changes in log J vs log V curves are similar to those for hole currents. The trap filled limit voltage is shifted toward higher voltages. A change in the slope of the curve is also observed. The sodium amalgam is oxidized very rapidly in air. To obtain quantitative results and to perform the photoenhanced current measurements, an electrode system is built so that the measurements may be performed in vacuum.

LABORATORY SPACE

This project is housed in the Facundo Bueso Science Building at the U.P.R. Rio Piedras Campus because of the space shortage in the PRNC Bio-Medical Building. The project began in January 1962, in a 360 square foot room. In February 1965, the project was moved into a room with approximately 900 square feet of floor space. Two offices with a combined area of approximately 240 square feet were also provided. The increase in space has made it possible to set up the equipment more advantageously and to add to the staff.



- (1) Dr. José A. Nassar working with the new well counter
(2) Dr. César Soto, with technician Leila Crespo de García,
working on blood samples in hematologic tests



CLINICAL RADIOISOTOPE APPLICATIONS

The Clinical Division of Radioisotopes teaches physicians and allied personnel to handle and use radioisotopes in nuclear medicine. This consists primarily of the diagnostic aspects of the various applications of radioisotopes to the study of human disease.

A basic course is offered twice annually for medical personnel wishing to qualify for a radioisotope license to engage in this type of medical practice. Activities offered to other trainees include extension of training, special projects or specialty courses.

The Division participates in teaching nurses, medical students and technologists by organizing visits to the laboratory, clinical conferences, consultations and clinic rounds and teaching courses.

EDUCATIONAL ACTIVITIES

The Division offers the following courses:

Basic Clinical Radioisotope Application Course

This 8 week course consists of clinical conferences which stress the usefulness of radioisotopes in the resolution of a diagnostic problem. Therapy with internal emitters is also included. Laboratory procedures are keyed to the clinical material which is selected to provide a wide variety of clinical states which come into the trainee's consideration, to cover general presentation of the various applications available in current practice in nuclear medicine.

Subject matter treated in this course includes: thyroid disorders, cardiovascular system, liver and kidney function, gastrointestinal absorption, hematological applications, analysis of fluid compartments and electrolyte turnover, tumor localization, organ visualization, and radioisotope therapy of thyroid disorders.

Trainees and teaching staff correlate points of clinical interest with the various tests performed. Teaching is based on demonstrations, laboratory performance of tests, discussions of results, conferences and audiovisual presentations.

The course is satisfied when the student completes at least 80 adequately performed diagnostic procedures and evaluates and treats 3 patients with thyroid disorders (Table 1).

Nuclear Medicine Course for Medical Technologists

This course, offered this winter for the first time to students of the Medical School of the University of Puerto Rico, consists of 9 sessions, of 4 hours each, in which clinical conferences and laboratory procedures of the major areas of diagnosis in nuclear medicine were presented. The Medical

Technology student is thus offered the opportunity to learn about this discipline and encouraged to extend training in this field.

Informal Courses

Practical training is offered for extended periods to students wishing to acquire more clinical and laboratory experience working with patients under the guidance of the laboratory staff. Opportunities are available in thyroid diseases, hematology and radioisotope localization studies.

There were 43 participants in the nuclear medicine course for medical technologists. They appear in Table 2. There were 7 participants in our other courses, who are listed in Table 3, and whose professional background and countries of origin appear in Table 4.

The various teaching diagnostic or therapeutic procedures done for or by the students appear in Table 5. Table 6 illustrates the service load of diagnostic and therapeutic procedures of the Division during past year.

RESEARCH IN PROGRESS

Evaluation of Antithyroid effect in hyperthyroid patients with Radioiodine tests

During the past year 13 new patients were included in this study. The radioiodine uptake at 24 hours averaged 77%, which under tapazole administration fell to 35.8% at 3 hours and to 17.4% at 24 hours. There were 12 patients under the effect of tapazole whose residual uptake values at 24 hours were above one fourth of the 3 hours value. By this criterion, only one patient would have been predicted sensitive to the drug. However when the 24 hour values post tapazole were compared with the 24 hour pre-tapazole uptakes, only 3 patients showed residual 24 hour uptakes above one fourth of the baseline values at 24 hours. When the KCNS discharge test was given to 7 patients, significant discharge of iodide was observed in all of them. By this approach all 7 patients showed considerable blockade of thyroid hormone synthesis and consequently, they are expected to respond well to drug therapy.

Thirteen patients were studied with propylthiouracil. 24 Hour baseline radioiodine uptake, compared to 3 and 24 hour uptake values under propylthiouracil effect, showed depression of radioiodine uptake by the thyroid gland, but not as marked as in the cases under the influence of tapazole. The patients had average baseline radioactive uptake values of 75.0%; under propylthiouracil the 3 hour and 24 hour uptake values averaged 46.0% and 39.9%, respectively. None of these patients could have been judged responsive to the drug on the basis of a comparison of the 24 hour uptake value under propylthiouracil with 3 hour uptake, also under this drug, or with the 24 hour baseline value. When the KCNS discharge test was given to 6 patients medicated with propylthiouracil, a strong response was observed in 3 of them. The greater detection of responders by the KCNS discharge method is related to the fact that the degree of thyroid blockade by the antithyroid medication is maximal at the time of the test (3 hours) and is much less at 24 hours. The medication is not repeated during the day for the performance of the 24 hour test so that a greater amount of organification may occur at this time. By reproducing the clinical situation with the administration of antithyroid medication throughout the 24 hour period, one would anticipate a similar effect as that observed at the 3 hour measurement. The next group of patients will be studied with this approach.

Organ Scan and Tumor Localization

Radioisotope localization of thyroid, brain, liver and kidney constitute

the major organs studied, with the exception of lung scanning studies which were done primarily to detect pulmonary embolism. Clinical and histopathologic correlation of the scan pattern, when positive or non-contributory, has been good in patients coming to operation, or in whom final diagnosis has been confirmed at autopsy, laboratory procedures or by clinical observation.

Effects of external irradiation on thyroid gland

Eight patients were followed after their thyroid glands were irradiated during the course of radiotherapy for extrathyroidal carcinoma. Radioiodine studies of uptake, PBI 131 Conversion Ratios and PBI 131 Plasma levels were performed several times during the period of observation which was of 1 year in 5 patients and 2 years in the remaining three. The 24 hour thyroid uptake of radioiodine showed a consistent tendency to depressed values except in two patients who had no change. Both the PBI 131 Conversion ratios and PBI 131 plasma levels showed low values before and after radiation, with some depression in the majority of patients, which does not seem significant. The average values of the tests before radiation and at the time of last observation were as follows:

	<u>Before Radiation</u>	<u>Last Observation</u>
24 Hour Uptake	16.8%	11.5%
PBI 131 Conversion Ratios	5.4%	3.7%
PBI 131 Plasma Levels	0.03% per liter of plasma	0.025% per liter of plasma

Results in this group would indicate that after the initial phase of depression reported in last year's annual report, there follows a rebound phase in which nearly pre-radiation values are approximated, which apparently is followed by another phase of moderate depression in most of the patients. Further follow up should prove helpful in establishing whether this depressed phase of thyroid function will eventually lead to a hypothyroid state or to recovery.

Experimental Hepatotoxicity in dogs with Thioacetamide (Dr. A. Rodríguez Ollerós)

During the past year the early lesion induced by thioacetamide in the liver of dogs was investigated by various tests. It was found that with the battery of tests of liver function the earliest biochemical alteration could be detected by the BSP test (Bromsulphthalein test) and by I-131 Rose Bengal liver scanning. Electron microscopy at this early stage of toxicity was also carried out and demonstrated the ultrastructural changes at the subcellular level, particularly the mitochondrial system, which is damaged early after the administration of thioacetamide and upon breakage is responsible for the release of liver transaminases.

Study of Renal Blood Flow:

Clearance studies of renal blood flow by the classical method using the well known standard technique of the paraaminohippuric acid clearance test have not been widely used in clinical medicine routinely as a diagnostic test of renal function because it is cumbersome and laborious to perform; yet, because of its exacting nature, it remains the method of choice as a standard reference. Recently since the advent of radioactive labelled substances appropriate for renal work, a group of clearance techniques have been proposed for the determination of renal blood flow. Diodrast and Hippuran labelled with radioactive iodine^(1,2) have been described as effective tools for this determination. Three techniques have been

described: (a) infusion method with catheterization of the urinary bladder; (b) infusion method without catheterization; and (c) a single injection technique which requires no catheterization, but which may be carried out either by multiple blood sampling or by assay of two bloods samples and an externally detected blood clearance curve. The methods for the determination of the effective renal plasma flow (ERPF) and for the glomerular filtration rate (GFR) based on the multiple sampling procedure were referred to in Schedule 189 FY 1968. In any of these methods, the blood activity curve is divided into two components which are analyzed in terms of the two compartment formula.

Effective renal plasma flow is being studied at this laboratory with I-131 hippurate using the method proposed by Blaurox et al. Patients with renal disorders are being referred by and studied in collaboration with the Nephrology unit of the University Hospital. The group also includes patients with normal renal function as controls and patients with a variety of thyroid disorders from our laboratory to assess the effect of thyroid dysfunction on renal circulation. A total of 112 patients have been examined; of which 15 are patients with normal renal function as controls, 27 are patients with varying degrees of renal dysfunction and 40 are patients with thyroid disorders (9 hyper and 31 hypothyroid individuals). Clinical and laboratory correlation of the degree of renal impairment detected by the radioisotopic determination will follow. The study of additional clinical material will also continue.

STAFF

Dr. J. O. Morales terminated his one-year period as a joint appointee of this Division and the School of Medicine.

Dr. Pedro Juan Santiago, Pediatrics, resigned as joint appointee of this Division and the School of Medicine. He continues on our staff as Chief Scientist Ad Honorem, specializing in pediatric hematology.

Dr. A. Rodríguez Olleros, Professor of Pharmacy at the University of Puerto Rico and the School of Medicine, was appointed Chief Scientist Ad Honorem of this Division.

SCIENTIFIC MEETINGS

Dr. Sergio Irizarry attended the Annual Meeting of the Society of Nuclear Medicine, St. Louis, Missouri, June 1968.

Dr. Aldo E. Lanaro attended the Second Latin American Congress of Biology and Nuclear Medicine, Mar del Plata, Argentina, November 1968. At this meeting Dr. Lanaro presented a talk on "The Teaching Programs of this Division and its Relation to Training in Latin America."

TABLE 1

Two Month Training Program on
Clinical Applications of Radioisotopes

UNITS OF STUDY	AVERAGE NUMBER OF PROCEDURES PER TRAINEE
Thyroid Function	35
Routine uptake measurements	(15)
Assay of radioactive thyroid hormone levels in blood	(15)
Modified tests of thyroid function	(5)
Dynamic functions of the hepatic, renal, and vascular system	20
Hematologic applications of radioisotopes	5
Tumor Localization studies	10
Gastrointestinal absorption	5
Electrolyte and fluid balance	2
Therapeutic procedures	<u>3</u>
TOTAL	80

TABLE 2

Basic Course in Clinical Applications of Radioisotopes

January 1 to February 23, 1968

- | | |
|----------------------------|-----------|
| 1. Zobeida de Jesús Malpas | Venezuela |
| 2. María de las M. Iñiguez | Dom. Rep. |
| 3. Ricardo Socolsky | Argentina |
| 4. José R. Mariaca | Bolivia |

July 15 to September 7, 1968

- | | |
|-------------------------|-------------|
| 1. Dinorah C. Rodríguez | Dom. Rep. |
| 2. Gerhard B. Ramírez | Puerto Rico |

Special Training Tumor Localization and Organ Visualization

September 1-30, 1968

- | | |
|---------------------------|---------|
| 1. Rodrigo Fierro Benítez | Ecuador |
|---------------------------|---------|

Orientation in Nuclear Medicine

November 12 - December 13, 1968

- | | |
|--------------------------|-------------|
| 1. Buitrago Luna, Carmen | Puerto Rico |
| 2. Burgos Pérez, Julia | Puerto Rico |

(Orientation in Nuclear Medicine, continued)

3. Cabrera Rivera, Olga	Puerto Rico
4. Colón Velázquez, Carlos M.	Puerto Rico
5. Correa Colón, Wanda S.	Puerto Rico
6. Cruz Colón, Gloria María	Puerto Rico
7. De Armas Vázquez, Lillian	Puerto Rico
8. Fernández Vives, Aida	Dominican Rep.
9. García Zabala, Elba	Puerto Rico
10. González Vázquez, Julia	Puerto Rico
11. Hernández Molina, Ana Nilsa	Puerto Rico
12. Jiménez Santiago, Ana	Puerto Rico
13. Juliá Reichard, Nora C.	Puerto Rico
14. Macaraig, Vilma	Puerto Rico
15. Martínez Robles, Elba	Puerto Rico
16. Medina Aguayo, Ana Celia	Puerto Rico
17. Medina Rivera, Ana Milagros	Puerto Rico
18. Méndez Rosa, Ileana	Puerto Rico
19. Monagas Acosta, Huguette	Puerto Rico
20. Nieves López, Noelia	Puerto Rico
21. Ojeda Reyes, Gladys	Puerto Rico
22. Ortiz Cruz, José L.	Puerto Rico
23. Padilla Berríos, Luz Marina	Puerto Rico
24. Pagán López, Alba Griselle	Puerto Rico
25. Pérez Oronoz, Gilda Ileana	Puerto Rico
26. Quiñones Ramos, Nilda Paula	Puerto Rico
27. Ramírez Acevedo, Rosa I.	Puerto Rico
28. Ramírez Díaz, Ivelisse	Puerto Rico
29. Ricart Quintero, Olga	Puerto Rico
30. Rivera Ferrer, Awilda	Puerto Rico
31. Rivera Lamberty, Myrna	Puerto Rico
32. Rivera Ortiz, Margarita	Puerto Rico
33. Rivera Rivera, Milagros	Puerto Rico
34. Saldaña, Irma Josefina	Puerto Rico
35. Santiago Becerra, José	Puerto Rico
36. Sellés Hernández, José	Puerto Rico
37. Sobrino Forteza, Wanda	Puerto Rico
38. Tapia Cruz, Gladys	Puerto Rico
39. Texidor Carmona, Migdalia	Puerto Rico
40. Trinidad Salgado, Myrna	Puerto Rico
41. Vallecillo Emanuelli, Priscilla	Puerto Rico
42. Verges Ramos, Hilda	Puerto Rico
43. Urdaz Alvarez, Vivian	Puerto Rico

TABLE 3

Number of Trainees

Type of Student	Number
M.D.	5
Medical Technicians	2
Medical Technologists	<u>43</u>
	50

Origin	Number
Puerto Rico	43
Latin America	7
Argentina (1)	
Bolivia (1)	
Dom. Rep. (3)	
Ecuador (1)	
Venezuela (1)	
	<u>50</u>

TABLE 4

Procedures completed, observed or discussed by students

TRAINEE	COURSE	ob- served	Done	Discussed at Clin.Sessions	TOTAL
Zobeida de Jesús Malpas	Clin. Applic. Rad.	114	139	139	392
María M. Iñiguez	" "	126	135	139	400
Ricardo Socolsky	" "	95	125	139	359
José R. Mariaca	" "	147	109	139	395
Dinorah C. Rodríguez	" "	88	135	162	385
Gerhart B. Ramírez	" "	85	136	162	383
Rodrigo Fierro Benítez	Sp. training Tumor Loc. and Organ Vis.	-	97	-	97
	TOTAL	645	876	880	2411

TABLE 5

Diagnostic and therapeutic procedures Carried Out During 1968

Thyroid Studies	-----	1938
Liver Studies	-----	132
Heart Studies	-----	31
Kidney Studies	-----	224
Gastro-Intestinal Studies	-----	21
Blood Studies	-----	18
Tumor Localization	-----	47
Training Procedures	-----	2591

TOTAL		5047



Radiotherapy technician Irene Lopez de Velázquez, R.N., positions a patient for treatment with an Eldorado "A" teletherapy cobalt-60 unit

RADIOTHERAPY AND CANCER

The Radiotherapy and Cancer Division has a three-fold objective: education, research, and service.

The Division functions as part of the radiotherapy department of the I. González Martínez Oncologic Hospital, adjacent to the Biomedical Building at the Puerto Rico Medical Center. The Oncologic Hospital provides some of the paramedical personnel, equipment and space, operating rooms, hospital beds, outpatient facilities, clinical laboratories, and medical services essential to the care of cancer patients.

The hospital renders services to over two-thirds of Puerto Rico's indigent cancer cases. Since May 1966, it has also provided the radiotherapy services to patients of the Puerto Rico Medical Center.

At the academic level, the Radiotherapy Division operates as the radiotherapy section of the University of Puerto Rico School of Medicine. It also works closely with the Cancer Control Program of the Puerto Rico Department of Health.

Partial support for this program is obtained from the National Cancer Institute training grant through the University of Puerto Rico School of Medicine.

EDUCATION PROGRAM

The educational program includes the radiotherapy residency program (long term training), short-term radiotherapy training course, in-service cancer training for medical students, in-service training for radiological physicists and radiotherapy technicians, and a series of lectures in radiotherapy and cancer offered to third-year medical students.

The radiotherapy residency program, designed to prepare qualified radiation therapists, 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. Residents acquire background in clinical oncology through 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 in solid sources (needles, tubes, wire), such as radium, strontium, cobalt, iridium, and cesium.

The short-term radiotherapy training 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 training for medical students acquaints 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 and radiotherapy technicians is provided as the demand calls for it. These persons are allowed supervised practice in the division's facilities.

The radiotherapy of cancer lecture course for third year medical students is offered yearly as part of the medical school curriculum. Twelve lecture hours highlight epidemiology of cancer, radiological physics, radiobiology, clinical radiotherapy, and radioisotopes in therapy.

EDUCATIONAL ACHIEVEMENTS

Formal programs and courses were offered regularly to physicians and medical students. These 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, Curiotherapy and hospital in-patient area, follow-up, and radiological physics). Resident physicians in the program also rotated through the Pathology Department of the Oncologic Hospital, the radioisotope courses of PRNC, and the Medical Sciences and Radiobiology Division for radiobiology training. See Table 1 for the regular activities of the Radiotherapy Division, Table 2 for the list of trainees, and Table 3 for service activities.

The following former trainees of this division are heading, or participating in, radiotherapy teaching programs in their communities: Dr. Mario Vuksanovic, University of Miami; Dr. Efrain Navarro, National Cancer Institute in México City; Dr. Modesto Rivero, National University in Caracas, Dr. Juan Reusche, Instituto de Radiología Cayetano Heredia, Lima; Dr. Oriel Alva, Roffo Cancer Institute, Buenos Aires, Argentina. Drs. Guillermo Gómez, Alvaro Ariza, and Alvaro Rosas participate in the training program at the Instituto Nacional de Cancerología in Bogotá, Colombia. Dr. Silvio A. Aristizábal participates in the training program at Vanderbilt University in Nashville, Tennessee. Dr. Stephen Brown participates in the training program at the University of Vermont in Burlington, Vermont. The following former trainees are directing radiation therapy programs; Dr. Ernesto Amadey, Corrientes, Argentina; Dr. Lucas Di Rienzo, Córdoba, Argentina; Dr. Arturo Valencia, Pereira, Colombia; Dr. Napoleón Matos, Lima, Perú; and Dr. Andrés Peralta, Santiago, Dominican Republic.

The radiotherapy staff also participated in the teaching programs of other divisions of PRNC and in the weekly Tumor Conference of the Medical School, in the weekly Tumor Conference of the San Juan City Hospital, and in the New Patients Conference of the Oncologic Hospital (Monday through Friday). The radiotherapy of cancer lecture course for third year medical students was presented again this year.

STAFF

At year's end, the Radiotherapy Division had four radiotherapists, one physicist, an assistant physicist, and a biostatistician. The services of a

research medical records librarian, three graduate nurses, three clerical persons, and a photographer-electronic technician, were also available. Personnel is still insufficient for the needs of the division, particularly at the radiotherapist level; the present patient and teaching load requires at least six radiotherapists.

TRAINEES

Short-term Radiotherapy Training

1. One month training for residents in Radiology at the Veterans Administration Hospital in the Bronx, New York by special arrangement with Dr. B. Roswit, Chief of Radiation Therapy Service at that Institution.

<u>Name</u>	<u>Country</u>	<u>Date</u>
Dr. David A. Chastanet	United Kingdom	January 1968
Dr. Justo M. González	Puerto Rico	February 1968
Dr. Bernard Ghelman	Brazil	March 1968
Dr. Po-Young Li	Formosa	April 1968
Dr. George Aramendía	Argentina	May 1968
Dr. Danuta Montorfano	Argentina	September 1968
Dr. Jong Ok Park	Korea	October 1968
Dr. In Sook Song	Korea	November 1968
Dr. Ramón Acosta	Puerto Rico	December 1968

2. Dr. G. Stephen Brown, U.S.A., fourth year resident at Penrose Cancer Hospital Colorado Springs, Colorado, spent March to June 1968 training in our program. Doctor Brown is a member of the faculty of the University of Vermont School of Medicine.

Long-term Training

Dr. Ildefonso Arenas-Bueno, from Spain, a fourth year radiotherapy resident who spent the first three years of training at the Penrose Cancer Hospital, Colorado Springs, Colorado, trained in this program from January 1 to December 31, 1968.

Dr. Silvio A. Aristizábal, from Colombia, spent three years of training as resident in radiotherapy at our Institution, from July 1965 to June 1968. Doctor Aristizábal is in his fourth year of training at the Radiotherapy Dept., Vanderbilt University Hospital, Nashville, Tennessee, where he also holds a position as Instructor in Radiotherapy.

In-service Cancer Training for Medical Students

<u>Name</u>	<u>Country</u>	<u>Date</u>
Lorenzo Lizardi	Puerto Rico	January 1968
Ana I. León Valiente	Puerto Rico	January 1968
Tomás Hernández	Puerto Rico	April 1968
Sonia Dones	Puerto Rico	June 1968
Arturo A. Ydrach	Puerto Rico	June-July 1968
Jerome Ngangane	Nigeria	June-July 1968
Omar Salazar	Cuba	June-July 1968
José M. Sobrino	Puerto Rico	June-July 1968
José F. Irizarry	Puerto Rico	June-July 1968
José R. Hernández	Puerto Rico	June-July 1968
Frank Rodríguez	Puerto Rico	June-July 1968
Francisco Robert	Puerto Rico	June-July 1968
Moraima M. Landrau	Puerto Rico	June-July 1968

(In-service Cancer Training, continued)

José Víctor Figueroa	Puerto Rico	July-Sept. 1968
Louis A. Schuller	New York	Sept.-Dec. 1968
Irving Klapp	New York	Sept.-Dec. 1968

RESEARCH PROGRAM

A. Current Research Projects at End of Year

1. Dose time relationships in the external irradiation of carcinoma of the uterine cervix: comparison of 4500R vs. 5000R (See annual report 1967, page 61).
2. Fractionation in radiation therapy of carcinoma of the uterine cervix: 3 vs 5 fractions per week (See annual report 1967, page 61).
3. Fractionation in radiation therapy of inoperable breast cancer: 1 vs 5 fractions per week (See annual report 1967, page 61).
4. Surgical adjuvant breast project (National Study) (See annual report 1967, page 63).
5. Radiotherapy for carcinoma of the prostate - Stage C (National Study) (See annual report 1967, page 63).
6. Study of the incidence of leukemia in patients with cervical cancer treated with radiation (National Study). (Discontinued-See annual report 1967, page 63).
7. Study of Optimal irradiation in carcinoma of the esophagus: A boost of irradiation 2 weeks post-radiotherapy.
8. Fractionation in radiation therapy of post-surgical breast cancer: 3 vs 5 fractions per week.
9. Study of tumor regression in carcinoma of the cervix.

B. Projects Terminated During the Year

1. Fractionation in radiation therapy of head and neck cancer: 3 vs 5 fractions per week. This project, previously described, aims at comparing tumor and normal tissue effect of irradiation of head and neck cancer using two fractionation regimes of the same weekly dose (3 vs 5 fractions). The study of the accumulated data will be carried out when the clinical experience of the individual tumor sites is analyzed. Over 300 cases were randomly included in the study.
2. Pilot Study of Split-Course Irradiation in Carcinoma of Base of Tongue. This project aims at a comparison of 4 radiation dose fractionation regimes for advanced carcinoma of the base of the tongue (lesions where the primary or metastatic tumors measured 5 cm or over). A total of 48 cases were randomly distributed for the following treatment modalities: 3000 R in 2 weeks, followed by a 2 or 3 weeks rest period, then a repetition of the irradiation (3000 R in 2 weeks) utilizing fractions of 500 R 3 times per week, 3000 R in 2 weeks, followed by a 2 or 3 weeks rest period, then a repetition of the irradiation (3000 R in 2 weeks) utilizing fractions of 300 R 5 times per week; 6000 R in 7 weeks with 170 R fractions 5 times per week and 6000 R in 7 weeks with fractions of 285 R 5 times per week. The study was analyzed concerning tumor control and

normal tissue damage and the results will be presented at a national scientific meeting in Philadelphia in April 1969.

3. Review of Clinical Experience with Carcinoma of the Uterine Cervix - Year 1962. This is a retrospective clinical study of tumor control and normal tissue damage among the patients with carcinoma of the cervix treated with irradiation at our department in the year 1962. The results of this study will be subsequently reported.

4. Study of Results of Treatment Techniques in Adenocarcinoma of the Endometrium. This is a retrospective study of treatment results of adenocarcinoma of the endometrium patients treated at the Radiotherapy Department of the I. González Martínez Hospital during 1955-1965. The combination of external irradiation followed by intracavitary curietherapy followed by surgery has given outstanding curability, particularly in Stage II patients where the disease has invaded the cervix. A scientific paper based on this work was presented at the Annual Meeting of the American Radium Society at Miami in April 1968, and it will be published soon in the American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine.

5. Review of Clinical Experience with Wilms' Tumor - Years 1941-1966. This is a study of the clinical experience with Wilms' Tumor at the I. González Martínez Oncologic Hospital during 1941-66. The best results were obtained by nephrectomy followed by irradiation to the tumor bed. A scientific paper based on this work was presented at the Annual Meeting of the Puerto Rico Medical Association and it will be published during the year 1969 in the Boletín de la Asociación Médica de P. R.

6. Review of Clinical Experience with Patients with Brain Tumors - 1941-66. This is a retrospective study of the clinical experience with brain tumors at the I. González Martínez Hospital conducted by Doctor Aristizábal, a trainee, who is expected to prepare a paper for publication based on this work.

7. Review of Clinical Experience with Cases of Carcinoma of the Pyriform Sinus, 1955-67. This is a retrospective study of the clinical experience with carcinoma of the pyriform sinus at the I. González Martínez Hospital during 1955-67 conducted by Doctor Arenas, a trainee, who is preparing a paper for publication on this work.

STAFF POSITIONS

1. During the year Dr. Victor A. Marcial held the position of Associate Director for Medical Programs while acting as Director of the Radiotherapy and Cancer Division. In addition, he was member of the Safety Committee of PRNC and Chairman of the Committee for Human Uses of PRNC. He held the following other appointments in Puerto Rico: Chief, Radiotherapy Department, Oncologic Hospital, Consultant for Professional Education of the Cancer Control Program of the P. R. Department of Health; Member of the Advisory Board of the P.R. Radiation Protection Program; Member of the Subcommittee for Clinical Services of the Puerto Rico Medical Center; Representative for PRNC at the Medical Policy Committee of the P. R. Medical Center; Member of the Medical Board of the University Hospital; Member of the Executive Committee of the Oncologic Hospital; President of the Medical Staff of the I. González Martínez Hospital; Member of the Cancer and Utilization Committee of the University Hospital, Member, Planning Committee, P. R. Regional Program for Heart, Cancer and Stroke. In addition, he held the following overseas appointments: Counselor for P. R. to the Interamerican College of Radiology; Counselor for P. R. to the Radiological Society of North America; Member of the U. S. Committee for Radiation Therapy Studies;

Member of the Task Force for Cancer Data Coding Member of the Cancer Commission, American College of Radiology; Member of organizing committee of the Tenth Interamerican Congress of Radiology to be celebrated in San Juan, May 1971; Co-chairman, Conference for Radiobiology as Applied to Radiation Therapy to be held at Carmel, California in September 1969; Project Director of the Split-Course Radiotherapy of Cancer - Clinical Trial - a national study.

2. During the year Dr. José M. Tomé held the position of Chief Scientist I at the Radiotherapy and Cancer Division of the Puerto Rico Nuclear Center, he was also a Member of the Board of Examiners of the Radiology Technicians; Member of the Library Committee of the I. González Martínez Hospital; Member of the P. R. Gastroenterology Society; and Member of the Education Commission of the Interamerican College of Radiology.

3. Dr. Jeanne Ubiñas held the position of Chief Scientist I at the Radiotherapy and Cancer Division of the Puerto Rico Nuclear Center. She was Member of the Planning Committee on Cancer and Heart Stroke, P. R. Department of Health; she also acted as Director of the Cancer Control Program of the Puerto Rico Department of Health.

4. Dr. Antonio Bosch held the position of Chief Scientist I at the Radiotherapy and Cancer Division of the Puerto Rico Nuclear Center and was Member of the Emergency Committee of the Puerto Rico Medical Center.

5. Dr. Guillermo Gómez-Cárdenas held the position of Visiting Radiotherapist at the Radiotherapy and Cancer Division until May 1968.

VISITING SCIENTISTS

March - Dr. Harold E. Johns, Director of the Biophysics Department of the University of Toronto, Canada, acted as consultant to the program under the Training Grant.

April - Dr. Chahin Chahbazian, Radiotherapist from the Penrose Cancer Hospital, Colorado Springs, Colorado - Consultant under the Training Grant.

June - Dr. L. G. Lajtha from the Christie Hospital and Holt Radium Institute, Manchester, England - Lectured on Stem cell studies at the Paterson Laboratories in Manchester.

November - Dr. Ruheri Perez Tamayo, Chief of the Radiotherapy Department, Ellis Fischel State Cancer Hospital, Columbia, Missouri - Consultant under the Cancer Control Program, P. R. Department of Health.

December - Dr. John E. Ulmann, Associate Professor of Medicine, University of Chicago, formerly with the Francis Delafield Hospital, New York - Consultant under the Training Grant.

SCIENTIFIC TRIPS OF STAFF

1. Dr. Jeanne Ubiñas: April 1-8, Visiting Professor at Ellis Fischel State Cancer Hospital, Columbia, Missouri. Four lectures were presented. November 20-23, Houston, Texas - M.D. Anderson Hospital and Tumor Institute. Attendance of "Symposium on Carcinoma of the Breast."

2. Dr. Antonio Bosch: November 20-23, Houston, Texas - M.D. Anderson Hospital and Tumor Institute. Attendance of "Symposium on Carcinoma of the Breast".

3. Mrs. M. M. Palacios de Lozano: January 7-13, Visit to the Radiological Physics Department of the M.D. and Tumor Institute, Anderson Hospital, Houston Texas; November 30-December 5, Attendance at the Annual Meeting of the Radio-

logical Society of North America, Chicago, Illinois; December 6-14, Visits to The Mayo Clinic (Rochester, Minnesota); University of Rochester (Rochester, N.Y.); Cornell University (Ithaca, N.Y.); Brookhaven Laboratories (New York, N.Y.); Naval Medical Center, Walter Reed Hospital and National Institute of Health (Washington, D.C.).

4. Miss Zenaida Frias: May 17, Attendance and participation in a meeting of participants in the Split-Course Project, National Cancer Institute, Bethesda.

5. M.D. José M. Tomé: November 14-17, Presentation of a paper at the Annual Meeting of the P. R. Medical Association; December 1-6, Attendance of the Annual Meeting of the Radiological Society of North America, Chicago, Illinois.

6. M.D. Víctor A. Marcial: April 1-7, Visiting lecturer at the Hospital San Jorge, Pereira, Colombia, with presentation of two lectures; April 8-13, Attendance of the Annual Meeting of the American Radium Society in Miami, Florida, with presentation of a paper; Sept. 25-30, participation in the "IX Jornadas Radiológicas Colombianas", Medellín, Colombia, with presentation of four papers and two round tables; Oct. 12, Presentation of a paper at the "Convención Anual del Colegio de Farmacéuticos de P. R.", Hotel Dorado Hilton, Puerto Rico; Oct. 31 to Nov. 4, Attendance of Annual Meeting of the Association of American Medical Colleges, Houston, Texas; Dec. 1-6, Attendance of the Annual Meeting of the Radiological Society of North America, Chicago, Illinois.

Other Activities:

Mrs. M. M. Palacios de Lozano participated in the Course for Students in Radiological Technology offered by the Division of Radiology of the P.R. Medical Center.

Dr. Víctor A. Marcial was named Member of the Advisory Committee for the Regional Cancer Program organized by the Tumor Institute of San Francisco.

CLINICAL RADIATION ACTIVITIES

A. Distribution by site of New Cases Treated - January to December 1968

ORAL CAVITY.....	59	FEMALE GENITAL ORGANS.....	233
Anterior 2/3 of tongue	28	Cervix uteri	185
Floor of mouth	19	Endometrium	25
Other	12	Ovary	17
OROPHARYNX	107	Other	9
Base of tongue	34	MALE GENITAL ORGANS	10
Tonsil	29	URINARY ORGANS.....	26
Other	44	Bladder	21
HYPOPHARYNX.....	33	Kidney	5
NASOPHARYNX.....	12	SKIN.....	103
RESPIRATORY SYSTEM.....	96	BRAIN AND NERVOUS SYSTEM.....	15
Paranasal sinuses	6	BONE AND CONNECTIVE TISSUE....	13
Larynx	40	LYMPHATIC AND HEMATOPOIETIC	
Bronchus and lung	41	SYSTEM.....	47
Other	9	Hodgkin's Disease	25
DIGESTIVE SYSTEM.....	112	Other	22
Esophagus	100	OTHER	32
Other	12		479
BREAST.....	98		517
	517	TOTAL	996

B. Teletherapy Applications.....28,799
(⁶⁰Co, X-rays, 137 Cs)

C. Intracavitary and Interstitial Therapy 240

D. Follow-Up..... 5,646

E. Consultation..... 418



- (1) One of three isolation nurseries. The soybeans are exposed to gamma radiation and the seedlings are screened for beneficial mutations. The artificial illumination is used to slow down the initiation of inflorescence during the winter season(short days)
- (2) For radiobiological experiment with soybeans, Dr. Ferrer equilibrates the seed moisture to a desired content over a constant relative humidity. The vapor pressure is conditioned by glycerol concentration



AGRICULTURAL BIO-SCIENCES

The purpose of the Agricultural Bio-Sciences Division remains two-fold: first, to train students in agricultural and biological research with emphasis upon nuclear science applications in these investigations; and second, to continue basic research programs which are particularly concerned with problems in tropical agriculture that can uniquely be studied by nuclear techniques.

EDUCATIONAL AND TRAINING ACTIVITIES

During 1968, the emphasis on training continued to be directed toward the graduate and post-graduate level. Such training was frequently related to the Division's basic research activities, which are outlined in a later section. As honorary members of the various science departments of the University, Division staff offered the following courses during the year:

Cytogenetics - Dr. F. K. S. Koo. Taught in the Biology Department in Rio Piedras. The cytogenetic effects of radiation was one of several subjects emphasized in lectures and laboratory exercises.

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

Food Chemistry - Dr. S. N. Deshpande. A new course, designed to initiate in the College a Food Science curriculum which may interest graduate students in research of radiation preservation of food.

Nuclear Techniques in Biological Research - Dr. J. Ferrer-Monge, Dr. S. N. Deshpande, Mr. J. Cuevas, and Dr. F. K. S. Koo. Covers the use of radiation and radioisotopes as tools in biological research.

Nuclear Techniques in Agricultural Research - Dr. S. N. Deshpande, Mr. J. Cuevas, Dr. J. Ferrer-Monge and Dr. F. K. S. Koo. Covers the use of radiation and radioisotopes as tools in agricultural research.

Special Studies in Biology - Dr. J. Ferrer-Monge. Provides supervised research in special topics of biology.

Biology: Research and Thesis - Staff. For students doing M.S. thesis research in biology

Agronomy: Research and Thesis - Staff. For students doing M. S. thesis research in agriculture.

In addition, the staff contributed lectures and laboratory sessions to the PRNC basic course in radioisotope techniques, the University's course in radio-chemistry, and PRNC courses in radiation biology and tissue culture.

Graduate Research

A number of graduate students were active in thesis research for M.S. degrees in biology or in agriculture under the supervision of Division staff members. Research topics reflected the broad interests of the Division. During 1968, five graduate students completed their theses.

A. Thesis research completed

1. Combined effects of chemicals and gamma rays on the production of chromosomal aberrations in Tradescantia paludosa - Edith R. 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, a synergistic effect was observed in the production of chromosome breaks and interchanges. It is of special interest to note that the increase in chromosomal interchanges in the combination treatment may be attributable to either an increase of interaction between chromosome breakages produced by two agents or an increase of number of breakages per cell, thus resulting in more interchanges. Research was performed under the direction of Dr. F. K. S. Koo.

2. Effects of ionizing radiation on O-diphenol: oxygen oxireductase - Aida G. 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. Research was directed by Dr. R. A. Luse.

3. Effect of gamma radiation on ripening of banana fruits - Joaquin Oliver-Campos (Puerto Rico). The extent of delay in ripening of Johnson and Montecristo variety bananas given low doses of gamma radiation has been determined. The interaction of radiation delay with subsequent artificial ripening by chemicals has also been studied. Thesis was directed to completion by Dr. R. A. Luse.

4. Preferred host plants of the sugarcane borer, Diatraea saccharalis (F)-Victoriano Quintana (Puerto Rico). Seasonal surveys of infestation by the sugarcane borer in field crops and the University's world grass collections have been made. Corn was found to be the host plant that most nearly satisfies the total requirements of the sugarcane borer. The other species in their relative order of general suitability as a host are teosinte, milo, sugarcane and Merker grass. The remaining species rank relatively low in overall suitability as hosts. Research was directed by Dr. D. W. Walker.

5. Cytogenetic effects of chronic gamma radiation on microsporogenesis and other inherent cytological anomalies in Palicourea riparia - Robert C. Venator (Puerto Rico). This under-story tree species 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 increased with dose and these aberrations decayed 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 polyspory. Research was performed under Dr. F. K. S. Koo's supervision.

B. Thesis research in progress

1. Effect of temperature on the mitotic cycle of Vicia faba - Carmen E. Cintrón (Puerto Rico). The most significant findings to date are: (1) with temperature increase from 0°C to 40°C, there is generally an increase in mitotic rate, (2) at higher temperatures certain cells are severely affected, thus lowering the mitotic index, and (3) at least for the Vicia faba Long Pod variety used, there is a high degree of variability in the mitotic index among the roots (seeds) at given temperature. Completion of this study is expected in March, 1969. (Advisor - Dr. J. Ferrer-Monge)

2. Influence of ionizing radiation on methionine utilization by Escherichia coli - Frederick E. Rushford (Puerto Rico). E. coli cultures exposed to various doses of gamma irradiation are being studied as to their ability to incorporate ³⁵S-labelled methionine into cell constituents. Thesis is expected to be completed in 1969. (Advisor - Dr. R. A. Luse)

Special Training

The Division participated actively in technical and scientific training programs. Training in radiation preservation of food was provided for an International Atomic Energy Agency Fellow, Miss Vachira Jiravatana of Thailand; technical assistance was given to Instituto Centro Americano de Investigación y Tecnología Industrial (ICAITI) of Guatemala. These are further examples of training offered by the staff to five other trainees (sponsored by ORAU, IAEA, the Peace Corps, and OAS) since 1965. Miss Jiravatana, a scientific staff member of the Office of the Atomic Energy for Peace in the Ministry of National Development in Thailand, joined the Division in August and will be with us for one year. She first received a technical orientation and studied all phases of fruit preservation by irradiation, including selection and pre-treatment of mangoes, experimental design, irradiation operation, dosimetry, post-irradiation storage, visual observation and testing by instrument, biochemical analyses, etc. She was then assigned to conduct independently specific research problems closely related to the needs of her homeland. By the end of 1968, Miss Jiravatana amassed extensive data on the shelf-life extension and biochemical changes in irradiated papaya fruits; she is to continue her studies of radiation effects on fruit respiration, flavor, enzymes, etc.

The second program, which receives financial support from the USAID, is the Technical Assistance Program specifically planned for ICAITI. Dr. Pedro Solé and Mr. Carlos Rolz, both Research Engineers at ICAITI and Professors of Chemical Engineering at the National University of San Carlos, spent three weeks each at PRNC in November to study the effects of radiation on papaya fruits. They investigated changes in respiration, flavor, and pectic enzymes using infra-red spectrophotometry, gas chromatography and other analytical methods. During the current fiscal year (FY 1969), PRNC staff members will travel to ICAITI to assist in establishing new food irradiation preservation programs, to serve as consultants, and to review progress. In addition, a radiation program for Central America will be planned, taking into consideration the needs of Guatemala and other Central American Common Market countries.

The Division staff continued to serve as Scientific Advisors at the USAEC "Atoms in Action" Exhibit in Latin America. In April-May 1968, three staff members participated in the Exhibit in Caracas, Venezuela. Their activities were as follows:

<u>Name</u>	<u>Subjects covered</u>
R. A. Luse	Food Preservation by Radiation and Radioisotope Techniques
D. W. Walker	Insect Control by Radiation Sterilization
Carmen Asencio	Food Preservation by Radiation

The time and effort spent encouraging scientific research in Latin America has brought positive results; agricultural and biological investigations initiated during the Exhibits have been continued and further developed in El Salvador, Guatemala, Costa Rica, Nicaragua, Ecuador and others. Work on numerous theses, originally outlined and supervised by our staff, has brought both students and faculty members into contact with nuclear techniques. It is felt that such research promotion in Latin American countries, and cooperation with these scientists, will continue to foster application of nuclear techniques in Central and South American agriculture.

RESEARCH ACTIVITIES

Basic research activity may be discussed under four main categories:

1. Sugarcane improvement. By means of automated analytical techniques, mass screening continued for variants having increased sugar content in the gamma and neutron irradiated and vegetatively-propagated progenies. Selected individual cane seed pieces have been grown in the field to provide material for confirmation analysis for sucrose content; the final selections will be evaluated in the Sugarcane Improvement Program of the University's Agricultural Experiment Stations. It is anticipated that induction of mutations of high sucrose content by gamma rays and neutrons offers one solution to declining yields in sugarcane, still the most important crop in Puerto Rico's economy.

Another project to increase sugarcane yield was the induction of mosaic virus resistance by radiation in several selections susceptible to this disease, but otherwise promising in many agronomical characteristics. In 1968, an improved method for artificial inoculation of mosaic virus was tested, but only a limited amount of material was screened for virus resistance, as the project was shelved in order to develop other programs in line with the Division's limited funds and personnel.

2. Environmental adaptation of crops by mutation breeding. The main objective of this new program is to adapt crops to tropical environmental stresses by mutation breeding. In the summer of 1968, preliminary tests for regional adaptability of chickpeas and a group of soybean varieties and selections from high protein crosses were made. In December, the first generation of gamma ray-treated soybeans (three varieties) was planted in isolated plots with supplemental lighting. This group of material is expected to provide the first massive source from which to select desirable mutant types (adaptive, day-length neutral, high yielding, high protein content, disease resistant, etc.), beginning in the summer of 1969. Other subjects for investigation in the near future include the physiology of environmental stresses as presented by high temperature, high rainfall and humidity, high and low light intensity, etc. This program has been carried out in cooperation with the University of Puerto Rico Agricultural Experiment Station.

3. Radiation Preservation of Tropical Fruits. Considerable effort was directed to the underlying processes associated with radiation preservation of tropical fruits. To continue the previous year's work on characterization of flavor components, mango and papaya macerates were extracted for the volatiles. For mangoes, solvent extraction with chloroform was attempted. The papaya macerates, on the other hand, were subjected to extraction by means of a complex apparatus for isolation of the volatiles. This assembly utilized the principle of flash evaporation and vaporization from a continuous thin heated film. Water and vaporized food constituents were recovered by condensation in a series of cold traps cooled by liquid nitrogen, dry ice and acetone.

Condensates were analyzed by gas liquid chromatography. Judging from retention times for standard carbonyl compounds, there was very strong evidence for the preponderance of carbonyl compounds in the condensates, followed by traces of esters and organic acids. To confirm the occurrence of carbonyl compounds, 2, 4, dinitrophenyl hydrazine derivatives were made from the condensate and subjected to separation by thin layer chromatography.

Studies of the ionizing radiation effect on the kinetics and molecular structure of pectin methyl esterase and its relation to mango fruit softening have been reported previously. In 1968, a group of selected subjects was again investigated with papaya fruits. It has been suggested that pectin methyl esterase may be present in a concentrated form. Increasing doses of radiation may cause disruption of cellular components, thus liberating the enzyme and increasing its activity. Results with papaya strongly suggest that ionizing radiation increased activity of the enzyme pectin methyl esterase; in the irradiated fruits after storage, the increase of enzyme activity was even greater. These results again support the view that cellular degradation caused by radiation and ripening liberates the enzyme and results in its higher activity. For the characterization of the pectic constituents, the papaya pulp was determined for total uronide content and methoyl content.

Financial support to the Radiation Preservation of Tropical Foodstuffs Program provided by USAEC Division of Isotopes Development was terminated in September. Since then, the Division has carried on research on papaya shelf-life extension, radiation effects on its nutritional components and respiration. Preliminary results indicate that a considerable retardation of ripening was attainable by gamma ray treatment at 25-50 Krad levels. This delay in ripening, coupled with the insignificant loss of vitamin C, suggests a very favorable response of papaya fruits to radiation preservation. Other results of the AEC-DID supported program are presented elsewhere in this Report.

4. Radiation Sterilization of Sugarcane Borer. Research carried out by Dr. D. W. Walker, with primary financial support provided by the USAEC Division of Biology and Medicine, is reported elsewhere in this Annual Report.

In addition to the Agricultural Bio-Sciences Division's basic research in agriculture, two projects of fundamental radiobiological importance are incorporated within the Division:

1. Resonance in Radiation Effects. Previous work with metalloenzymes indicates an energy dependence of damage (inactivation) related to the constituent metal. At equal dose, 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. To determine more precisely the nature of damage produced by x-irradiation at the iron K-edge, a very pure preparation of bovine liver catalase was irradiated and studied by the latest techniques of protein chemistry to determine the structural changes in the enzyme induced by such radiation. Results indicate that there was an energy dependence in the inactivation mechanism with maximum effect slightly above the K-absorption edge of the target atom. The inactivation of the catalase molecule was mainly due to the effects on the iron centers, resulting in the release of free iron from the inactivated molecule and the impairment of the integrity of the heme molecules. Data from the spectrofluorometric analysis and iron release studies strongly indicate that the energy absorbed by the iron atom transferred to other linkages in the porphyrin molecule. The amount of iron released followed the total dose of irradiation, but the fluorescence for porphyrin was not detectable until the dose reached 500 kilorads. The gamma inactivated samples increased in optical density in the protein absorption region, indicating a molecular aggravation different in nature from the monochromatic x-ray irradiated sample.

Further studies of the low-energy monochromatic x-ray effect on genetic material labeled with 5-bromodeoxyuridine again confirm that photons with the K-absorption edge energy of the target atom bromine are capable of inducing mutations at a much enhanced rate not known with any other radiations including high-energy photons. In the past year emphasis has been placed on the completion of the action spectrum studies for mutation induction in Escherichia coli and Corynebacterium equi. The workability of irradiating bacteria with high intensity by means of fluorescence line irradiation has been investigated. Radiation action on molecular lesion induction has also been studied. It is generally believed that, among the x-ray induced DNA strand breaks, only the single strand breaks are amenable to repair, double strand breaks are considered lethal, and the repair efficiency for the x-ray induced lesion is very low as compared with that for the UV-induced lesion. These generalizations are being investigated with low-energy monochromatic x-rays.

2. Radiobiological studies of seeds. To further a world-wide program on seed irradiation organized by the International Atomic Energy Agency, the Division has begun investigations of the gamma radiation effect on soybean seeds with different moisture contents. Apparatus for attaining the moisture equilibrium in the seed at any desired levels has been constructed. Preliminary results indicate that soybean is relatively sensitive to radiation as measured by its growth inhibition at the seedling stage. Studies with soybeans and other seeds using neutrons will await the completion of the remodeling of the neutron irradiation facility.

In addition to its own research activities, the Division has promoted cooperative investigations with scientists of other institutions. Several programs were initiated in 1968, including (a) differential adsorption of C-14 labeled herbicides by different types of Puerto Rican soils; (b) H³-thymidine labeling study of chromosomes treated with radiomimetic chemicals; and (c) breaking of dormancy and mutation induction by radiation in the root crop yautias (Xanthosoma atrovierens).

STAFF

There were many changes in the Division's roster in 1968. In July, Dr. Luse departed (on leave of absence for two years) for Vienna, Austria, where he assumed his duties of First Officer in the Genetics and Plant Breeding Section of the FAO/IAEA Division of Atomic Energy in Food and Agriculture and the responsibility for the mutation breeding program for high protein crops and the neutron irradiation program. Also in July, Dr. Kamath resigned to take a position at the University of Iowa, Iowa City, as associate professor. In December, Dr. Walker was granted a one-year leave of absence to serve as an International Atomic Energy Agency Technical Expert assigned to the Atomic Energy for Peace Program at the Nuclear Center in Bangkok, Thailand, to investigate rice stem borer sterility. On the plus side of the balance, Miss Angelica Muñiz joined the Division in September and participated in resonance radiation effect studies in genetic systems. She received her M.S. degree in Biology at the University of Puerto Rico in the summer of 1968.

Dr. Ferrer and Dr. Deshpande were granted joint appointments by the College of Agriculture and Mechanical Arts, U.P.R. in Mayaguez. Dr. Ferrer is Professor of Biology in the Biology Department and Dr. Deshpande is Assistant Professor of Chemistry in the Chemistry Department. Also, Mr. Cuevas received an Ad Honorem appointment as Assistant Professor in the Department of Horticulture, CAAM in Mayaguez.

Two Division members completed graduate studies at the University of Puerto Rico, Mayaguez. Mrs. Edith Irizarry received her M.S. degree in Biology in June and also her promotion to Research Associate I at PRNC; Mr. Victoriano Quintana passed his final examination for an M.S. degree in biology in October and left for Spain to enroll in the School of Medicine at the University of Salamanca.

Dr. Luse was elected Chairman-Elect 1968 of the Puerto Rico Section of the American Chemical Society. Mr. Cuevas was appointed Permanent Secretary of the Sociedad Americana de Ciencias Agrícolas.

On April 28, Dr. Luse accompanied Dr. Gomberg and Mr. Barceló to Colombia to conduct an on-site survey of the humid, low land area lying directly east of the Andes (the Llanos Orientales) to determine what research required highest priority for the development of Llanos agriculture. The second trip to the area was made on July 22-28. Discussions were held with the scientists at the Colombian Institute of Nuclear Studies (IAN) and the Colombian Institute of Agriculture and Animal Husbandry (ICA) and with staff members of the USAID mission in Bogota on the feasibility of agriculture development in the Llanos.

On October 6-10, Dr. Koo visited the Instituto Centro Americano de Investigación y Tecnología Industrial in Guatemala City, Guatemala where he made final plans for the ICAITI staff training program in food irradiation preservation at PRNC and our staff visits to ICAITI.

Several Division staff members attended scientific and technical meetings during 1968. Dr. Luse was an invited participant in a Panel on "Enzymological Aspects of the Application of Ionizing Radiation to Food Preservation" sponsored by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture in Vienna, Austria (April 8-12), delivered a paper on "Radiation inactivation of enzymes important in fruit ripening," and served as chairman of a group drafting recommendations on Food Irradiation. He also attended the IAEA Panel on "New Approaches to Breeding for Plant Protein Improvement" at Svalof, Sweden on June 17-21. On July 7-12, Mr. Cuevas traveled to Trinidad to participate in the 6th Annual Meeting of the Caribbean Food Crops Society. He presented a paper entitled "Preservation of tropical foodstuffs by irradiation." Dr. Koo was invited participant to the Conference on Food Production and Economic Development held in August at the University of Puerto Rico, Mayaguez, and presented a paper on "Soybean protein production improvement in the tropics." This Conference was sponsored by the University and Dr. Graham served on the Organizing Committee. Dr. Graham attended the 8th Annual AEC Food Irradiation Program Contractors' Meeting on October 16-17 in Washington, D. C. Dr. Koo participated in the meeting of the Working Group for the Study of Food Preservation by Radiation sponsored by the Inter-American Nuclear Energy Commission of the Organization of American States in Montevideo, Uruguay during the period December 16-20, and presented a paper on "Puerto Rico Nuclear Center participation in food irradiation program development in Latin America." In December, Dr. Walker attended the Annual Meeting of the Entomological Society of America in Dallas, Texas, where he presented a paper on "Inherited sterility in the sugarcane borer."

Dr. Deshpande attended an intensive short course on "Molecular characterization of polymers" offered by the American Chemical Society at Vassar College, N.Y. on June 3-7.

SUGARCANE BORER CONTROL PROGRAM

The sugarcane borer, Diatraea saccharalis (Fab.) (Crambidae: Lepidoptera) causes direct destruction of plant tissues, often completely destroying young plants, as well as causing sugar inversion in cane stalks. This pest also allows secondary invasion by fungi and bacteria, which reduce sucrose yield. This pest is of major importance in most sugar producing areas of the world; it causes an estimated annual loss of 2.5 million dollars to Puerto Rico's sugarcane crop alone.

Principal research objectives include determining whether Diatraea saccharalis can be rendered sterile by gamma irradiation and studying the bionomics of this species with reference to phases of the life cycle that are applicable to the development of a program of mass-release of sterile adults in Puerto Rico. This project is part of a cooperative effort with the UPR Agricultural Experiment Station.

Since the previous report (Annual Report, PRNC, 1967), work has been concentrated in two main areas: (1) developing of the island test in Vieques, P.R. and (2) inherited sterility.

Approximately 300 adults are harvested daily in the Mayaguez insectary. This line was obtained from the Louisiana State University laboratory in Baton Rouge from Dr. S. D. Hensley. This strain has undergone more than 100 generations in the laboratory on a wheat-germ diet developed by Hensley, Long and Yadov as a modification of the original wheat-germ diet developed by Vanderzant and Adkisson. Adults are vigorous, and of long life. The females lay approximately 350 eggs each, and are well-adapted to a laboratory rearing regime. Larval life-span is approximately 25 days and the pupal stage requires 6-7 days for completing development. Larval and pupal mortality is low, and the frequency of diapause in our laboratory has been less than 5 percent. These factors taken together have given us the assurance that we will be able to produce a sufficient number of sterile adults to complete an island test involving 1000 to 2000 acres of sugarcane.

Food cost remains relatively high; approximately \$2000 per million adults. Labor costs are higher than food costs under the present production schedule but the two will be approximately equal when rearing is on a larger scale. Total rearing costs of insects to be released on a mass-scale will be on the order of 0.5 cents each. Thus we are very interested in achieving the maximum efficiency from our release methods. Inherited sterility is a potential solution to this problem.

Inherited sterility is being investigated in the laboratory and in field cages. The ultimate objective of this work is to achieve a high population level of F₁ adults that are sterile. This might be possible by liberating sufficient numbers of P generation sub-steriles that outbreed with the normal population and produce a large number of F₁ larvae that survive to adulthood, but who are sterile F₁ adults. The amplification ratio is expected to be on the order of 10 times, i.e. ten times as many sterile F₁ adults as were released as P generation sub-steriles.

Laboratory tests confirm that sterility factors are heritable through successive generations in some sublimes when there was continual outbreeding. This hypothesis will be tested under field conditions in a cage.

Host preference was measured using: (1) gravid females to test oviposition preference, (2) first stage larvae for attractiveness and acceptance and (3) development and feeding of third stage larvae in the preferred hosts. This work constituted the M. S. thesis program of Victor Quintana.

Conclusions

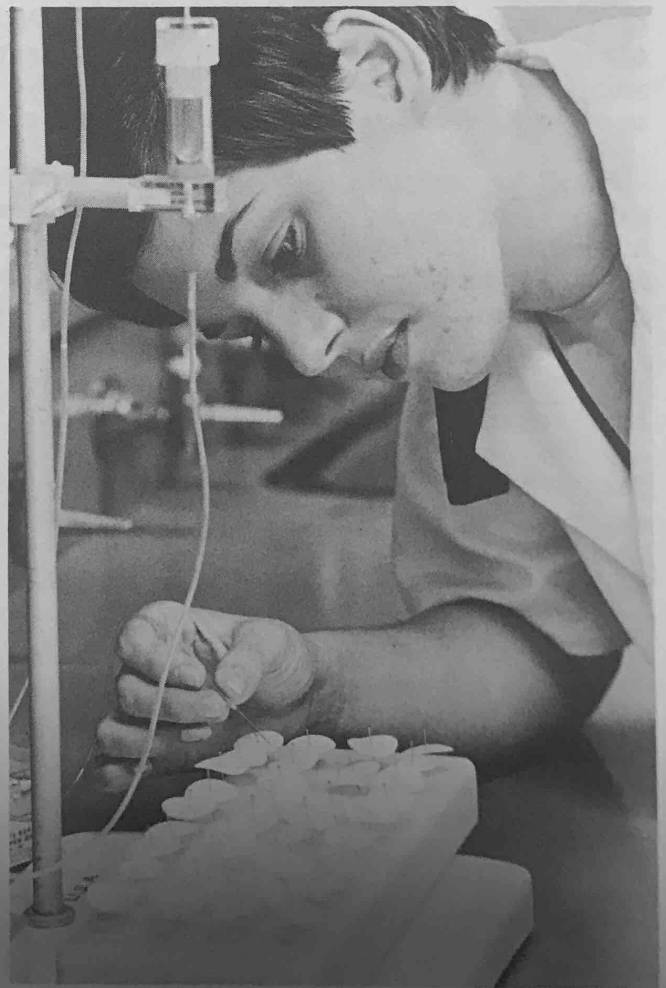
Corn was the most suitable host for first stage larvae as measured by attractiveness and acceptability. Corn was also nutritionally the best plant as measured by the rapid growth and rapid maturation rate of third stage larvae in the nutrition tests. Corn also ranked as the best in oviposition response when many alternate choices had been offered. These tests indicate that corn is the host plant that most nearly satisfies the total requirements of the sugarcane borers.

In relative order of general suitability as a host after corn the species can be ranked as follows: teosinte (Euchlaena mexicana), milo (S. vulgare), sugarcane (Saccharum officinarum), and Merker grass (Pennisetum purpureum).

The remaining species rank relatively low in overall suitability as hosts, but many of them are important oviposition sites and sources of larval food.

A mass-release program of sterile adult sugarcane borers will have to consider areas where cultivated and wild host plants are growing. These plants are a reservoir of this pest and will cause a re-infestation into commercial cane fields.

Preparing for liquid scintillation counting, Miss Angélica Muñiz is delivering fractions onto paper discs following sucrose density gradient centrifugation of E. coli DNA in a study to determine the effect of low-energy monochromatic x-rays on DNA strand breakage



PRESERVATION OF TROPICAL FOODSTUFFS BY GAMMA IRRADIATION

Studies of radiation preservation of fresh mango fruits were continued. The Eldon, Rubi, Sensation, Keitt and Kent varieties, considered to have high marketing potential in the near future both locally and on the U.S. mainland, were obtained from the Fortuna Substation of the University of Puerto Rico Agricultural Experiment Station. Measurement of shelf-life extension (retardation of ripening) and biochemical assays of changes in food quality were made for these exotic varieties.

As described in previous reports, mature green mangoes (physiologically fully developed, but green) were picked the day before irradiation. They were selected so as to exclude those which were bruised or otherwise defective and were then gamma irradiated at 25, 50, 75, 100 and 150 kilorad doses.

The irradiated and non-irradiated fruits were stored in a constant temperature room at 68°F and 80% relative humidity. At appropriate intervals, depending on how the ripening of the fruits progressed, individual fruits were withdrawn and assayed for sugar, starch, ascorbic acid, total carotenoids, and titratable acidity. The progress of ripening was also noted and observations made for rotting or other deteriorative processes. On cutting the fruits, observation was made for blackening or pithing due to the irradiation treatment.

Eldon mangoes treated with 50-150 kilorads of gamma radiation showed a delay in ripening of about 5 days as compared to the non-irradiated controls. After the 7th day in storage, only the 75 kilorad level effected a delay in fruit ripening. At 150 kilorads, the fruits showed skin discoloration; ripening and softening seemed to have been accelerated.

Sensation variety mangoes treated with 50-75 kilorads showed a storage life extension of 7 days as compared to the non-irradiated controls. After 12 days of storage, fruits treated with 100 and 150 kilorads doses seemed to ripen faster than the controls. Those treated at a level of 150 kilorads were soft and revealed no significant loss of ascorbic acid (vitamin C). There was some decrease in the level of total carotenoids at the 75-150 kilorads levels and after 11 days of storage the sugar to acid ratio of fruits treated at 50 and 75 kilorads was appreciably lower than that of the control.

At doses of 50 and 75 kilorads of gamma radiation, Rubi mangoes showed a ripening delay of five days. Generally, the biochemical analyses for this variety showed the same trends as for the Sensation variety.

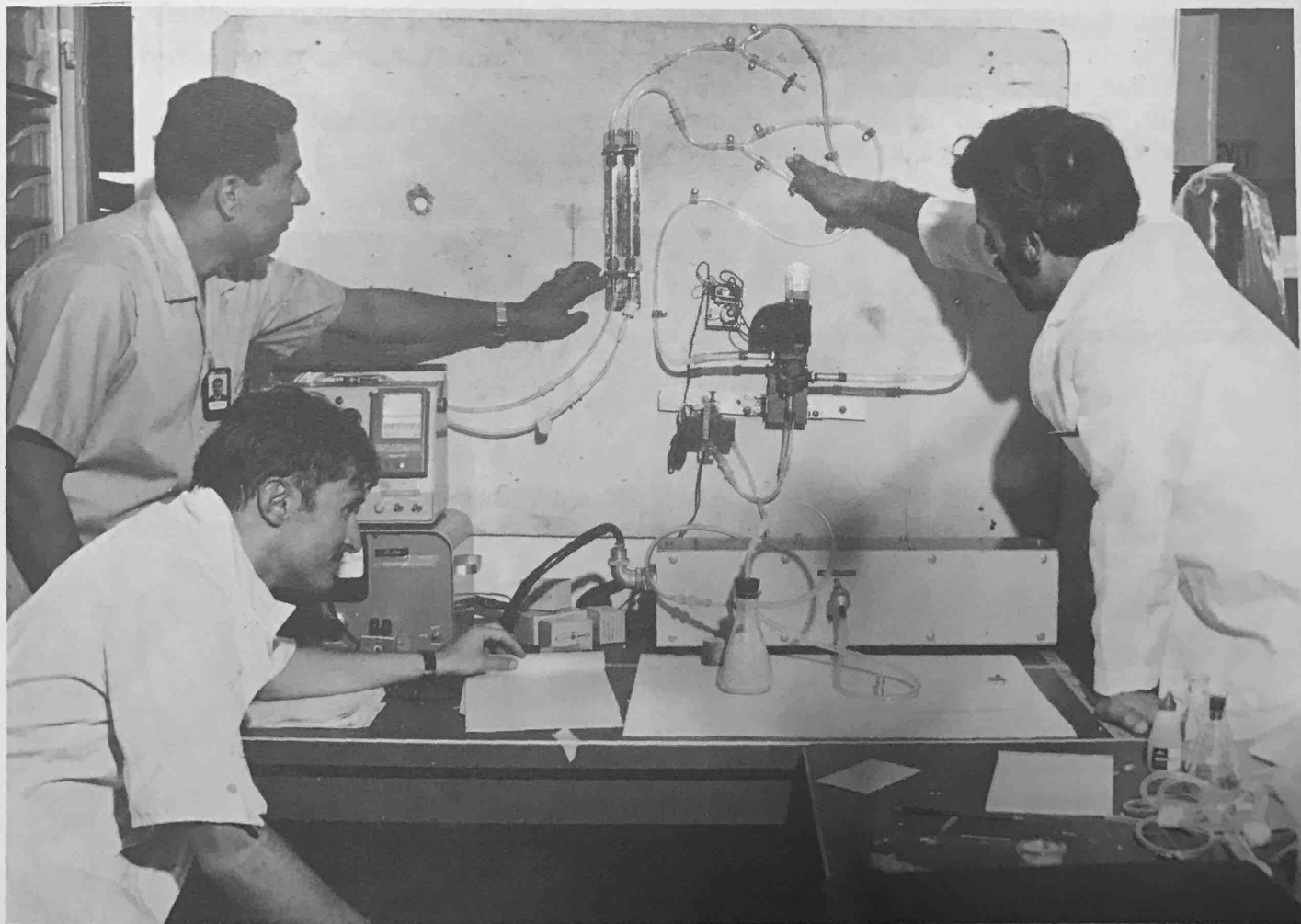
Keitt variety showed a delay in ripening of 6 days, even at the 25 kilorads level of irradiation. The Kent variety showed a delay in ripening of 2 days at doses of 50-100 kilorads.

Both the Rubi and Keitt varieties were much higher in titratable acidity and lower sugar content than other varieties. These, as well as the Sensation variety, were rather low in ascorbic acid as compared to the Mayaguezano variety. From the data gathered over the past 3 years, it seems reasonable to conclude that the smaller Mayaguezano mango is much richer in ascorbic acid and sugar, but lower in titratable acidity, than the larger, more exotic varieties.

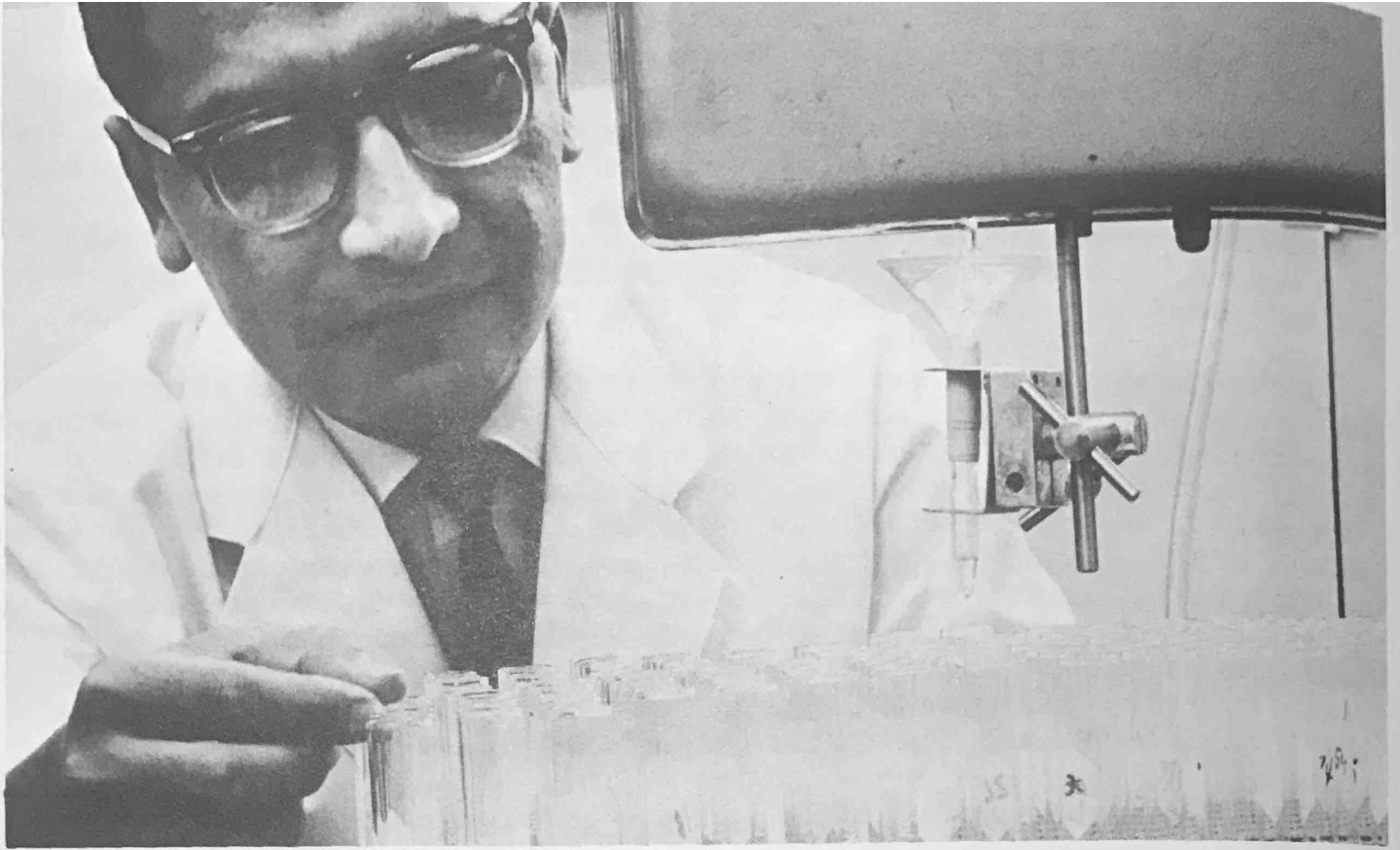
Simulated shipping studies were again conducted this year on irradiated Keitt mangoes. Irradiated fruits and non-irradiated controls were marked as to dose and packed in a crate 20" long x 16" wide x 12" high (inside dimensions).

A temperature recorder was then placed among the fruits. The crate was then carried in the car from Mayaguez to San Juan (about 105 miles) and returned, once a day for 5 consecutive days and then the fruits were stored at 68°F. Shipping in this manner provided the vibration and jarring likely to be experienced during commercial fruit shipment and handling. Immediately after irradiation, at the end of the shipping period, and at subsequent periods during the storage at 68°F, individual fruits from each treatment were examined for appearance and ripening, and analyzed chemically. The data obtained from these studies re-confirm that vibration and exposure to high temperature during shipping speeded ripening in all fruits, but the radiation treatment caused a delay in fruit ripening. There was no significant loss of vitamin C.

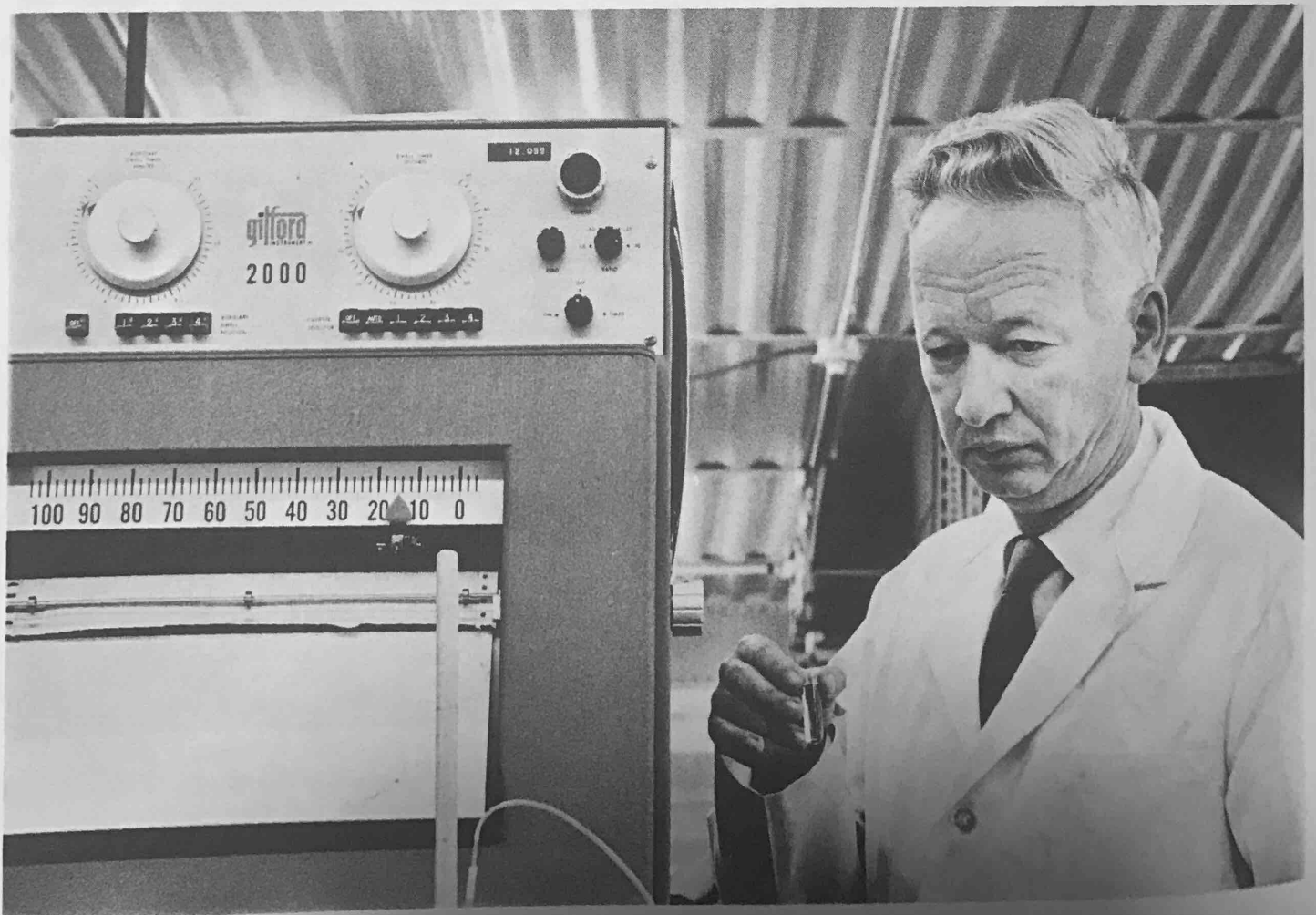
USAEC financial support for this project terminated at the end of September, 1968.



Mr. Cuevas(left) and two visiting scientists, Dr. Solé(right) and Mr. Rolz(center) from ICAITI, Guatemala, make final check on the infrared spectrophotometric set-up for respiration experiment of irradiated papaya fruits



- (1) Mr. Roger Ramos Aliaga of Perú, uses chromatographic column fractionation to observe the effect of irradiation on polynucleotides which produce interferon
- (2) Dr. Raymond A. Brown conducts sucrose density gradient analysis with the Gilford Spectrophotometer



MEDICAL SCIENCES AND RADIOBIOLOGY DIVISION

The Medical Sciences and Radiobiology Division offers training and research opportunities in fundamental nuclear energy aspects of biology, radiation biology, biochemistry, molecular biology, 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.

This report covers educational, training and research activities involving Division personnel. Also included are projects sponsored by the AEC Division of Biology and Medicine:

1. Studies on the host-parasite relationship in Schistosomiasis mansoni: Radiation effects and application of radioisotope techniques
2. Radiation activation of latent virus in wild arthropods and vertebrates
3. Radiation effect on the host-parasite relationship in Trypanosoma cruzi infections (Proposal in study in the DVM)

EDUCATIONAL ACTIVITIES

The following courses were offered during 1968:

1. Tissue culture and radioisotope techniques at cellular level. Offered under the direction of Dr. Ramiro Martínez-Silva. Participants were: Miss Rachelle Evenchick (7/22-8/30/68,) U.S.A., and Dr. Abdel Arandia Patraca (4/19-8/27/68), Mexico.

2. Graduate Course in Virology. Sponsored by the UPR School of Medicine's Department of Microbiology. Offered at PRNC under the direction of Dr. Julio I. Colón, Associate Scientist (ad honorem) & Professor of the School of Medicine.

The participants were:

<u>Name</u>	<u>Country</u>	<u>Name</u>	<u>Country</u>
Felicita Gotay	Puerto Rico	Carlos Roberto Lau	Puerto Rico
Víctor M. Gotay	Puerto Rico	José A. Licha	Puerto Rico
Héctor Gotay	Puerto Rico	Enrique Reynold López	Puerto Rico
Angela T. Hernández	Puerto Rico	Rachel López de Suárez	Puerto Rico
Oristóbal Jiménez	Puerto Rico	Agapito López Rivera	Puerto Rico
Citrajanes Lall	Guyana	Charles Lowry Phillips	United States

3. Radiation Biology (PRNC 510). Given for academic credit by Doctors Jorge Chiriboga, Ramiro Martínez-Silva, and José N. Correa, members of this Division. The following students attended: Aquiles Santana, from Colombia, Luis A. Rodríguez, from Ecuador, and Ramón Cruz Ortiz, Daniel Torres Ortiz, and Heriberto Torres Castro, from Puerto Rico.

4. Special Training. The following persons received training in the topics listed:

<u>Name</u>	<u>Country</u>	<u>Field</u>	<u>Training</u>	<u>Dates</u>
Dr. Oscar Juliao	Colombia	Virologist at the National Institute of Health in Bogotá	Microautography	7/18-24/68
Marcela Estremadoyro de Rendón	Perú	Med. Technologist	Tissue culture Radioisotopes techniques	5/58-10/18/68
Neftale Katz	Brazil	M.D.	<u>Schistosoma mansoni</u> (Use of radioisotopes for testing drugs)	10/7-11/18

5. Special Training in Radiobiology for Radiotherapists. Special training in basic radiobiology, with lectures and laboratory exercises, was offered to Dr. Silvio Aristizabal of Colombia by Division staff members Dr. Ramiro Martinez-Silva, Dr. Julio I. Colón, Dr. Jorge Chiriboga and Dr. José N. Correa.

6. Orientation on PRNC Program in Tropical Nuclear Biology and Medicine Course. The following postgraduate fellows, who attended a Tropical Medicine Course at Louisiana State University were given a special orientation: David T. Dennis, M. D.; Gerald V. van der Vlugt, M. D.; Clarence A. Parker, Ph. D.; Clayton R. Page, Ph. D.; John W. Rippon, Ph. D.

7. Orientation in tissue culture and radioisotope techniques. Given to the following professors from the Faculty of the Central University in Venezuela: Miss Mercedes U. de Montalvo, Miss Rosaura Belto, and Mrs. Mireys de Gugig.

8. Summer studies. Gerald M. Kidder and Roger Arnold Pedersen from the Graduate Program of the Biology Department of Yale University studied molecular biology here in the summer, using radioisotopes of marine specimens. They were referred to PRNC by the School of Medicine (UPR).

9. Thesis research. The following students continued their thesis research:

<u>Name</u>	<u>Country</u>	<u>Degree</u>	<u>Source of Sponsorship</u>	<u>Title</u>
Carmen Rivera	P. R.	Master Microbiology	NIH	Effect of irradiation on interferon levels
Roger Ramos Aliaga	Perú	Ph. D. in Biochemistry	PAHO and PRNC	Labeling of cocaine and metabolic studies

COOPERATIVE TRAINING AND RESEARCH

1. Division staff members assist in the teaching activities of the following divisions: Physical Science, Health Physics, and Clinical Applications.

2. Research cooperation with Agricultural BioSciences Division. Techniques for analyzing both isopycnic and sucrose density gradients have been established in the laboratory and applied to several problems. Working with Dr. Koo, isopycnic centrifugation has been used to determine the degree and homogeneity of substitution of 5 bromo uracil for thymidine in E.Coli DNA. Sucrose density gradients will then be used to compare the effects of X-ray irradiation upon the substituted and normal DNA. There is particular interest in what the molecular effect will be of the increased energy absorption produced by the heavy atom substitution.

3. School of Medicine UPR.

a. Department of Microbiology - Dr. Julio I. Colón, Virologist, continues as an "ad honorem" member of the PRNC staff.

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

c. Department of Biochemistry and Nutrition - Dr. E. Toro Goyco, Associate Professor of Biochemistry and Nutrition, continued to study the biochemistry of Schistosoma mansoni eggs; he is receiving help from the Division.

d. Dr. Jorge Chiriboga, Professor of Biochemistry (ad honorem) at the UPR School of Medicine, has lectured at the Department of Biochemistry.

e. Garrido Annex: An agreement has been made between Dr. Ramos Morales, Head and Director of the Schistosoma Program of the Department of Medicine, and Dr. Chiriboga, through which the bilharzia laboratory of the Department of Medicine will be used for PRNC research programs. In return PRNC will provide antigens for clinical and epidemiological studies of bilharzia.

4. U. S. Public Health Service. Cooperation on Schistosomiasis research with the group headed by Dr. Frederick Ferguson has continued. Schistosoma cercariae in large numbers were labeled with Se⁷⁵ to study the cercariophagic activity of guppy fish under laboratory and field conditions. It is hoped that these studies will eventually contribute to the better understanding of the ecology of this disease. Mrs. Wilda B. Knight of USPHS is assigned to PRNC.

5. Veterans Administration Hospital. A project on the resistance of mice to Schistosoma mansoni has terminated; the results have been presented for publication. Dr. Rafael Menéndez Corrada, Associate Chief of Staff of the Veterans Administration Hospital, and members of our Division, have begun a time lapse cinematography project on the penetration and development of T. cruzi in DC₂ cells developed in our laboratory. 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.

6. Schistosomiasis Laboratory. Dr. Neftale Katz, the Universidad Federal of Minas Gerais, Belo Horizonte, Brasil, spent two months at PRNC with the Schistosomiasis group.

7. Walter Reed Army Institute of Research. Dr. Lawrence S. Ritchie of the Walter Reed Army Institute, who has been assigned to the Schistosomiasis group of PRNC until June, was appointed Chief Scientist II in the Medical Sciences and Radiobiology Division.

8. Medical Research Center. The Division engaged in a cooperative program with Miss Encarnita Conde, from the Medical Research Center, in the mechanism of action of hemolymph of Biomphalaria glabrata in Schistosoma mansoni parasite in vitro, using radioactive techniques. The Division also provided biological materials for the Schistosoma research of the Medical Research Center.

9. Visiting Scientist from Oak Ridge National Laboratories. Dr. Raymond A. Brown, visiting scientist from the Biology Division of Oak Ridge National Laboratory, arrived in May 1968 to work with the Medical Sciences & Radiobiology Division.

RESEARCH ACTIVITIES

Research is related to radiobiological training activities offered at animal, cell, and subcellular levels. Special emphasis is given to studying the effects of radiation on the host-parasite relationship in various biological systems using tissue culture, arthropods, snails, and other animals. An account of current research, organized by sections, follows:

N-DEMETHYLATION OF COCAINE AND NUTRITIONAL STATUS (Thesis of Mr. Ramos Aliaga)

a. Chromatographic differentiation of cocaine metabolite in mice liver and intestines mouse using the N-demethylase enzymatic system. When cocaine was incubated with microsomal fraction + 9000 x g soluble supernatant along with TPNH₂ regenerating system + Mg in the presence of O₂, stoichiometric amounts of norcocaine and formaldehyde were found as the only metabolites in the liver. With intestinal mucosa, using the same system, benzoilecgonine — not norcocaine or formaldehyde — was found to be the metabolite.

Metabolites were identified by paper chromatography and thin layer chromatography in a number of different solvents. Standards synthesized in the laboratory were compared with enzyme-produced metabolites to prove identity.

Rf of the Metabolites of Cocaine

	Solvent I		Solvent II		Solvent III	
	Standard:Sample**	Standard:Sample	Standard:Sample	Standard:Sample	Standard:Sample	Standard:Sample
Cocaine	.66	.67	.71	.67	.89	.73
*Norcocaine	.20	.20	.34	.35	.72	.73
*Benzoilecgonine	.70		.74		.79	.78
*Ecgonine	.12		.26		.56	

* Prepared in the laboratory.

** Extracted from the incubated media and chromatography.

b. Distribution of N-demethylase of cocaine in different tissues of different animals. Brain, kidney, liver, heart, an intestinal mucosa were tested in the same fashion to see if N-demethylation of cocaine to produce formaldehyde and norcocaine occurred only in the liver. The enzymatic system was a mixture of microsomes + soluble fraction and TPNH₂ regenerating system.

Different rates were observed in different animals but in all them the liver was the only one that had the enzymatic system for N-demethylation of cocaine. The intestinal mucosa system produces benzoilecgonine, but not norcocaine.

c. Enzymatic induction of cocaine N-demethylase by the substrate. Two groups of animals, one with a normal defined diet (23% protein), and another in which the diet was 5% corn protein, were subdivided: one in which the diet contained 15 mg of cocaine/10gm of food and the other served as control. After four weeks, all groups were sacrificed and the rate of N-demethylation of cocaine was tested in vitro. In the group that received a normal diet and cocaine, a significant increase in the enzymatic rates in the liver was observed (P .05), but no increase was observed when a low corn protein diet was used.

d. Effects of cocaine on the incorporation of C¹⁴ from methionine-C¹⁴ into choline in rats with different nutritional states. An experiment was designed to test the hypothesis that cocaine could serve as a methyl donor in animals fed cocaine. Because cocaine labeled in the tertiary N-demethyl was not

available, cocaine was used as competitor for methione labeled in the methyl groups and the specific activity of choline measured.

The experiment was conducted in animals with different diets which were sacrificed at different times. Four hours after the injection of methionine, with one exception, cocaine decreased the specific activity of choline in animals with normal diet. However, this effect was not found in animals that had a corn diet (5% protein) (See Table 1)

e. Effect of cocaine in the metabolism of pyruvate $1C^{14}$ and acetate $1C^{14}$ by liver in vitro and in vivo in different nutritional states. Cocaine has a partial inhibitory effect on the utilization of acetic and pyruvic acid in vitro by liver homogenates ($^{14}CO_2$ evolution). 12/ug of cocaine in the media produced almost a 50% reduction in the decarboxylation rate of pyruvic acid. In the case of acetic acid, cocaine in some cases reduced the rate to 83.3% of the controls. When cocaine was utilized in vivo and the liver homogenate of the animals tested in vitro the rate of acetic acid utilization ($C^{14}O_2$) was diminished but the rate of pyruvic was not affected in the majority of the cases. (Table 1)

TABLE 1

INCORPORATION OF METHYL GROUPS FROM METHIONINE INTO CHOLINE (PHOSPHATIDYL CHOLINE) IN LIVER OF RATS FED IN DIFFERENT NUTRITIONAL STATES*

Specific activity in c.p.m./mg. of choline**

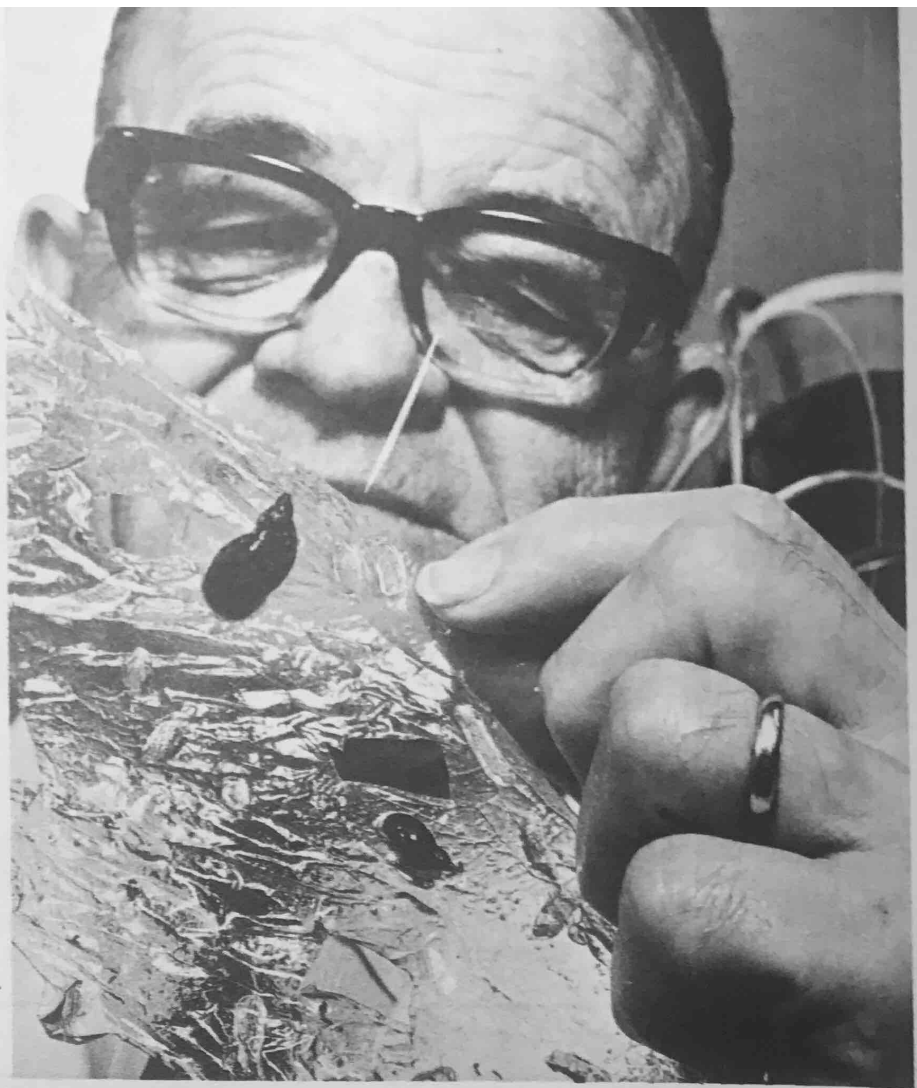
Rat No.	P U R I N A D I E T				.5% CORN PROTEIN DIET	
	1 Hour after Injection		4 Hours after Injection		4 Hours after Injection	
	Without Cocaine	With Cocaine	Without Cocaine	With Cocaine	Without Cocaine	With Cocaine
1	86,053	101,821	1,966	1,657	287,107	287,834
2	75,182	101,924	49,595	1,927	243,077	257,926
3	91,162	36,500	46,634	1,374	217,679	223,999

* Methionine labeled with C^{14} in methyl group used as precursor

** The values are averages of duplicate determinations after the intraperitoneal injection of 10/uc of the L-methionine $C^{14}H_3$ per rat

(1) Dr. Ritchie observes two specimens of Lymnae columella, snail vector of the liver fluke (Fasciola Hepatica) of cattle and sheep and numerous clutches of snail eggs on plastic sheeting

(2) two Fasciola hepatica worms dissected from the greatly distended common bile duct. One worm lies across the "high-lighted" opening of the sac at end of tweezers and the other lies on the right lobe of the liver. The distended gall bladder is conspicuous



SCHISTOSOMIASIS PROJECT

MAINTENANCE AND USE OF THE LIFE CYCLE OF *S. MANSONI*

New aquaria for snail-culture with continuous flow of water have been developed. The amounts of copper in the water have been checked repeatedly by flame photometry. That a hazard exists is apparent, but this has been controlled by crude charcoal filtration; commercial filters are now being installed. We have demonstrated some advantages of applying the food formula for the snail onto paper toweling, drying it, and then placing pieces of the paper of appropriate size in the aquaria.

A system for collecting cercariae for preparing antigens has been developed that allows concentration of great numbers with relative ease and with minimal hazard for the operator.

In March (1968), our infection of mice with *S. mansoni* began to fail; the number of worms was almost reduced to zero in most groups. This situation persisted for about 6 months for all exposures. The cercariae were penetrating, and exposed mice kept in other buildings acquired good infections. It was noted that high concentrations of ammonia were present in the air of the infected animal room due to decomposition of mouse urine; air conditioning had recently been installed. The windows were then kept open to improve air circulation. When the infections showed improvement, we experimentally exposed newly infected mice to ammonia fumes. The resulting infections were normal, leaving us without an explanation for the infection failure. Our worm recovery rates are now commonly 40 to 50% of the cercariae used in exposures. Uniformity of individual worm burdens seem to have improved.

Infected mice and rats are being used in increasing numbers for immunological studies, particularly to explore the mechanism of acquired resistance against *S. mansoni*. Worms are being collected and lyophilized for use in preparing antigens.

INVESTIGATIONS ON *FASCIOLA HEPATICA*, LIVER FLUKE OF CATTLE AND SHEEP

Fasciola hepatica causes a loss of revenue in the cattle and sheep industries in many parts of the world. Reports from Perú, reveal that the beef-dairy and wool industries in the Andes mountains have been virtually ruined by this parasite. The occurrence of infection is reported to be 80-90% in sheep and cattle. Moreover, as many as 20% of school children in 10 of 11 schools were found to be infected.

A research program applying the mass of information on schistosomiasis is under consideration on various aspects of fascioliasis, and the life cycle of *F. hepatica* has been established in our laboratory.

The snail vector (*Lymnaea columella*) has been successfully cultured. Tray aquaria with continuously changing water provide mature snails in one month and specimens about 25 mm. long in 3 months. The phenomenon of crowding may have

TABLE 1.

-Comparison of radioactivity of guppies that were fed labeled cercariae in light and darkness-

Trial No. & Condition	No. fish tested	Cercariae		Radioactivity (cpm) of fish after "n" hours			
		No. per fish	cpm	+ Bkd	1 Hr	4 Hrs	24 Hrs (%)
1. Light	24	500	10	342		2605	3039 (61)
Dark	24	500	10			1798	1920 (38)
2. Light	20	250	8	87		692	782 (39)
Dark	20	"	"			117	215 (11)
* 3.(a) Light	24**	250	12	108	62	606	738 (27)
Dark	24**	"	12		7	96	424 (14)
(b) Light	24**	"	12	92	441	1167	1324 (44)
Dark	24**	"	12		125	248	940 (31)

+ Background activity, subtracted from data.

* In trial No. 3b, the fish were fed normal cercariae repeatedly prior to test (i.e. they were familiarized); the guppies in trial 3a, had not encountered cercariae prior to test.

** Only 12 fish were observed after 1 hour of feeding.

TABLE 2

-The effect of prior feeding of normal cercariae (familiarizing) before determining cercariophagic activity of guppies with labeled cercariae-

Trial No. & Condition	No. of Fish	Cercariae		Mean activity (cpm) of fish after "n" hours			
		No. per fish	cpm	+ Bkd	1 Hr.	4 Hrs.	24 Hrs.
1. Familiarized	24	1400	6	360	404	--	573
Not Familiarized	24	1400	6		55	--	313
2*(a) Familiarized	24	500	12	108	441	1167	1364
Not Familiarized	24	500	12		62	606	738
(b) Familiarized	24	500	12	108	125	248	940
Not Familiarized	24	500	12		7	96	424

* Trial 2.(a) & 2.(b) were run simultaneously; 2.(a) in light and 2.(b) in total darkness.

+ Background radioactivity was subtracted from the data presented.

been manifested by a tendency for snails to crawl out of the aquarium when their number is excessive. The food formula used for Biomphalaria glabrata (vector of schistosomiasis) serves well for lymanaea.

To expose snails, eggs of F. hepatica were obtained from livers of infected cattle. Most snails were infected after exposures to 3-5 miracidia. Cercariae began to emerge from the snails after 50-60 days and encysted almost immediately to form metacercariae.

Infections in mice provide eggs for completing the life cycle in the laboratory.

The duration of infectivity of the metacercariae is being determined by exposing mice periodically. The latter cannot tolerate infections of more than 2 worms. The course of the infection in this host is being determined in comparison with published reports. Studies on biological potential of the snail are in progress. Sero-diagnosis and vaccination with metacercariae attenuated by irradiation will get early consideration.

The combined importance of schistosomiasis and fasciolosis, and the possible danger from paragonimiasis in part of South America, appear to warrant the establishment of a Pan American Control and Research Center for snail borne diseases.

THE CERCARIOPHAGIC ACTIVITY OF GUPPY FISH (LEBISTES RETICULATA) DETERMINED WITH RADIOACTIVE CERCARIAE

This study was designed to quantify cercariophagic activity of guppies using cercariae labeled with radioselenium (^{75}Se). Attempts were made to show whether the cercariae were consumed through a predacious act or if ingestion coincided with respiration.

Most guppies, regardless of sex or maturity, became radioactive after being fed cercariae. There were marked individual differences and heavily gravid females usually did not become active. The number of cercariae per unit of volume was varied by feeding the same number in different volumes, and different numbers in the same volume. In both cases a gradient of radioactivity in the fish occurred for counts taken after 24 hours, at which time the guppies had acquired 20-50% of the total activity of the cercariae. The counts for the fish were relatively low after one hour, but increased considerably after 2 and 4 hours.

Guppies took up radioactivity more slowly in darkness than in the light (Table 1). After one hour, fish in total darkness were essentially normal, and after 4 hours the fish in the light were 5 to 6 times as active as those in the dark. This difference was greatly reduced after 24 hours, suggesting the guppies developed a means of detecting the cercariae in the dark.

Guppies that were familiarized by repeated feedings of normal cercariae prior to giving them labeled cercariae, became radioactive much more quickly than fish that had not encountered cercariae previously (Table 2). After one hour the difference was 7 to 1; however, it was only 2 to 1 after 24 hours.

Selected fish with highest radioactivity were decapitated and the intestines were removed from the body. The difference between head and body was size-related while the relatively small intestine had about half the combined activity of head and body. Thus it appears that cercariae passed through the digestive system and that products thereof were absorbed.

The evidence clearly indicates that guppies are predacious.

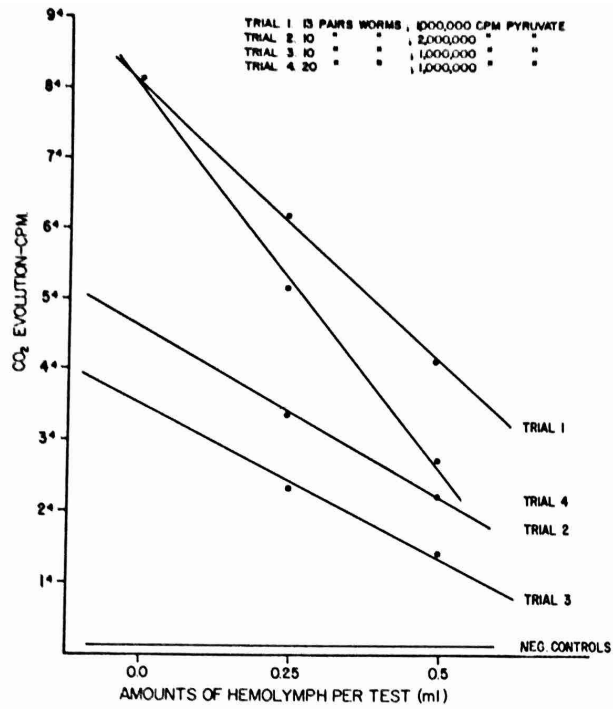


Fig. 1 The in vitro effect of the hemolymph of *B. Glabrata* on the metabolism of *S. Mansoni* worms

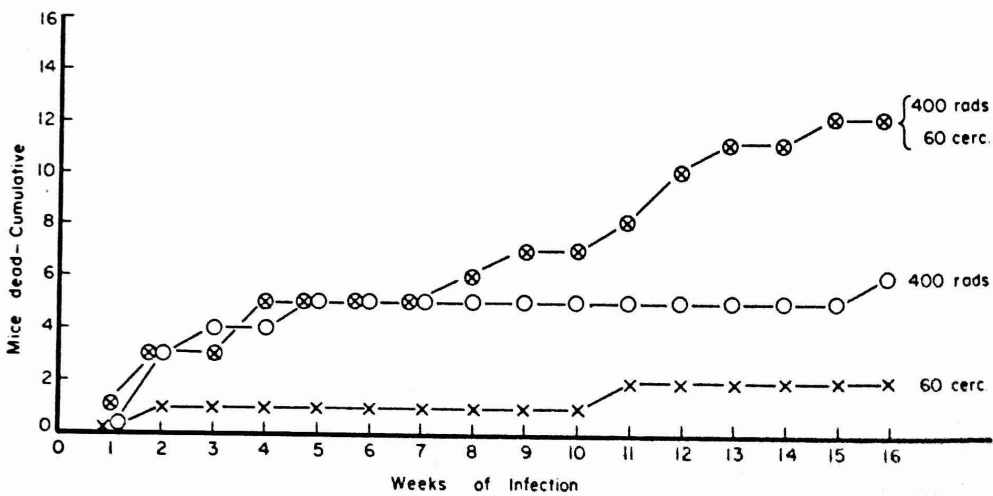
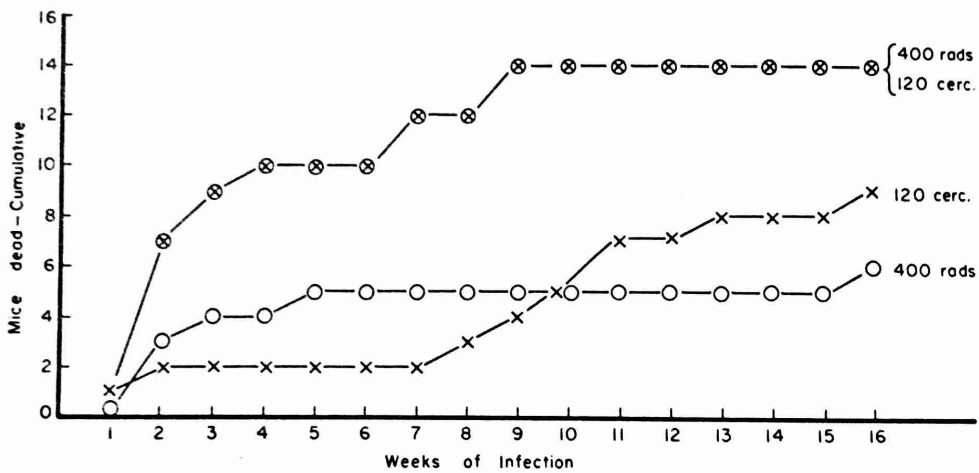


Fig. 2 Effect of whole body radiation on host-parasite relationship involving mice infected with *S. Mansoni*: Mortalities

THE EFFECTS OF WHOLE-BODY RADIATION (^{60}Co) ON THE HOST-PARASITE
RELATIONSHIP INVOLVING MICE INFECTED WITH SCHISTOSOMA MANSONI

Acquired resistance against S. mansoni has been conclusively demonstrated, but the mechanism involved remains uncertain. Involvement of antibodies is doubtful, while resistance mediated by cells is a possibility. Moreover, a combined effect of both these factors must be kept under consideration. In order to disassociate these 2 possible mechanisms, whole body radiation of the host was used to depress humoral antibody formation, whereas cellular components such as macrophages and reticular cells are relatively more radio-resistant.

When mice were irradiated with 400 rads and then exposed to cercariae in varying numbers, there was a trend for animals exposed to intermediate doses (60 and 120 cercariae) to show higher mortalities than animals so infected but without irradiation (Fig. 1). This finding may merely relate to combined sub-lethal effects of irradiation and the pathology of the infection, which jointly overwhelmed the host, or irradiation may have reduced the development of resistance, allowing for more worms or greater egg production. This aspect of the problem was studied.

Mice were irradiated and infected the same day and sacrificed after 28 and 49 days. There were no differences in the worm recovery rates for irradiated and non irradiated animals. Male and female worms were both a little longer in irradiated animals at 28 days, while the reverse was true at 49 days; however, the differences were small. Egg numbers in both the liver and intestine were higher in the irradiated animals.

The granuloma in irradiated mice were twice the diameter of those in the non irradiated controls. The larger size was due to the fibrous component, while the cellular infiltration around the granuloma was reduced in the irradiated animals.

IMMUNOLOGICAL STUDIES WITH SCHISTOSOMIASIS

General experience in immunology suggests that cellular immunity or delayed hypersensitivity, rather than humoral antibodies, is the effective system in host parasite interactions. However, there is little experimental work investigating the cellular aspects of immunity in Schistosomiasis.

Experiments have been initiated in an attempt to demonstrate passive transfer of immunity to schistosomiasis in mice using cell suspensions from immune animals. For many reasons the experiments are difficult and lengthy. For a concerted attack on the problem it is essential that one have an in vitro system for studying the problem. Attempts are being made to develop such a system. Preliminary experiments suggest that a practical system can be obtained incubating together adult worms and lymphocytes purified by sedimentation from spleen cell suspensions. When these are incubated overnight in tissue culture medium, the adherence of the lymphocytes to the worms appears to be correlated with the immunological status of the animals which supplied the lymphocytes. With the immune lymphocytes, there appears to be a reduction of about 50% in the glucose metabolism of the worm. Attempts will be made to quantify the lymphocyte adherence and improve other features of the test.

THE EFFECT OF SNAIL HEMOLYMPH UPON THE METABOLISM
OF SCHISTOSOMA MANSONI

The observation of Oliver-González that mice could be cured of Schistosoma mansoni infections by snail hemolymph led us to investigate the effect of this material upon the metabolism of C^{14} pyruvate by the worms using the techniques outlined above (Fig.2). It was established that the hemolymph inhibited the

metabolism of C^{14} pyruvate by the worms and that inhibitory activity was stable upon storage at 4°C. Separation of the hemolymph into low and high molecular weight fraction by ultrafiltration and dialysis, respectively, established that the inhibitory activity against the metabolism of pyruvate resided in the low molecular weight material, and not in the high molecular weight fraction. Molecular sieve chromatography using P-2 acrylamid gel established that the molecular weight of the active principal was between 500-1000. However, tests with infected mice demonstrated that both the dialyzed hemolymph and whole hemolymph reduced the worm burden of the snail by over 90%, whereas the ultrafiltrate gave a 22% reduction. It appears that one must invoke some mechanism other than that of an anti-metabolite to explain the effect of snail hemolymph upon mice infected with Schistosoma mansoni.

DESTRUCTION OF SCHISTOSOMA MANSONI IN MICE BY THE HEMOLYMPH OF ITS SNAIL VECTOR, BIOMPHALARIA GLABRATA

After Oliver-González observed that mature worms of S. mansoni in mice were killed by the hemolymph of its snail vector (1968, Proc. Soc. Exper. Biol. & Med. 128, 1029), we demonstrated that the rate of metabolic degradation of pyruvate by the worms was less when they were incubated with the hemolymph and the ultrafiltrate thereof, but not by the dialyzed hemolymph. With these three materials, we carried out in vivo tests in mice, following the experimental plan of Oliver-González.

Whole hemolymph (16.66 mg. protein per ml) and dialyzed residue were injected into mice with 50-day infections by the i.p. route daily for 5 days in amounts of 0.1 and 0.05 ml. (diluted 1:5 or 1:10 c saline); and 0.2 ml. of the ultrafiltrate was injected on 3 consecutive days.

Worm counts were made 1 to 20 days after injections in comparison with mice injected with saline. With the whole hemolymph, worm burdens were reduced about 50% after 5 days and 90% after 20 days, confirming the results of Oliver-González. The dialyzed hemolymph reduced the worms 88 and 99%, respectively, after, 10 and 20 days from the last injection. In case of the ultrafiltrate the worm burden was reduced only a little (22%) 15 days after the last injection (Table 3). These results with the dialyzed hemolymph and the ultrafiltrate are the reverse of those obtained in biochemical tests.

TABLE 3

EFFECTS OF HEMOLYMPH OF BIOMPHALARIA GLABRATA SNAILS ON INFECTIONS OF SCHISTOSOMA MANSONI IN MICE

	Mean Number Worms Recovered "N" Days After Injections				
	1-3	5	10	15	20
WHOLE HEMOLYMPH					
Injected mice		46 (5)	54 (10)		6 (4)
Control mice		86 (5)	86 (8)		58 (7)
Reduction (%)		47 %	37 %		90 %
DIALYZED PROTEIN RESIDUE (LABELED <u>c</u> ^{59}Fe)					
Injected mice	34 (3)		3 (2)		0.25 (4)
Control mice	25 (5)		25 (5)		25 (5)
Reduction (%)	none		88 %		99 %
ULTRA FILTRATE					
Injected mice	27 (5)			21 (13)	
Control mice	30 (5)			27 (8)	
Reduction (%)	10 %			22 %	

Figures in parenthesis indicate the number of mice involved.

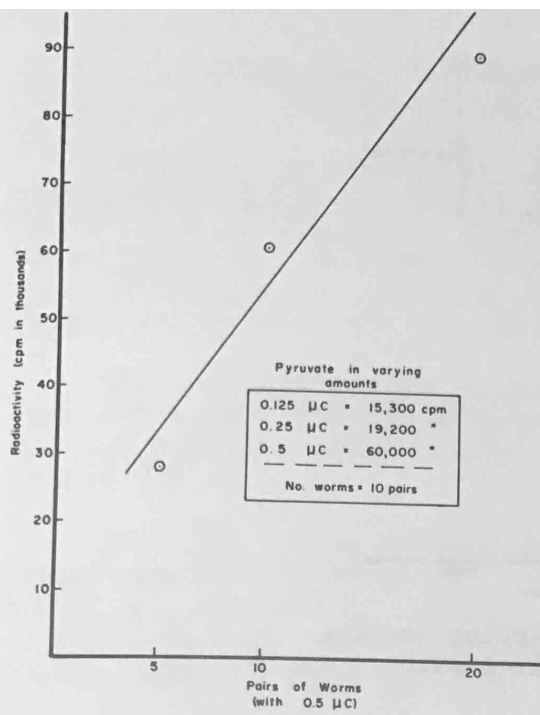


Fig. 3 Metabolic release of CO_2 from pyruvate 2- ^{14}C by varying numbers of worms

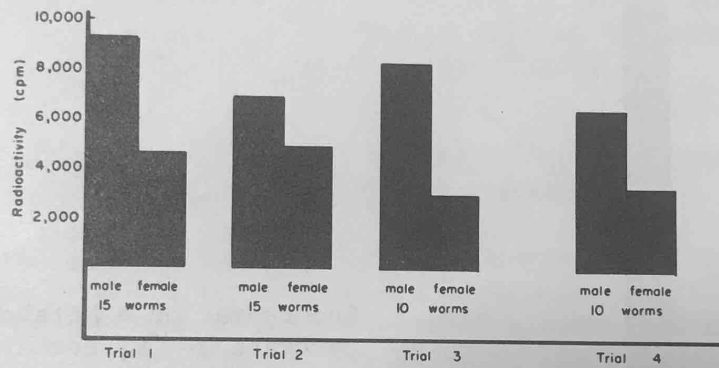
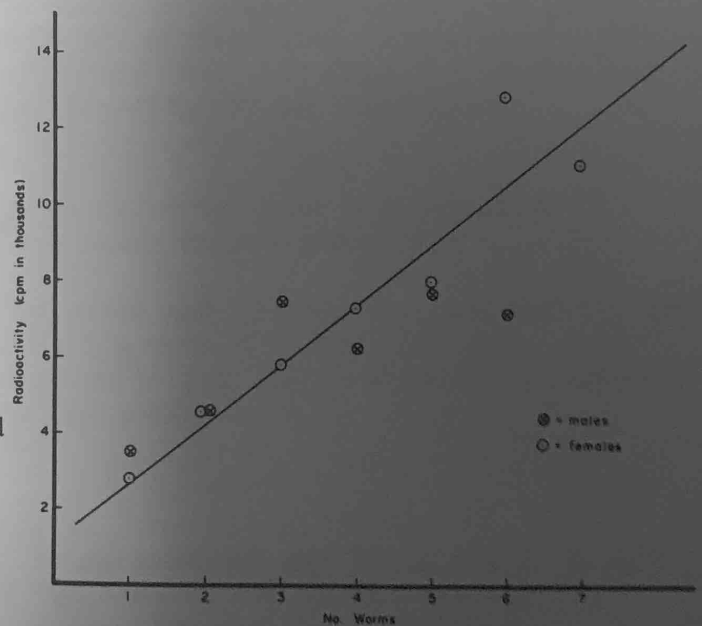


Fig. 4 Degradation of Pyruvate 2- ^{14}C by male and female worms of S. Mansoni

Fig. 5 Amounts of lactic acid produced when varying numbers of male and female S. Mansoni were incubated with ^{14}C glucose for 3 hours at room temperature



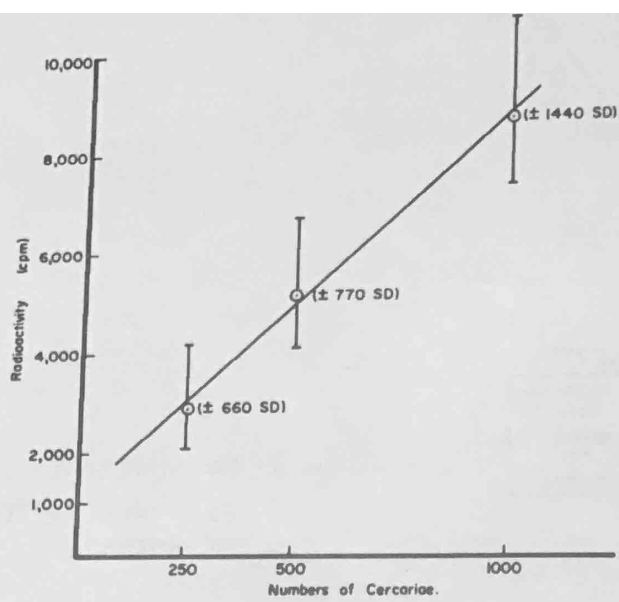


Fig. 6 CO₂ evolution by varying numbers of cercariae incubated with pyruvate 2-¹⁴C

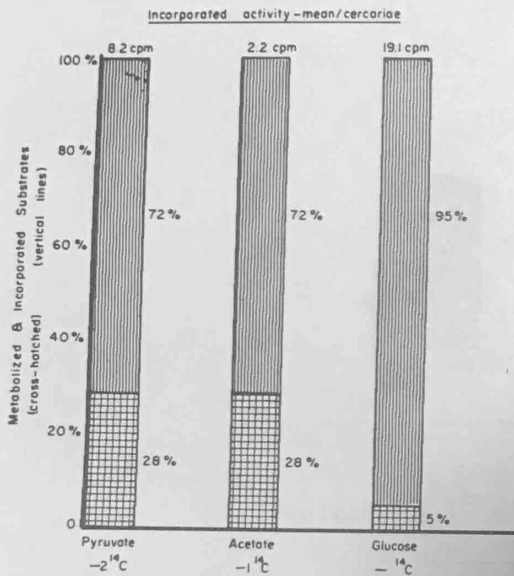


Fig. 7 Incorporation & Metabolism of pyruvate-2-¹⁴C, acetate-1-¹⁴C and glucose-¹⁴C by cercariae of S. Mansoni

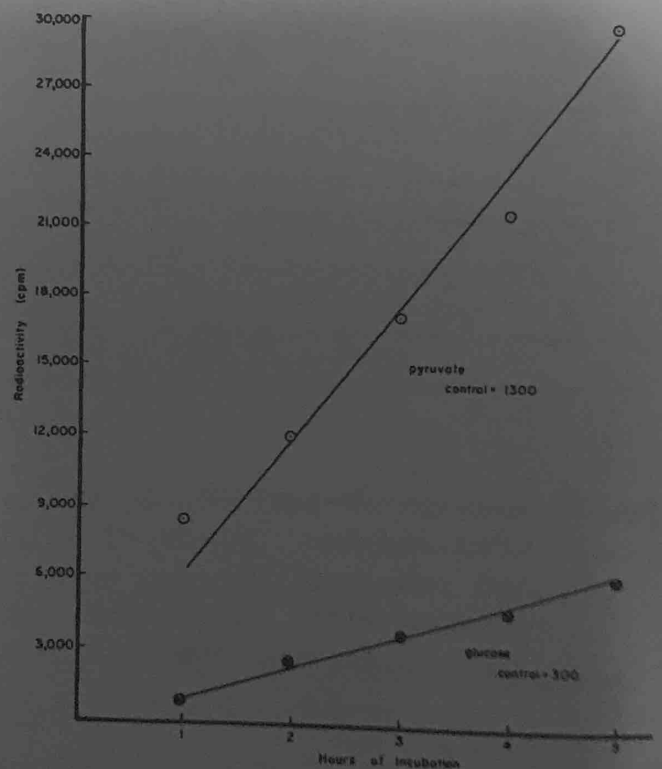


Fig. 8 Kinetics of the degradation of pyruvate 2-¹⁴C and glucose by cercariae of S. Mansoni

METABOLIC UTILIZATION OF PYRUVATE 2-¹⁴C AND GLUCOSE ¹⁴C
BY MATURE WORMS OF SCHISTOSOMA MANSONI

Metabolic utilization of pyruvate and glucose by mature worms of Schistosoma mansoni was determined by means of radioactive substrates. Measurements of labeled CO₂ were made by the method of Chiriboga & Roy (1962, Nature 193, 684), modified. Amounts of lactic acid resulting from glycolysis were also quantified. Varying numbers of worms were incubated with each substrate, and relative amounts used by male and female worms were determined.

When the same numbers of worms were incubated with pyruvate and glucose, each with a radioactivity of about 0.5 μc, CO₂ evolutions of 80,000 and 26,000 cpm were obtained, respectively. Dead-worm controls with glucose gave counts of only 247.

Five (5), 10 and 20 pairs of S. mansoni worms metabolized pyruvate in proportionately larger amounts, the CO₂ releases giving 27,700, 60,700, and 90,300 cpm, respectively (Fig.3). When the pyruvate activity was varied, using about 0.125, 0.25, and 0.5 μc, the corresponding counts were 15,300, 19,200 and 60,700 (cpm); for dead-worm controls, the count was 988.

In four trials, each with 3 or 6 replicates (flasks), the CO₂ recovery from male worms incubated with pyruvate was about double that for females (Fig.4). In contrast, the degradation of glucose to lactic acid was found to be about equal for the sexes, and with varying numbers of worms of each sex, a gradient of lactic acid production was obtained (Fig.5).

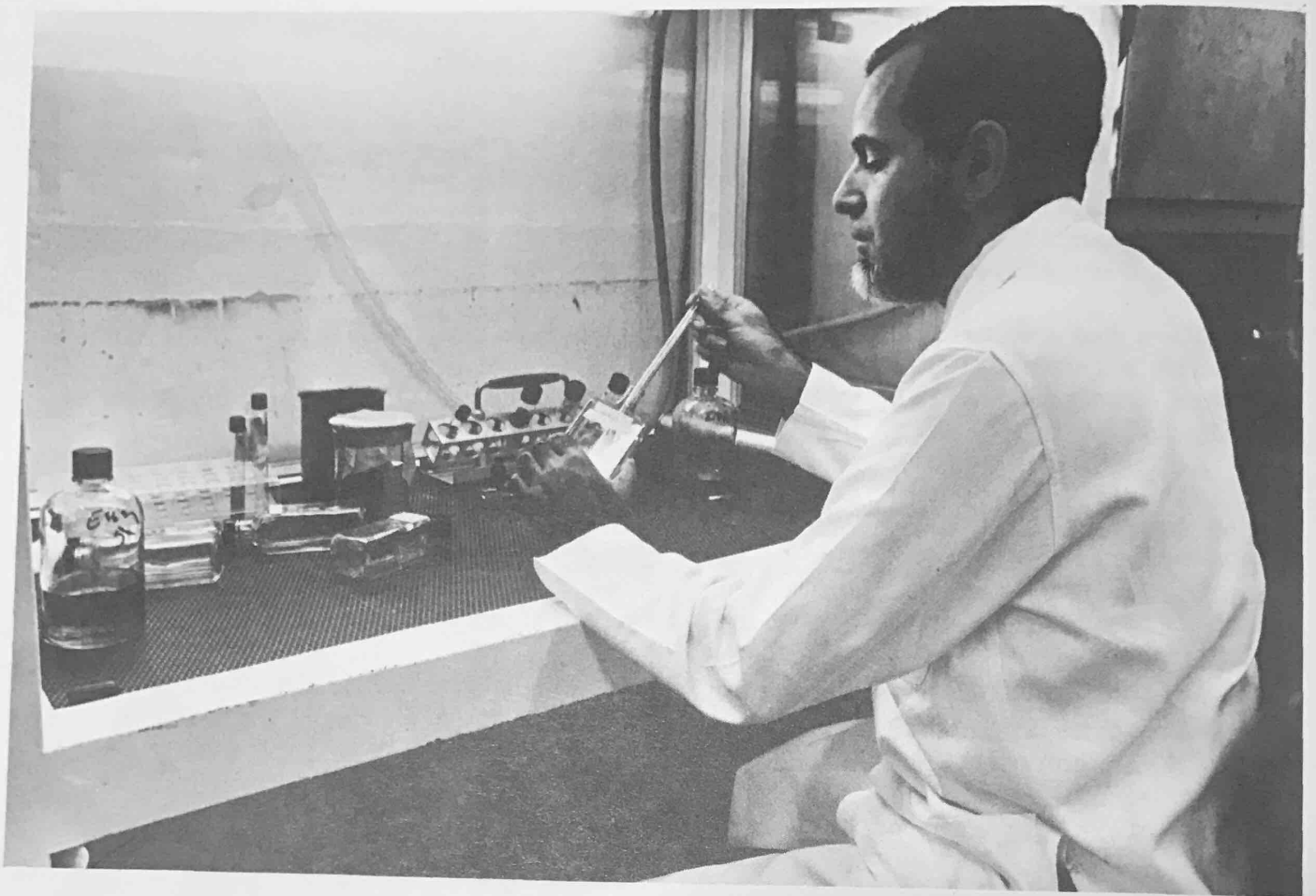
UTILIZATION OF PYRUVATE 2-¹⁴C, ACETATE 1-¹⁴C, AND GLUCOSE ¹⁴C
BY CERCARIAE OF SCHISTOSOMA MANSONI

Bruce (personal communication) found that cercariae of Schistosoma mansoni, while free-living, incorporated and metabolized radio-labeled pyruvate and glucose. Certain aspects of this problem have been studied in our laboratory, using pyruvate 2-¹⁴C, acetate 1-¹⁴C. To date, the investigation has involved only the evolution of CO₂, but it is intended to determine whether the cercariae degrade glucose to lactic acid.

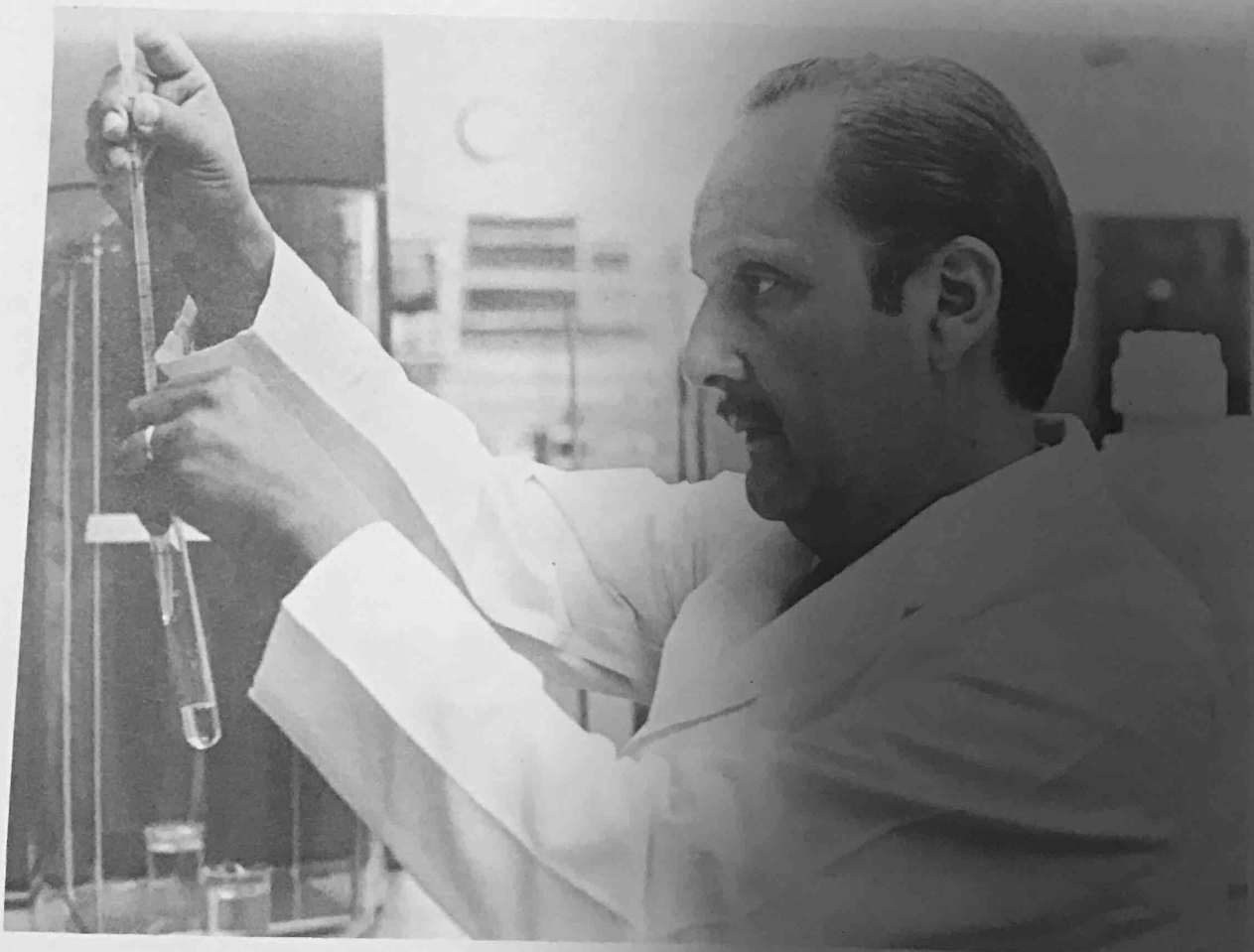
It was shown experimentally that 1.0 and 0.5 μc of glucose or pyruvate were optimal for testing. A direct relationship between number of cercariae and the amount of CO₂ released from pyruvate was demonstrated (Fig.6). Larger amounts of CO₂ were released from pyruvate and acetate than from glucose. On the other hand, more glucose accumulated in the cercariae (Fig.7), suggesting that metabolic degradation of glucose by cercariae may be "incompleted" (anaerobic).

With temperatures of 10, 20 and 30°C, cercariae gave a progressive increase in CO₂ evolution, while at 40°C a sharp decrease occurred.

When cercariae were incubated in pyruvate and glucose for periods of 1 to 5 hours, proportional increases in labeled CO₂ were obtained (Fig.8). This observation was checked by allowing cercariae to age for corresponding periods before they were incubated (1 hour) with the substrate.



- (1) Dr. Ramiro Martínez Silva inoculates tissue culture with T. cruzi
- (2) Dr. Jorge Chiriboga studies radiation effect on polynucleotides which produce interferon in tissue culture



TRYPANOSOMA PROJECT

1. Effect of whole body irradiation on Trypanosoma rangeli infection in mice. It was shown in the 1967 Annual Report that whole body irradiation (400 rads) administered to mice infected with T. cruzi produced greater parasitemia, more extended organ invasion and death of the animals at an earlier period than those non-irradiated. It was of interest to see whether similar effects were induced in mice inoculated with Trypanosoma rangeli, a parasite often found together with T. cruzi in human beings.

New-born mice were divided in two groups, one receiving 400 rads, the second one without radiation. Half of the irradiated mice were inoculated by the intracerebral (i.c.) route with a culture of T. rangeli. The remainder were inoculated by the intraperitoneal (i.p.) route. The non-irradiated mice were inoculated in a similar manner.

One month after inoculation, the mice were sacrificed and parasites were sought in the spleen, liver, and heart, which were found negative. These results suggest that the host (mice) cells do not become more susceptible to infection with this parasite.

2. Induction by radiation of attenuated mutants of Trypanosoma cruzi. To determine whether radiation would induce genetic alterations resulting in an attenuated strain that could be used as vaccine, a culture of T. cruzi (strain Bertoldo) was exposed to the Co⁶⁰ source. The cultures were in the logarithmic phase of growth and the doses administered were 100, 1,000, 10,000 and 50,000 rads. After radiation, the trypanosomes were inoculated i.p. in groups of 21 day old mice; the medium was replaced by a new one and further incubated at 26° during 24 days, when the irradiation was repeated. In all, the different doses were administered 4 times at daily intervals. The mice were searched for trypanosomes in blood and when found a hemoculture was made in liquid medium. Subcultures were made twice in Yaeger's medium and then titrated for virulence and infectivity in mice and tissue culture. Four weeks later, the mice were challenged by the i.p. route with 1,000 blood forms of T. cruzi (Tulahuen strain). (See table)

These data seem to indicate that better protection can be obtained when the microorganisms have been subjected to frequent exposures.

Twenty four days after inoculation i.p. of 0.1 ml of these cultures, the mice were challenged i.p. with 10^5 blood forms of the virulent Tulahuen strain with the following results:

CULTURE NUMBER	RADIATION EXPOSURE		PROTECTIVE INOCULUM			CHALLENGE	
	Doses	Times	Concentration	Infectivity	Lethality	Inf.	LCT
1	1,000R	1	2.26×10^7	1/4	0/4	4/4	1/4
2	1,000R	2	1.82×10^7	1/6	0/4	6/6	3/6
3	10,000R	3	0.81×10^7	5/6	0/6	6/6	1/4
				2/4	0/4	4/4	4/4
				0/6	0/6	6/6	2/6
4	50,000R	3	2.42×10^6	2/6	0/5	4/4	2/4
				1/5	0/5	5/5	2/5
5	1,000R	4	2.06×10^7	2/6	0/6	6/6	4/6
				1/6	0/6	1/6	2/6
				1/6	0/6	6/6	5/6
6	10,000R	4	2.85×10^7	1/5	0/5	5/5	2/5
				1/6	0/6	6/6	3/6
				1/5	0/5	4/4	1/4
7	10,000R	4	3.41×10^7	3/5	0/5	5/5	5/5
				2/6	0/6	6/6	2/6

3. Sensitivity of T. cruzi-infected cells to Trypsin. The metabolism of T. cruzi-infected cells is very different from normal cells. It is of practical interest to compare metabolic pathways of normal and infected cells, which makes it necessary to obtain almost pure populations of both types. A group of DC2 cells were infected with a fluid medium containing 10^6 Trypanosoma cruzi. Starting the 4th day after inoculation, a set of 4 tubes was separated and treated for 2 minutes at room temperature with trypsin and with Hank's saline solution. This treatment was repeated at different time intervals with the following results:

Rate of Infection by T. cruzi in Cells Treated with Trypsin

Day	TREATMENT			
	None	0.25% trypsin	0.12% trypsin	0.025% trypsin
5	4.3%	2.3%	3.7%	4.6%
6	13.9	2.5	10.6	5.6
7	12.1	3.2	5.4	6.7

Further studies are under way in order to separate and transfer the infected cell population, since it has been observed that infected cells can attach to the glass and even divide.

4. Dynamics of T. cruzi infection in different cell lines. Temperature is one of the factors accounting for intracellular growth of T. cruzi. An experiment was designed to see whether other factors (type of cell, strain of T. cruzi) could play an important role. DC₂ cells (reported in PRNC Annual Report 1965) and monkey kidney cells were infected with T. cruzi culture. The tubes were divided in two groups, one inoculated at 26°C and the second one at 37°C. Every 24 hours one tube of each group was removed from the incubator and examined microscopically for the presence of Leishmanial forms inside the cells, with the following results:

Intracellular Infection in 2 Line Cells Incubated
at Different Temperatures

Day	TYPE OF CELLS			
	Monkey Kidney Cells 26°	Monkey Kidney Cells 37°	DC ₂ Cells 26°	DC ₂ Cells 37°
1	0	0	0	0
2	0	0	0	+
3	0	0	0	+
4	0	0	0	+
7	0	+	0	+
8	0	+	0	+
9	0	+	+	+
10	0	+	+	+
11	0	+	+	+
14	+	+	+	+

These results show that there exist differences of susceptibility among the different cell lines. They also suggest that the principal factor affected by the temperature is the period of adsorption penetration.

5. High temperature effect on T. cruzi infection at cellular level. DC₂ cells, Clone I, were inoculated with T. cruzi. After inoculation, and every 24 hours thereafter, 4 tubes without inoculum and 4 of the inoculated group were transferred to a 40°C incubator. After 7, 5, 4, 3, 2, and 1 days of incubation at 40°C a subculture of these tubes was made; one was incubated at 26°C and the other at 40°C. Neither showed growth. No intracellular parasites were observed even when the tubes were brought back to 37°C for further incubation.

To complement this experiment, 18 roller tubes were inoculated with T. cruzi and incubated at 37°C and those showing intracellular infection were transferred to 40°C. After 7 days of incubation at this temperature, 2 tubes were removed daily and brought back at 37°C. The tubes removed from 40°C after 7 days incubation had large amounts of trypanosomes, but those incubated longer were negative. To see whether those tubes had intracellular parasites, the cells kept at 40°C during 7, 8, 9, 10, and 12 days were trypsinized, gently ground in a tissue grinder and titrated in DC₂ cells.

Day After Inoculation Showing Trypanosomes

Days at 40°C	DILUTIONS OF THE INFECTED MONLAYER						
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
7	6	6	6	6	12	16	0
8	6	19	12	0	0	0	0
9	6	6	11	0	0	0	0
10	16	23	0	0	0	0	0
12	16	23	0	0	0	0	0

These results seem to indicate that there is not complete inactivation of the intracellular parasites, even if production of forms reaching the extra-cellular medium is practically abolished.

6. Titration of T. cruzi infected mouse tissues in DC2 cells. To establish the sensitivity of DC2 cells to parasites present in tissues, a mouse infected i.c. with T. cruzi strain was sacrificed 14 days later. The organs were removed, ground and prepared in various dilutions. Two tubes were inoculated with 0.1 ml of each dilution and incubation carried out at 37°C.

Day After Inoculation Showing Trypanosomes in the Medium

Organ	DILUTION OF ORGANS						
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
Brain	4	4	7	8	9	13	Neg
Liver	4	4	4	10	18	Neg	
Spleen	4	4	7	8	9	17	Neg
Blood	4	4	4	6	20	Neg	Neg
Heart	4	4	4	8	9	Neg	Neg

This method of quantitating trypanosomes in a tissue system is very accurate and it is being tested in the effects of radiation, drugs, etc., affecting the host-parasite relationship.

7. Effect of Poly IC on Trypanosoma cruzi infection in mice. Poly I.C., polynucleotide, induces interferon in animals and cells against different viral agents. In order to determine whether this substance could affect the infection by Trypanosoma cruzi, five groups of mice (29 days old) received 500γ of Poly I.C. I.C. in 0.5 ml Via i.p. route. Five groups of identical litters were held as control. One day later both groups were inoculated i.p. with 0.25 ml of T. cruzi strain, in dilutions 10² to 10⁶. Poly I.C. was administered 3 times a week, for 2 weeks, in 100γ doses.

Effect of Poly I.C. in Mouse Lethality by T. cruzi

DILUTIONS OF T. CRUZI

	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
Mice with Poly I.C.	5/5	4/4	3/5	1/3	0/4
Mice without Poly I.C.	5/5	5/5	5/5	3/5	0/5

A small degree of protection seems to exist in the group given Poly I.C. and further experiments are under way to ascertain if this is significant.

8. Effect of Poly I.C. on Trypanosoma cruzi infection in cells. A similar experiment was set up in cells. Poly I.C. was administered to DC2 cells in amounts of 100; after 24 hours a titration of T. cruzi was made. The medium was changed 3 times a week and at these intervals 100 γ of Poly I.C. were added. Observations were made daily by inverted microscopy.

Intracellular Multiplication of T. cruzi in Cells
Treated with Poly I.C.

	DILUTIONS OF T. CRUZI					
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
Cells + Poly I.C.	+	+	+	+	+	+
Cells No Poly I.C.	+	+	+	+	+	+

The effects of Poly I.C. on the growth of T. cruzi in vitro was studied. To NNN medium, 10, 100, and 500 γ of Poly I.C. with T. cruzi was added and then inoculated.

Effect of Poly I.C. on the Growth of T. cruzi in NNN
Medium and its Infectivity

	POLY IC (IN γ)				
	1	10	100	500	None
Growth	+	+	+	+	+
Titer in cells/ml	10 ⁵	10 ⁵	10 ⁵	10 ⁶	10 ⁵

9. Adsorption and penetration of T. cruzi into cells. To determine the time necessary for T. cruzi to penetrate cells, DC2 cells were grown on coverslips in Petri dishes in CO₂. Once confluent, they were inoculated with 10⁷ (culture forms) T. cruzi and incubated again at 37°C. At specified times thereafter 2 coverslips were removed, one was put into a Leighton tube and EBM with 5% calf serum added; the other one was washed 3 times with Hank's saline solution containing 1% of an anti-T. cruzi serum, and then placed into a Leighton tube with Hank's and antiserum for 10 minutes, washed for the last time and incubated at 37°C with EBM. The medium was changed 3 times a week and the 7th day the coverslips were removed, fixed with methylalcohol and stained with Giemsa.

Number of Infected Cells in Monolayer
Inoculated with T. cruzi and Washed at Intervals Thereafter

	<u>Hours Incubated Before Washing</u>									
	0	1	2	4	6	8	12	16	20	24
Washed	0	6	9	N.D.	27	170	251	379	367	358
Not washed	31	69	66	123	389	N.D.	330	325	334	341

Adsorption occurs almost immediately after inoculation, but penetration of the parasites seems to have an optimum between 12 and 16 hours. Experiments being conducted indicate that the period of adsorption and penetration depends on the form of the parasite (in vitro culture or tissue culture) and strain used.

10. Cell receptors for T. cruzi. To test whether DC2 cell receptors were destroyed by T. rangeli, cells were inoculated with 10^7 forms from Yaeger's medium and incubated at 37°C during different periods of time. The monolayers were washed 3 times with Hank's saline solution and then inoculated with 10^6 and 10^5 of T. cruzi with 2 tubes left as controls. The cells were incubated at 37°C during one week with medium changed every 2 days.

Intracellular Infection by T. cruzi in Cells Previously Inoculated with T. Rangeli

Days with T. rangeli	Number of T. cruzi	Intracellular Infection
1	10^6	+++
	10^5	+++
	0	---
2	10^6	+++
	10^5	+++
	0	---
3	10^6	+++
	10^5	+++
	0	---
4	10^6	+++
	10^5	+++
	0	---
5	10^6	+++
	10^5	+++
	0	---

These results show that cell receptors are not affected by T. rangeli.

11. Trypsin action on cell receptors. The medium of DC2 cell growth in roller tubes was changed and replaced by a new one to which trypsin was incorporated at a final concentration of 0.0025%. The cells were further incubated at 37°C and at different intervals inoculated with 10^5 T. cruzi from tissue culture. Two tubes were left without inoculum as control of the trypsin on the cells, two additional tubes were used as control of the parasites. After 24 hours of trypsin action, the cell sheets were washed with Hank's new medium added, and incubated further at 37°C .

Action of Trypsin on Cell Receptors for T. cruzi

	HOURS OF ACTION			
	0	1	2	18
Cells treated and inoculated	2/2 (5)	2/2 (5)	2/2 (5)	2/2 (5)
Cells treated	0/2	0/2	0/2	0/2 (5)
Cells inoculated	2/2 (5)	2/2 (5)	2/2 (5)	2/2 (5)

No trypsin action was observed on the cell receptors by the different periods, at least with this concentration.

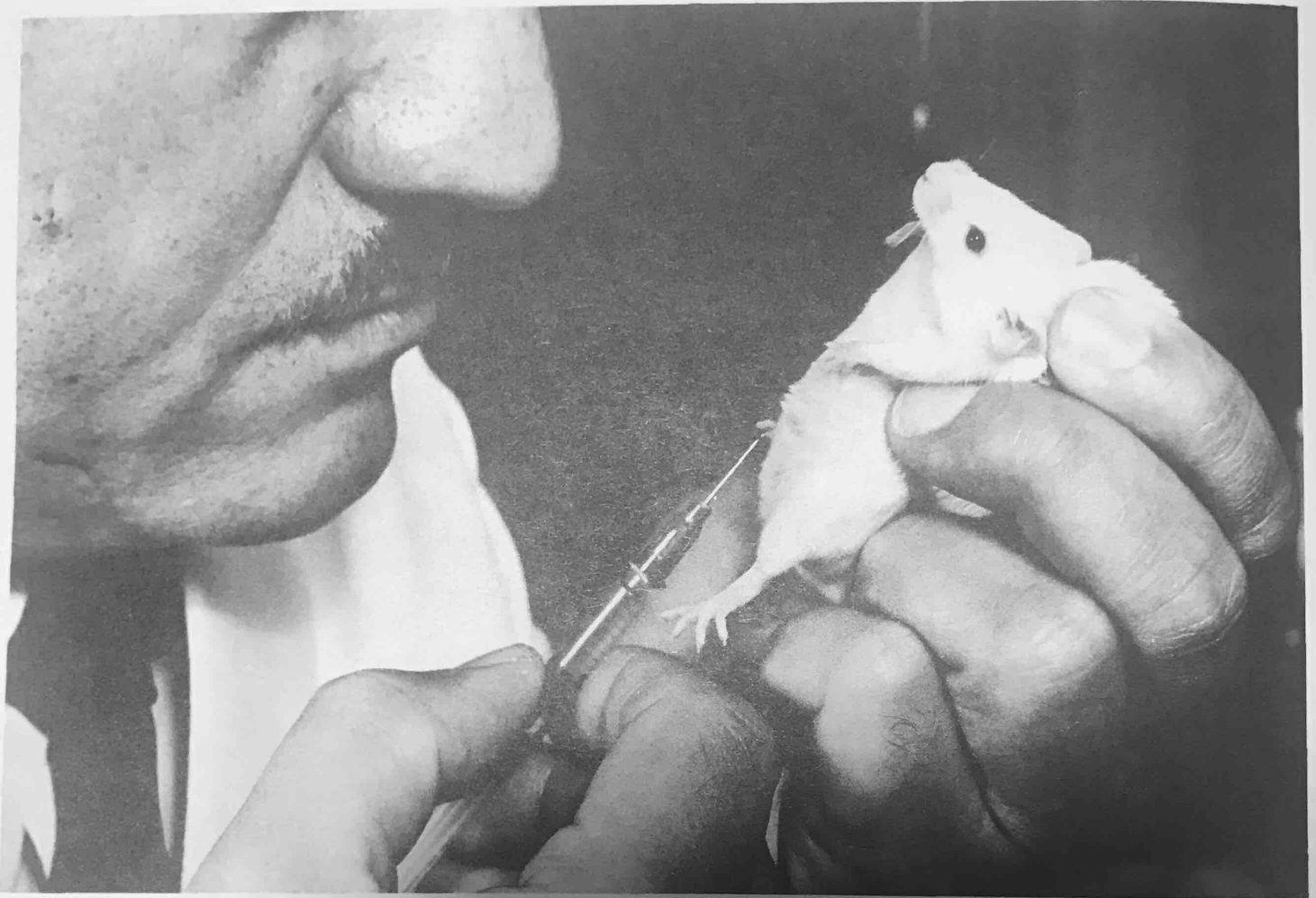
12. Resistance of T. cruzi to temperature. To determine the resistance of T. cruzi to different temperatures, blood of an infected mouse with a concentration of 12×10^7 parasites per ml. was diluted 1/4 in Yaeger's medium with 50 units of heparin. This mixture was divided in 3 lots, one left in refrigerator at 4°C, the 2nd, at room temperature (21°C + 2°C), and the third one at 37°C. At different intervals, 0.1 ml. of the blood was taken and titration made in DC2 cells. The results:

Effects of Temperature on the Inactivation of T. cruzi

Days	TEMPERATURE		
	4°C	21°C	37°C
1	10^6	10^6	10^6
2	10^6	10^6	10^6
3	10^5	10^6	10^6
4	10^4	10^6	10^6
7	10^2	10^6	10^6
10	10^0	10^6	10^4
16	10^0	10^6	10^4
21	10^0	10^4	10^0

The great resistance of T. cruzi to temperatures prevalent in tropical regions would allow the sending of blood samples to a central laboratory to process specimens for diagnosis of the disease as reported in this report.

13. Separation of DNA components of T. cruzi infected cells by centrifugation. Work has been started to separate the several components of trypanosomal DNA by isopycnic centrifugation in Cs_2SO_4 . There is reason to believe that it may be possible to separate the trypanosomal DNA from that of the tissue culture host cell. If this proves to be true, the use of thymidine incorporation and irradiation for a study of host parasite interaction at the cellular level will be very valuable.



Dr. Julio I. Colón inoculates intraperitoneally a white adult mouse with a virus sample

VIRUS PROJECT

RADIATION ACTIVATION OF LATENT VIRUSES IN WILD ARTHROPODS AND VERTEBRATES

A. Effect of Gamma irradiation on the infection of mice and rats with Cocksackie virus. Adult white mice and adult wild rats (Rattus rattus) normally resistant to coxsackie infection, were exposed to whole body gamma radiation from a 1,510 curie cobalt source. Immediately afterward, they were inoculated with coxsackie virus type A10. Non irradiated controls were also inoculated.

Non irradiated inoculated controls were also included in the experiments. These animals were bled daily and the virus content of the blood was determined in suckling mice.

Virus inoculations in unirradiated mice and in the group of mice irradiated with less than 400 Rads caused no death over the observation periods of 21 days. Death among the mice irradiated with 400 Rads or more and inoculated with 400 Rads or more 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 subgroups were compared, but the death rate increases and the average of death decreases with the increase of radiation, regardless of the presence of virus. There was not a synergistic effect of virus and gamma radiation when only death of the animals was the criterion. However, when infectivity was the criterion, a consistently higher concentration of virus was demonstrated in the blood and tissues of the irradiated animals. 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, 800 Rads the viremia lasted from 4 to 8 days, depending on the radiation dose. The evidence accumulated so far indicates that the virus is indeed multiplying in the irradiated animals.

Results obtained with wild rats are very similar to those obtained previously with adult white mice. Rats irradiated with 400 Rads and inoculated with virus showed a significantly high concentration of virus in the blood from the first to the 7th day. Rats irradiated with 800 Rads and inoculated with virus showed viremia on the 1st day which lasted for more than 7 days. Although not at very high titer, virus seems to persist longer in animals irradiated with 800 Rads.

B. Activation of coxsackie virus by radiation.

1. In Adult Mice. A selected number of mice of similar age (28-30 weeks) and weight average of 22 grams were divided into 7 groups of 26 mice each. They were irradiated as follows: Group I was kept as unirradiated control, Group II with 100 Rads, Group III with 200 Rads, Group IV with 300 Rads, Group V with 400 Rads, Group VI with 500 Rads, and Group VII with 600 Rads. Each group was then divided into 2 sub-groups, each one containing 13 mice. One sub-group was kept as irradiated control and the other was immediately inoculated i.p. with 10^6 LD50 suckling mice coxsackie A10 virus. As previously reported, and again in this experiment, animals that were irradiated and inoculated with the virus showed a viremia that lasted for several days, depending on the radiation dose. No viral activity was found in the irradiated uninoculated group. After 16 days, animals were bled again and no viral activity was found in the blood. 22 days after irradiation and inoculation, no viral activity was found in the blood or in selected organs of animals that were sacrificed. A second dose of irradiation (400 Rads) was given to the surviving mice. After 48 hours the animals 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 spleen, brain, heart, liver, and pancreas from the groups receiving 400, 500, and 600 Rads. In order to determine if the virus isolated from these organs was the same virus, neutralization tests were done. Only the original virus, coxsackie A10, was found. 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 apparently in any of the organs tested. Either the active virus has been present in very low concentration, and was not detectable by our methods, or the virus, in a latent state, was activated by radiation.

2. In Wild Rats. Adult wild rats (Rattus rattus) from El Verde Forest with a weight average of 147 grams and unknown age were brought to the laboratory on September 12, 1967. The animals were bled immediately to test for coxsackie A10 virus or neutralizing antibodies against it. Only one animal showed virus in the blood; it was sick with diarrhea and died 2 days later. The virus isolated from this animal was identified as coxsackie A10, the same virus isolated by Weinbren in 1965 from rats in the same area. Only rats that showed no coxsackie virus type A10 or antibodies were used in these experiments.

The selected animals were divided into 6 groups of 7 rats each. Group I was not irradiated but inoculated i.p. with $10^{6.7}$ LD50 suckling mice coxsackie A10 virus. Group II was irradiated with 400 Rads and immediately inoculated with virus. Group III was irradiated with 400 Rads but not inoculated with virus. Group IV was irradiated with 800 Rads, and immediately inoculated with virus. Group V was irradiated with 800 Rads but not inoculated with virus. Group VI was neither irradiated nor inoculated. As previously, irradiated rats showed a viremia that lasted for at least 7 days. No viremia was found on the 16th day after the treatment. After a 25-day observation period following the original treatment, no viral activity was found in the blood of the surviving animals nor in the organs of those sacrificed. 15 days after the rats were given a second irradiation dose (800 Rads) they were bled and sacrificed. Coxsackie virus type A10 was again isolated from the pancreas of Group II rats (irradiated and inoculated with virus). This again indicates activation of latent infection by radiation.

These experiments demonstrated that radiation enhanced the growth of virus in the tissues of adult mice and adult wild rats: (1) a significantly greater amount of virus in the blood of the irradiated animals was demonstrated as early as the first day in both the mice and the wild rats and consistently thereafter, depending on the radiation dose; (2) the virus persisted in appreciable amounts in tissues of the inoculated-irradiated mice, and a very small amount for only one day in the inoculated-unirradiated wild rats; virus was reactivated in animals that have been irradiated, inoculated, and irradiated again with a second dose of gamma radiation. The results obtained are in agreement with those obtained by Cheevers using x-radiation in mice, with the exception of a moderate increase in mortality rates and greater viral activity shown in tissues of the unirradiated inoculated controls shown in his experiments. This could be due to the different types of coxsackie virus used in the experiments (he used the Power strain of coxsackie virus) or to the fact that he was using 4-5 week old mice while in our studies we used 28-30 week old mice. One day old mice are very susceptible to coxsackie virus infection; susceptibility decreases with age, and adult mice are very resistant to the infection. Resistance, unaccompanied by previous experience with the virus and hence unassociated with antibodies immunity, is affected by age, sex, genetic background, state of nutrition, hormonal balance and other factors. Radiation probably affects some of these parameters, making the animal susceptible to the infection.

C. Isolation of Virus from Immune Animals after Gamma Irradiation

1. Immune Adult Mice. Adult mice (CF 1) were immunized with coxsackie A10 virus by inoculating i.p. 0.2 ml. of a virus dilution containing $10^{7.7}$ LD50 suckling mice on three occasions at 7 day intervals. Inoculated animals developed neutralizing antibodies to a significantly high titer (1/128). No active virus was isolated from these mice at any of the times tested. On the 52nd day after the last virus inoculation, the animals were divided into 3 groups: (1) Immunized not irradiated; (2) immunized irradiated with 400 Rads and (3) immunized irradiated with 800 Rads. Non-immune animals of the same age were also divided into 3 groups to serve as controls; (1) non-immune not irradiated; (2) non-immune irradiated with 400 Rads and (3) non-immune irradiated with 800 Rads. The animals were bled immediately after irradiation and after 48

hours, whereupon the animals were sacrificed and several of the organs harvested and tested for the presence of virus. Coxsackie virus type A10 was isolated from mice that were immunized and irradiated with 400 Rads. No viral activity was found in the immunized and not irradiated, or in the not immunized irradiated. The animals from which the virus was isolated had demonstrated neutralizing antibodies. Here we have the case in which a virus persists in an immune host.

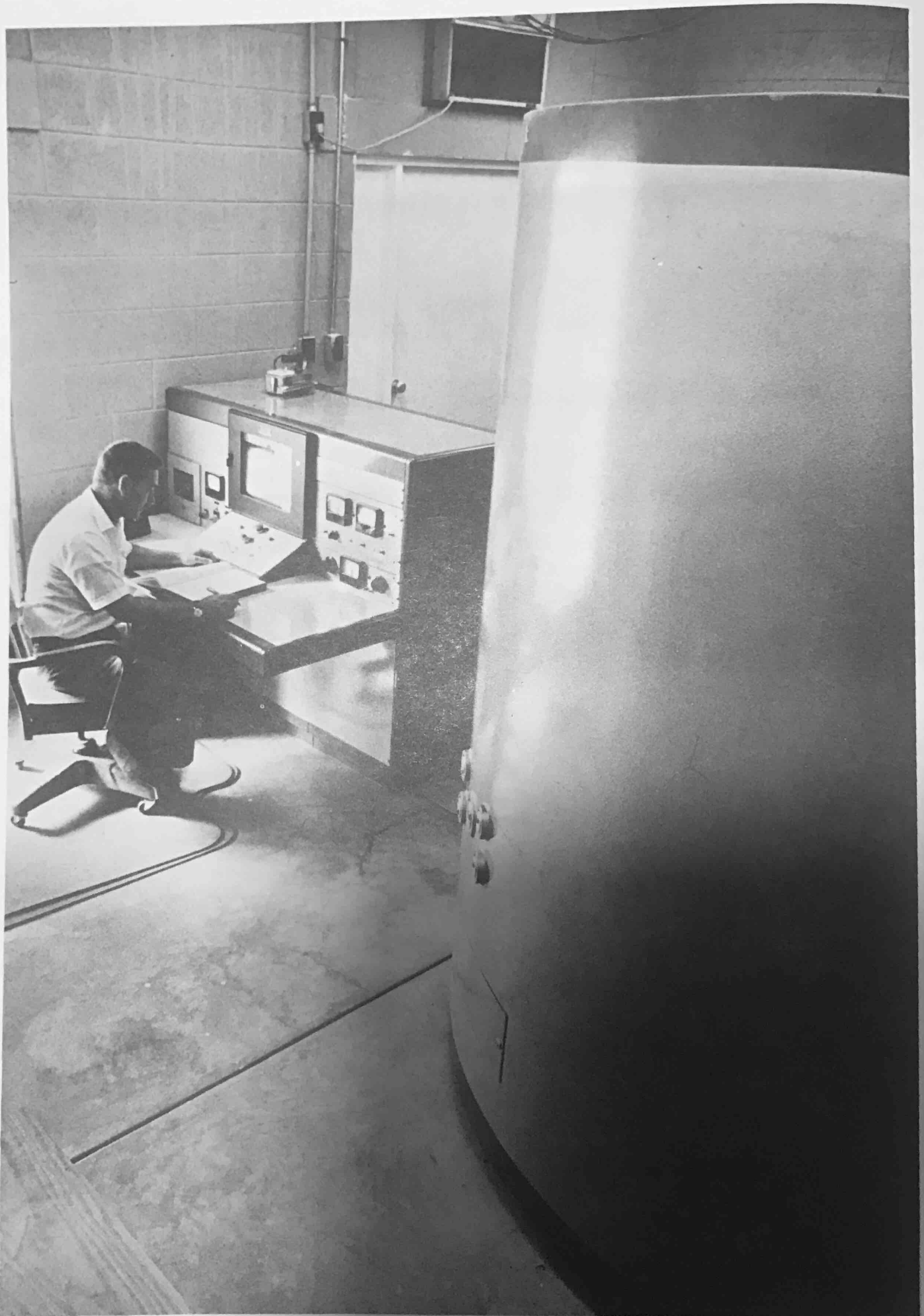
2. Immune Newborn Rats. Newborn rats inoculated i.c. with coxsackie virus type A10 developed a very intensive viremia for 72 hours. After the viremia, the rats remained alive and after 18 days antibodies were recovered from the serum. No active virus was found the 6th day. The mother of these newborn rats had no neutralizing antibodies. The rats were divided into two groups: (1) irradiated with 800 Rads and (2) immune not irradiated control. After irradiation, coxsackie virus was isolated from the immune-irradiated rats while none was isolated from the immune-not irradiated.

In a number of viral and rickettsial diseases, Just as in some bacterial and fungal diseases, one attack almost invariably causes lifelong immunity; examples of this are smallpox, measles, mumps, polio, yellow fever, and epidemic typhus. Most person who recover from these maladies continue to have demonstrable specific circulating antibodies for many years afterward. One might account for persistent immunity on the basis of repeated contacts with the viral agents. On the other hand, this explanation does not account for persistent antibodies in persons who recover from yellow fever or typhus and then live for many years in areas where the disease is not endemic. It is often assumed in such instances that the agent persists in the recovered individual. In the experiments that have been described we have shown that the virus does persist in the immune animals and that it can be activated by gamma radiation.

The mechanism by which these pathogens survive in the immune host is not understood. They may exist intracellularly, where they are protected from the usual humoral or phagocytic defense mechanisms of the animal or they may exist in a form that is not affected by these defenses.

D. Enhancement of Interferon Production by Gamma Irradiation in Chick Embryo (Thesis of Mrs. Carmen Rivera).

The effect of gamma radiation on the production of interferon (IF) by 10 day-old chick embryos induced by Newcastle disease virus (NDV) was studied. Embryos were treated as follows: Group I received no irradiation and no virus; Group II 400 Rads; Group III 800 Rads, Group IV no irradiation and 10^6 pfu NDV. The virus was inoculated immediately after irradiation. Separate pools of allantoic fluid and embryos were made at 14, 24, and 48 hours after irradiation and infection. The IF was assayed by the plaque inhibition test of sindbis virus in chick embryo fibroblast. Only small amounts of IF were produced at 14 hours in Group VI. The allantoic fluid and the embryos of groups I, II, and III did not show IF at any of the time tested. The allantoic fluid and the embryos of groups IV, V, and VI at 24 hours had 80, 2500, and 1280 units of IF per ml, respectively. The amount of IF at 48 hours in Groups V and VI was 2560 and 1280 units of IF, respectively. All embryos in Group IV died between 24 and 28 hours. Embryos in groups V and VI began to die at 48 hours. This partial protection could be related to the high concentration of IF present in embryos. Some complexed polynucleotides induced the production of IF and, since irradiation liberates nucleic acid from cells, nucleic acid could be involved in this enhancement. Irradiations also break the permeability of the cell membranes; this damage could make more cells capable of producing IF accessible to the virus.



Division Head, Richard Brown Campos at the L-77 Reactor Console

REACTOR

The Reactor Division provides 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-60 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 operated routinely two shifts per day, five days a week, accumulating a total of 2970.116 megawatt-hours. A total of 773 side-of-core irradiations were performed. In the gamma pool, 419 irradiations were carried out.

The L-77 reactor was used for training and student experimentation throughout the year. The pile oscillator experiment was successfully completed and served as basis for the thesis of a graduate student.

Work on the one Kilowatt reactor slowed down, pending revision of the Safety Analysis Report. This work will be continued next year, at which time all necessary documentation will be prepared and submitted for consideration.

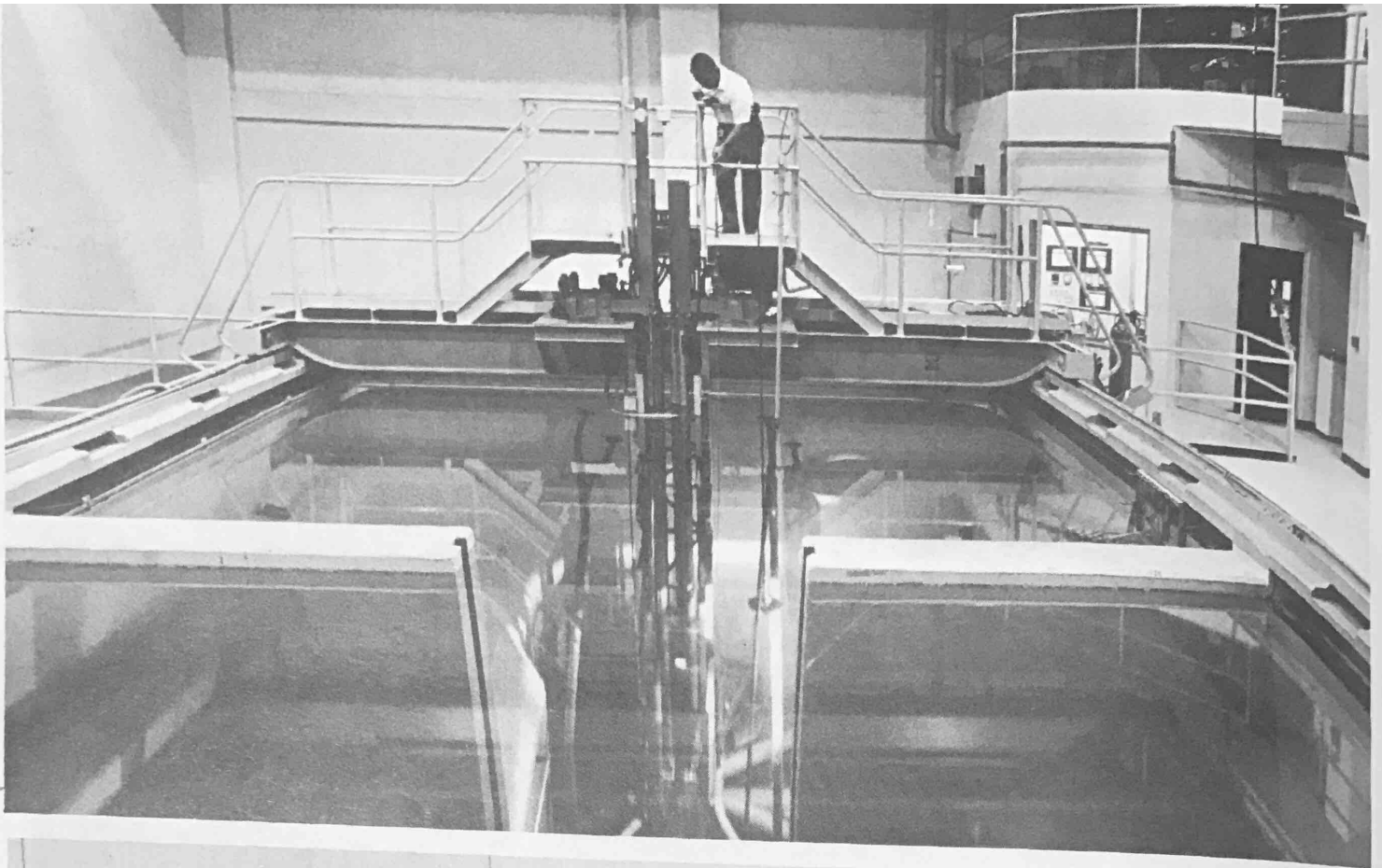
The operating limits for the one megawatt pool type reactor were revised and brought up to date. Test procedures were prepared and implemented to comply with most of the limits set forth in the document. Procedures will be prepared for the remainder of the limits during the coming year.

Plans and negotiations are underway to convert the one megawatt reactor to a more powerful, versatile unit. The present reactor will be converted to a two megawatt thermal constant power reactor with a pulsing capability of two thousand megawatts. The reactor to be installed will utilize modified (FLIP) Triga type fuel of zirconium hydride with erbium as a burnable poison, and be stainless steel clad. Conversion and full power operation is expected to take place in the spring of 1970.

EDUCATIONAL ACTIVITIES

Mr. Fernando Lopez Carrasco, from Mexico, who served with the Reactor Division after completing his M.S. Degree, left the division in May, at which time he received a certificate indicating that he had served and was qualified as a Reactor Supervisor.

The Reactor Operator Refresher Course, initiated in 1967, continued during the year at a slower pace and will continue through next year.



- (1) Reactor pool and core support structure
- (2) the one megawatt reactor console

One operator trainee was qualified as a reactor operator in June after receiving intensive training. The position of Chief Reactor Operator was created and filled by one of the operators so as to serve as the night shift supervisor.

STAFF

Mr. Pedro Cruz Gonzalez, formerly Research Associate II, has been appointed Reactor Supervisor.



Operator observes the one megawatt reactor console and instrumentation rack



Miss Heidi Pabón Pérez sets up x-ray detection equipment used with diagnostic and therapy x-ray units to measure direct beam and stray radiation

HEALTH PHYSICS

The Health Physics Division, which deals with health and safety problems, operates at both Rio Piedras and Mayaguez with two main functions: it provides the services needed for safe operation of the Puerto Rico Nuclear Center and implements the regulations; it contributes to PRNC's educational and research programs.

The services (see Table I) include consultation and supervision, in all matters concerning safety and especially in radiation safety.

To implement and enforce safety regulations, the Division instituted regular safety inspections in addition to existent monitoring practices and established safety committees with members within each Division. Indoctrination on safety, especially on radiation protection, is offered to PRNC personnel either through special courses or through the safety coordinating committee.

The education and research program includes:

- 1) Courses offered at UPR, Mayaguez, and UPR, San Juan, and the Medical Center, Rio Piedras in basic Radiation Protection at the graduate level for students not specializing in the field.
- 2) An M.S. degree program in Health Physics at UPR, Mayaguez. This program has been offered since 1959. Two students were enrolled during 1968.
- 3) A new one-year program leading towards the M.S. degree in Radiological Health at UPR, San Juan campus. This program is offered by the Department of Preventive Medicine and Public Health in conjunction with the Puerto Rico Nuclear Center. Fifteen new courses were designed to meet the needs of the students especially from Latin America. Six of them were offered during the Fall Semester 1968. Four students were enrolled, two of them from Latin America.
- 4) Advice and supervision of student research theses.
- 5) Special training in Applied Health Physics, and
- 6) Basic research

Special emphasis was given this year in improving the PRNC safety standards and in developing the new program in Radiological Health. The progress so far in both areas is quite satisfactory.

TABLE I

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

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

*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 full program of 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 completely capable 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 was placed in one of the hot cells which was modified into a calibration room for higher range calibrations.

The film badge service laboratory was moved to the installation at Cornelia Hill. A dark room was constructed for this purpose. The program operates satisfactorily in the new location. Plans are underway to incorporate a digital voltmeter in the circuit of the densitometer. This adaptation will speed up the film density reading. Space is also provided for the Nuclear Accident Dosimetry program. The relocation will be done as soon as the emergency power lines are completed. A complete revision and updating of the NAD program is planned for next year.

The radiation safety program at the Oncologic Hospital has greatly improved. 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 for new hospital personnel.

Table II

Health Physics Services 1968

Film Service to PRNC and BONUS for the year 1968:

	<u>Beta Gamma</u>	<u>Neutron</u>	<u>Totals</u>
PRNC	4,909	694	
BONUS	974	815	
Total	<u>5,883</u>	<u>1,509</u>	7,392

Radiation Survey meters calibrated:

a) gamma	306		
b) neutron	26		
Total	<u>332</u>		332

Area Monitoring Samples Analyzed

a) smears	1,821		
b) water	65		
c) air	45		
Total	<u>1,931</u>		1,931

Environmental Surveillance samples analyzed:

a) water	48		
b) air	25		
c) soil	15		
d) vegetation	12		
Total	<u>100</u>		100

Review of questionnaires for reactor experiments:

254	254
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Review of requests for use of irradiation facilities other than reactor:

37	37
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Review of requests for radioisotope procurement:

99	99
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Medical Dispensary - No. of cases seen:

a) minor accidents	49		
b) physical exams	107		
Total	<u>156</u>		156

The new procedures for the procurement, production and use of radioactive materials are being followed satisfactorily.

A new 4500 Ci ^{60}Co source, was ordered from ORNL to replace the old 1500 Ci ^{60}Co source in Río Piedras. A permit to transport the source was obtained from the Department of Transportation. The old source was transferred in Mayaguez and was placed in the reactor pool next to the gamma room. The gamma room will be used as a new radiation facility for moderate dose rates.

A special three-room storage building is being built behind the animal house. Radioactive materials, flammables and other hazardous materials will be stored there. The building will be ready in March 1969.

Plans to construct a special irradiation room for the Texas Nuclear Neutron Generator were made. The safety features for this room are being examined.

An emergency plan in case of any catastrophic event in Mayaguez was prepared. The plan, a general one, relates PRNC problems and competencies to the surrounding community.

Two committees were organized to deal with all safety problems. The committees have branches in Mayaguez and 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, followup of recommendations are implemented. Indoctrination of personnel is offered in two ways: first, through safety institutes in Mayaguez and 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.

The Industrial Safety and Fire Protection program has greatly improved in Mayaguez and Río Piedras during the year. Personal safety and Fire fighting equipment are provided by the division as needed.

EDUCATION AND TRAINING

The education program has two main objectives. The first is to provide graduate programs 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 for PRNC personnel, and courses for students who will be working with radiation sources, but who do not intend to specialize in Physics.

A new Radiological Health program was developed by the Division through the School of Medicine, Department of Preventive Medicine and Public Health (PMPH) in addition to the existing Health Physics program offered by the Department of Biology, UPR Mayaguez campus.

Two students were enrolled in the Health Physics program UPR, Mayaguez; five were accepted in the Radiological Health program PMPH-San Juan, two of whom are from Latin America (see Table III). One student from Mexico has completed his training in Applied Health Physics. The program was arranged to meet the specific needs of the student (to be applied in his country).

Three courses, one at UPR Mayaguez, and the others at the School of Medicine San Juan, are regularly scheduled for students not specializing in the field.

Table III
List of Students

Name	Country	Program or Course
1. Efigenio Rivera	Puerto Rico	M.S. in Health Physics
2. Amalia Vélez Paradis	Puerto Rico	Dep. of Biology, UPR-Mayaguez
1. Luis Rodríguez	Ecuador	M.S. in Radiological Health; Dep. of Preventive Medicine and Public Health, UPR-San Juan
2. Luis Aquiles Santana	Colombia	M.S. in Radiological Health; Dep. of Preventive Medicine and Public Health, UPR-San Juan
3. Daniel Torres Ortiz	Puerto Rico	M.S. in Radiological Health; Dep. of Preventive Medicine and Public Health, UPR-San Juan
4. Heriberto Torres Castro	Puerto Rico	M.S. in Radiological Health; Dep. of Preventive Medicine and Public Health, UPR-San Juan
5. Agnes Weisz	Israel	M.S. in Radiological Health; Dep. of Preventive Medicine and Public Health, UPR-San Juan
1. Ignacio Maldonado Rico	Mexico	Applied Health Physics
1. Alice Ortiz de Caraballo	Puerto Rico	M.S. in Radiobiology
2. Michael Gileadi	Israel	M.S. in Public Health
1. Luis David Bernier	Puerto Rico	PMPH 561
2. F. J. Fernández	Puerto Rico	Principles of Radiological Health
3. F. Folch	Puerto Rico	" " "
4. N. A. Lores	Puerto Rico	" " "
5. J. A. Negrón	Puerto Rico	" " "
6. R. Orta Allende	Puerto Rico	" " "
7. R. Rodríguez	Puerto Rico	" " "
8. A. J. Santiago	Puerto Rico	" " "
9. R. Sánchez Valentín	Puerto Rico	" " "
10. Jesús López García	Puerto Rico	" " "

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. Laboratory on Radiation Detection was included in the Mayaguez course, while the 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 PRNC "Radioisotope Techniques" course offered four times this year.

One staff member of the Division was enrolled in the M.S. in Radiation Biology program, UPR Mayaguez and one in the M.S. in Public Health program PMPH, UPR San Juan.

A one week intensive course was given by the US PHS-NCRH at PRNC Mayaguez. The course was organized by the Health Physics Division in conjunction with the Department of Health, Commonwealth Government of Puerto Rico, and was entitled "Management of Radiation Accidents." Twenty students attended, ten of them PRNC personnel.

A similarly organized course entitled "Radiological Health" was given for nurses and policemen. Sixty-five attended.

Thesis Research Status

Gamma-Ray Spectra Around the PRNC 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 Vélez. 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 size of the patients, and the particular X-ray unit used, kVp, mA settings and the filtration used are considered. The entrance and the exit dose were determined, utilizing thermoluminescent dosimetry techniques. The data will be published as a PRNC report.

The New Program In Radiological Health

The new program in Radiological Health is offered through the School of Public Health, which is considered by many leading universities in USA to be the natural environment for a program in Radiological Health. All courses aside from the Public Health courses are new ones offered by PRNC, and are specifically designed for the students of Radiological Health.

The academic prerequisites for enrollment in the program are a B.S. and 6 credit hours each of university level physics, chemistry, biology, and mathematics.

The duration of the program is one full year, including 2 months of field practice. The thesis was eliminated (as it is in many leading universities) and field practice was introduced since it appears to be more useful to the students. However, if there is a future need for a more sophisticated degree (including thesis) a course can be offered to students who prefer an academic career. During the summer the facilities of the Bio-Medical Building-PRNC Rio Piedras are made available for Hospital Physics-minded students, while the

nuclear reactor and other facilities at PRNC-Mayaguez are available for students interested in Health Physics. This training is arranged according to the needs of the students and their country.

There is also a possibility for future expansion of the program so that it may serve as a core for an M.S. program in Radiation Biology and in Hospital Physics.

Most of the instruction is given in Spanish, (approximately 75%) with textbooks in English. This makes the program especially attractive for students from Latin America.

The Curriculum

The curriculum is designed to provide integration of related disciplines as the most effective way to create radiological Health Specialists. A minimum of 40 credit hours of course content is required. Table IV is a list of the courses offered. Full description of the courses is given elsewhere.

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 uW 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. No further progress was made this year. There are plans to continue this work next year.

The neutron dosimetry project. This work has been discontinued; 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 will be reactivated next year. The purpose is to study the heat inactivation pattern of 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 was completed and reported elsewhere. 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.

Table IV

List of courses offered for the M.S. Program in Radiological Health

The following are Required Courses:			Credits*	
PRNC	501	Radiation Physics	2	I**
PRNC	505	Radiation Chemistry	2	I
PRNC	510	Radiation Biology	2	I
PRNC	515	Radiation Effects on Mammals and Humans	2	II
PRNC	520	Radiation Detection	2	I
PRNC	525	Radiation Dosimetry	2	II
PRNC	530	Radiation Hazards and Protection	2	II
PRNC	535	X-Ray Protection	1	II
PRNC	540	Decontamination & Waste Management	1	I
PRNC	545	Laws and Regulations on Radiological Health	1	II
PRNC	565	Basic Nuclear Electronics	2	II
PMPH	470	Environmental Health	3	I
PMPH	556B	Indust. Hygiene and Indust. Accident Prevention	2	I
PMPH	450	Biostatistics	2	II
PRNC	599	Field Practice	4	S
The following are Elective Subjects:				
PRNC	550	Radioactivity of the Environment	2	I
PRNC	555	Safety in Reactor Operations	1	II
PRNC	560	Reactor Technology	2	II
PMPH	476	Seminar	1	I,II
PMPH	489	Basic Epidemiology	2	
PMPH	420	Fundamentals of Public Health Administration	2	
PMPH	430	Social and Cultural Aspects of Public Health	2	
Phys.	325	Atomic Physics Laboratory	3	
Phys.	326	Nuclear Physics Laboratory	3	
Biol.	231	Genetics	4	
Biol.	351	Cellular Physiology	4	
Biol.	372	Nuclear Techniques in Biological Research	4	
Chem.	221	Chemical Analysis	4	
Chem.	465	Radiochemistry	4	
Math.	152	Statistical Analysis	3	
Math.	203	Mathematical Analysis	3	
Math.	204	Mathematical Analysis	3	
Math.	307	Ordinary Differential Equations	3	
Met.	101	Introduction to Meteorology	3	
Met.	103	Introduction to Climate	3	

* One credit is equivalent to 18 hours of lectures or at least 36 hours of laboratory work.

** I = first semester, II = second semester, S = Summer

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 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. The results are reported by the Radiotherapy Division.

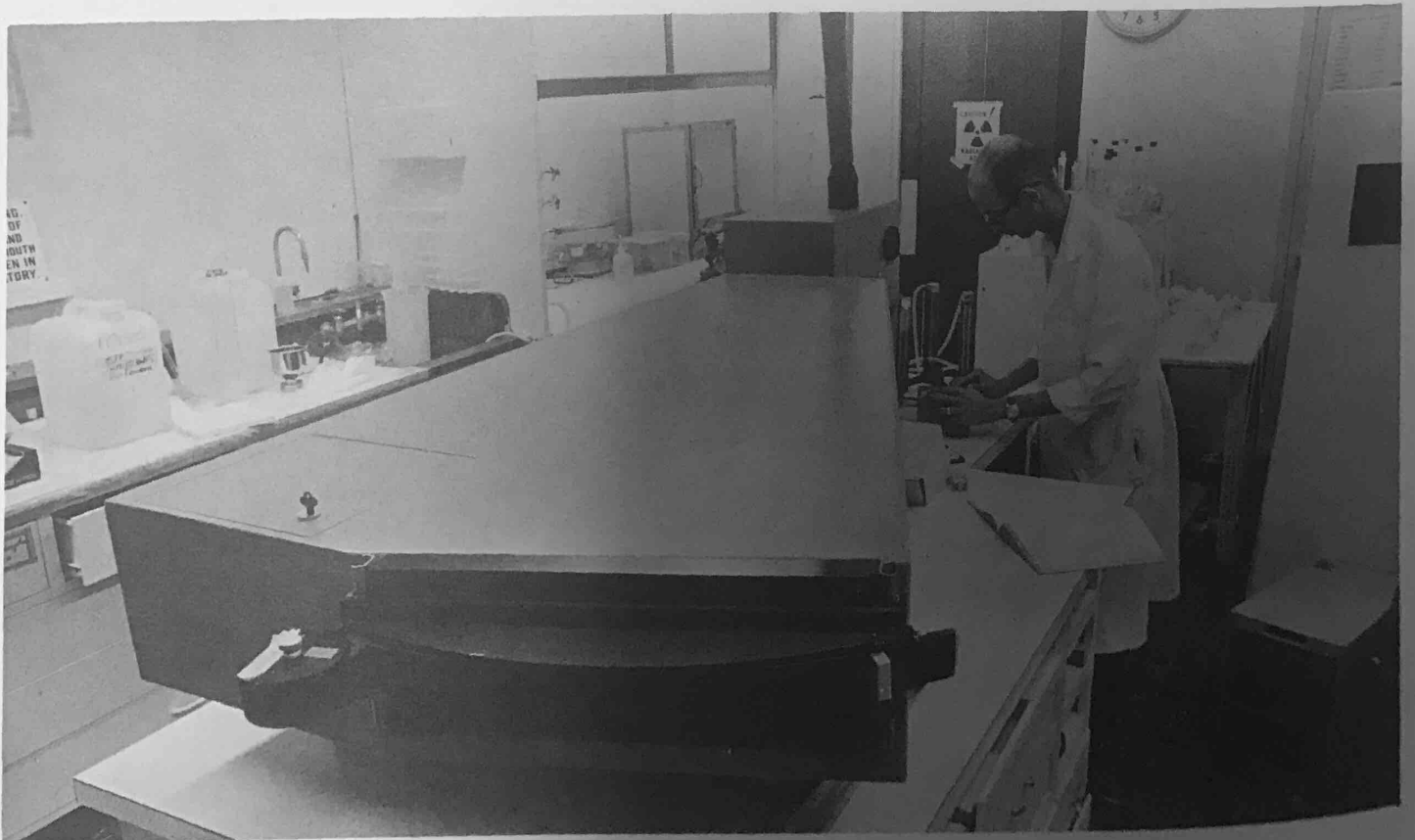
Population exposure project. A study was undertaken on population exposure in Puerto Rico. Efforts were directed in measuring the gonadal dose to patients undertaking medical X-ray exposures. It was felt that this kind of exposure may be more hazardous to the public in Puerto Rico than any other exposure.

The work was divided in two areas. Measurements of the exposure dose delivered to the patients during routine chest X-ray exposures were performed. The gonadal dose was then calculated. Secondly, direct measurements of the exposure dose and the gonadal dose delivered to the patients during routine abdominal X-ray exposures were performed.

Thermoluminescent (TLD) dosimetry techniques were used. The age and the size of the patients and the particular X-ray unit used, Kvp, mA settings and the filtration used were considered. The first part of the work consisted of data compiled throughout the western part of the island and included statistical variations. The results will be published as a PRNC publication.



- (1) Miss Norma Nazario sends a sample for non-destructive manganese analysis via the pneumatic rabbit system used to transport them into PRNC's reactor for short irradiations with thermal neutrons
- (2) Mr. Raul McClin Escalera prepares a sample for the arc spectrograph



MARINE BIOLOGY

The work in the Marine Biology Program at PRNC includes both field and laboratory studies. Special emphasis is placed upon field sampling and measurements to determine biological and environmental mechanisms which influence the transport and distribution of trace elements and their radionuclides in shallow water ecosystems.

The aims of the program have not been changed in the last year. Methods of approach do change, however, to take advantage of new analytical techniques and to resolve questions raised by recent investigations in this and other laboratories in radioecology field studies.

The program was started in January, 1962 to provide background information for use in the application of the specific activity approach in predicting environmental hazards from radionuclides. The purpose of the program was, and continues to be, the development and use of field and laboratory techniques for measuring the amounts of selected trace elements including Be, Ca, Sr, Sc, Mn, Fe, Co, Ni, Cu, Ag, Zn, Cd, Hg, Al, C, Si, Pb, N, P, Br, I, rare earths and U in rocks, soils, river and marine waters, marine organisms and river and marine sediments. The methods developed in this work are directly applicable to the use of the specific activity approach for marine contamination problems. These methods have been used during the past two years in a feasibility study for a sea-level isthmian canal.

The program is composed of six projects which are coordinated and integrated into a team approach to environmental and ecological measurements including:

1. Analysis for selected trace elements by flame spectrophotometry, atomic absorption spectrophotometry, x-ray emission spectrography, neutron activation, colorimetry, fluorescence emission, polarography and arc spectrography.
2. Measurements of concentration factors and turnover rates in selected organisms for given radionuclides in different forms.
3. Basic marine ecology investigations.
4. Measurements of biological productivity and energy transfer between trophic levels.
5. Background measurements in physical, chemical and geological oceanography.
6. Determination of distribution patterns of "light" and "heavy" rare earths in ecosystems including river and marine water and sediments, marine plants and the hard and soft parts of marine invertebrates.

The main geographical area of study is on the west coast of Puerto Rico. In this area the outflows of three rivers empty into a 20 mile stretch of coast. The Culebrinas river to the north drains an area containing large amounts of limestone. The central river, the Añasco, drains an area of volcanic origin in which are located hydrothermal deposits of copper sulfide. The Guanajibo to the south drains a mountainous region containing large amounts of serpentinite which is especially rich in cobalt and nickel. Although all rivers contain the same group of trace and major elements, their ratios of abundance vary greatly, with each watershed supplying its characteristic assemblage to the marine organisms in the near-shore waters.

A second area of study is on the south coast at Phosphorescent Bay in which investigations upon the seasonal changes in trace element content in the phytoplankton, zooplankton, water and sediments have been started.

The production of radioactive materials continues to accelerate. The number of reactors constructed for electrical power and desalination of sea water for irrigation or drinking increases yearly and the use of nuclear explosives for excavation and other peace-time applications is in the planning stage. Both reactors and nuclear explosions are capable of producing large amounts of radionuclides and some of these, accidentally or intentionally, will be introduced into the marine environment. In addition, the use of reactors for the propulsion of ships, rockets and other vehicles will continue to provide occasional injections of localized, but relatively large, amounts of radioactive material into marine areas as a result of accidental damage or destruction of the reactors. Smaller amounts of fission products and neutron-induced radionuclides are released periodically into or near harbors by nuclear propelled ships. With continued advancement in engineering design this source of contamination may be reduced or eliminated but at the present time it must be considered. With the increasing number of countries developing nuclear weapons the possibility of the use of nuclear weapons for attack or retaliation does not diminish and the wide scale use of nuclear explosives in an ensuing all-out struggle can not be discounted. In an action of this kind a large fraction of the terrestrial areas now used for the production of human food could receive additions of radionuclides including Sr^{90} and Cs^{137} in sufficient quantities that man could be forced to turn, in part at least, to marine sources of protein. Strontium-90 and Cs^{137} are subjected to sufficient isotope dilution in sea water that they would not likely become significant contaminants of marine foods. Other radionuclides, however, including Be^7 , Ba^{140} - La^{140} , Zn^{65} , Y^{91} , Ce^{144} , Pr^{144} , Pu^{239} , Pb^{203} , Pb^{210} , Zr^{95} , Nb^{95} , P^{32} , W^{185} , Mn^{54} , Mn^{56} , Fe^{55} , Fe^{59} , Co^{57} , Co^{58} , Co^{60} , Ru^{103} , and Ru^{106} - Rh^{106} could be introduced into estuarine and other near-shore areas by direct fallout, runoff from rivers or by addition of contaminated ocean waters. All of these radionuclides are subject to rapid sedimentation in shallow waters and, thus would be tied up in "reservoirs" of activity and be continually incorporated into human food organisms of marine origin. Radionuclides deposited in areas of upwelling along the west coasts of South America or Africa would also be trapped in cyclic circulation systems so that build-up of radionuclides in the rich populations of plankton and fish would occur with time. Some nuclides would undergo radioactive decay and not be of consequence as contaminants; others, because of their chemical characteristics, would not be incorporated into food webs leading to man. At least one-half of the radionuclides listed above, however, have relatively long physical half-lives and are accumulated by marine organisms to levels that they could contribute significant radiation doses to populations using them for food.

The more populated areas of the world, in general, border the major seas and oceans; man's principal point of contact with the marine environment is in the shallow near-shore areas. In these marine regions are located most of the world's harbors, the nursing grounds for many larval and immature forms of

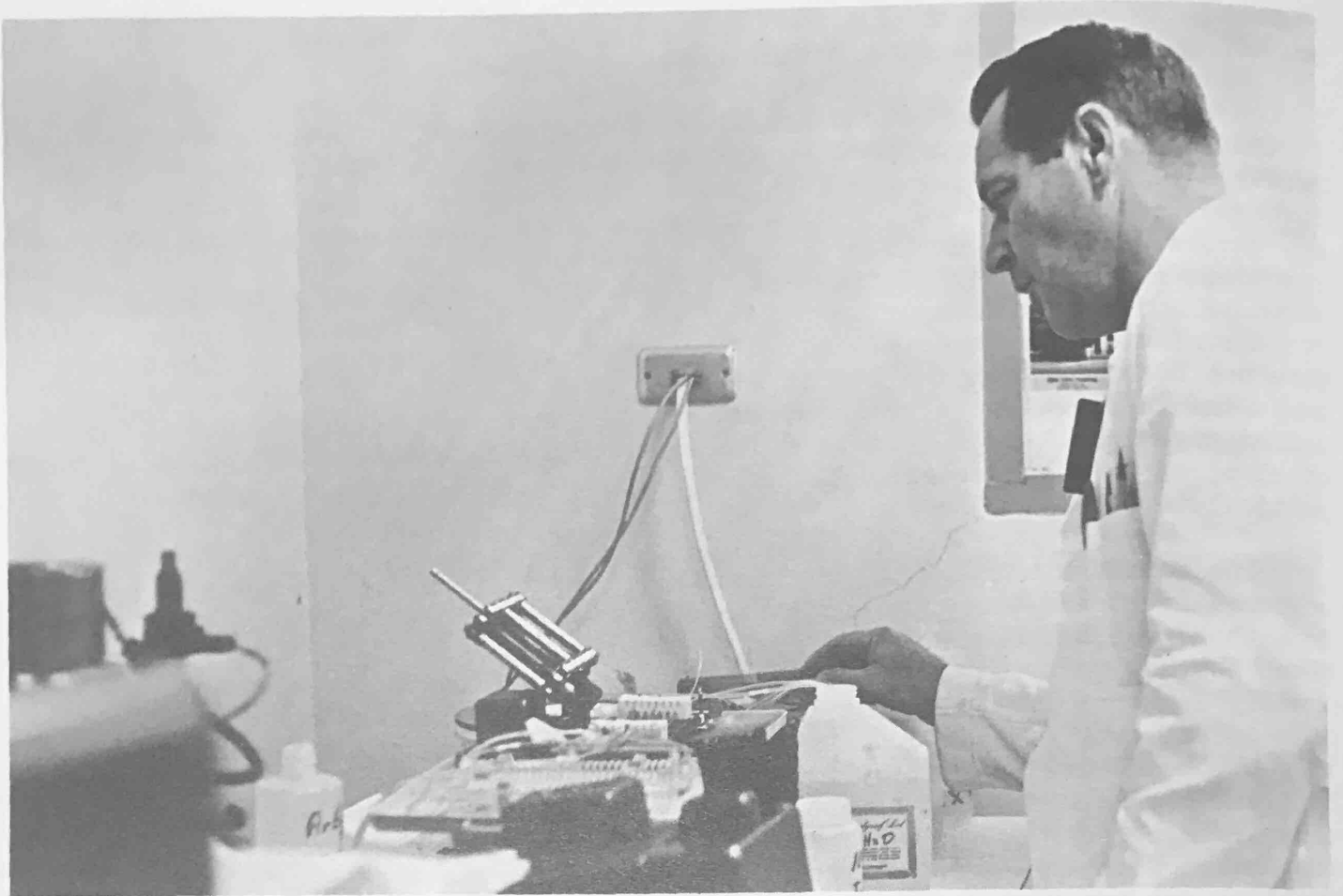
commercially important marine food animals, as well as the adults of others including algae, molluscs, crustacea and bottom fish.

A knowledge of the biogeochemistry of trace elements in the near-shore areas is thus of critical importance to man, as well as of academic interest to the researcher, since radioactive isotopes of the same elements are apt to become incorporated into food webs from which human food is derived. Some disagreement now exists among various investigators concerning the relative roles of inorganic reactions and biological influences upon the distribution and retention of trace elements and their radionuclides in estuarine and other near-shore marine waters. The distribution patterns of many of the trace elements in the shallow waters have not been measured and little is known of their rates of transfer between compartments constituted by fresh and marine waters and sediments and the contained organisms. Even less is known concerning the chemical and physical forms of elements in these waters and the relative availability to organisms of the particulate, chelated or ionic fractions.

The dominant mechanisms which control the distribution patterns of non-conservative trace elements and their radionuclides in the open ocean differ from those which operate in the near-shore regions. In most estuarine, and other shallow parts of the sea, the biomass and biological productivity are usually large as a result of nutrient additives from the land or from upwelling of deep waters. In addition, the waters are mixed throughout their depths to the bottom and are in contact with bottom sediments which are often resuspended by tidal currents and waves resulting from winds. Thus, the near-shore areas constitute regions of intense biological, physical and chemical activity in comparison with the open seas where the distribution patterns of added radionuclides are controlled mainly by water currents and density gradients except for those radionuclides added as particles large enough to be subject to gravity. In these regions biological effects upon the distribution of most elements are small except for limited transport downward of phosphorus, nitrogen, silica, lead, barium and rare earths. Biological transport of these elements is effected mainly through their incorporation into faecal pellets by zooplankton with subsequent transport to the bottom. Significantly all of these elements would exist as relatively insoluble salts in faecal pellets.

In the near-shore areas the sediments appear to exert the major influence upon the distribution patterns of several biologically important trace elements and their radionuclides. These sediments also influence the distributions of some radionuclides whose stable counterparts have no known biological function although they are accumulated significantly by some marine organisms. (These elements include (1) Biologically important P, Cu, Zn, Mn, Fe, Co, (2) non-biological Ru, Zr, Nb, Pb, Ag, Ni, Sc, Re, Cd, Al.) Many of these elements, added to estuarine regions by terrestrial runoff, are precipitated or coprecipitated in areas of mixing of fresh and saline waters. Plant detritus in the bottom sediments of these areas often contain several hundred times as much iron, manganese and scandium as is found in the source plants on land.

Investigations designed to determine the distribution patterns of stable trace elements in the soils, land plants, river waters and sediments, marine waters and sediments and the marine organisms may be used to measure and define the relative influences of physical, chemical and biological mechanisms which control the distribution of radionuclides introduced into the same system. In the Marine Biology Program these distribution patterns are being made. For all biological samples the amounts of trace elements are reported on the bases of wet, dry and ash weights and per gram of carbon and nitrogen and phosphorus and per 10,000 calories. In this way the transport of trace elements through food webs may be related to transport of organic material and energy through the same system.



Working in the Marine Biology laboratory in Mayaguez: top-Research Associate Russel W. Davis, below-Program Head Dr. Frank Lowman

DEVELOPMENT OF ANALYSIS METHODS

During the past year additional methods for neutron activation analysis of water, plants, animals, sediments, rocks and soils have been developed. Non-destructive analysis of sediments, soils and rocks may be done for aluminum, sodium, manganese, iron, cobalt and scandium and in some samples, lanthanum and copper. Separation procedures for geological samples using carbonate fusion followed by solution, adjustment of pH and extraction with ammonium pyrolydine dithiocarbonate (APDC) allows the analysis of copper, nickel, bismuth, and cadmium with simple chemistry.

A method for analyzing uranium content of biological samples of marine organisms has been developed utilizing neutron activation followed by chemical separation. Two hundred milligrams of irradiated (Neutron flux 2×10^{12} n/cm²/sec) ash is quickly dissolved and the uranium is coprecipitated with 3 successive ferric hydroxide precipitations. The precipitate is washed, dissolved in 5% HNO₃ and extracted into ethyl acetate. The extract is dried under a stream of air and the 0.07467 Mev photopeak of U²³⁹ (T_{1/2} 23.5 min.) compared with that of a 10 ug uranium standard. The yield is about 90%.

A method has been developed for measuring small amounts of silver in biological samples. Silver has been reported to be relatively volatile at ashing temperatures. In tests made on exoskeleton, muscle, gills, hepatopancreas, and GI tract of spiny lobsters raised in sea water spiked with Ag¹¹⁰, however, the loss of this radionuclide from ashing at 450°C for 24 hours was less than 1%. In the analysis for stable silver one gram of biological ash is sealed, under vacuum, in glass capsules with alternate capsules containing 5 μg of Ag on low-ash filter paper. The samples and standards are irradiated for 10 days (160 hours). After a few days silver carrier and a minimum amount of 8N HNO₃ is added to each sample which is dissolved by heat and diluted with water. Silver is precipitated as the chloride and the precipitate is dissolved in NH₄OH and precipitated as the sulfide which is washed with water and dissolved in boiling HNO₃. The solution is scavenged with ferric hydroxide, the precipitate rinsed and added to the supernate and the pH adjusted to about 3 with HNO₃. The silver is extracted with dithizone in carbon tetrachloride. The sample is dried and counted for the 0.6576 and 0.8845 Mev photopeaks. Yield is determined by neutron activation of carrier silver.

Total particulate and "soluble" zinc, manganese and scandium in sea water may be analyzed with a relatively simple procedure. Twenty liters of filtered sea water (0.45 μ) are acidified with 100 ml of HNO₃ and carrier-free Zn⁶⁵, Mn⁵⁴ and Y⁸⁸ added for the determination of chemical yields. The sea water is scavenged with purified ferric hydroxide (100 mg Fe) at pH 9 during an 8 hour period. One half of the precipitate is irradiated 4 to 16 hours and allowed to decay for 3 weeks. The 0.8896 and the 1.1206 photpeaks of Sc⁴⁶ are counted by coincidence spectrophotometry and compared with a Sc standard.

The other half of the ferric hydroxide precipitate is dissolved in 8 N HCl and the iron extracted with isopropyl ether. The acid layers are retained and diluted to 10 ml with distilled water. One ml of the solution is irradiated 4 minutes along with a 10 μg Mn comparator standard. The solution is scavenged twice with ferric hydroxide. The 0.8468 Mev Mn⁵⁶ photopeak is compared with that of the standard. Chemical yield is later determined from the Mn⁵⁴.

Zinc is determined by extracting the element twice with Dowex-50 from 5 ml of the acid solution used for the manganese analysis after it had been adjusted to pH7. The stable zinc is analyzed by atomic absorption spectrophotometry and the yield is determined from the Zn⁶⁵ spike.

Particulate zinc, iron, manganese, calcium and strontium retained on micropore filters (pore size 0.45μ) may be determined by atomic absorption spectrophotometry after ashing and dissolution of the samples. Scandium is measured by neutron-activation analysis as described above.

A new method has been developed for the measurement of particulate phosphorus retained on micropore filters. The filter paper is compacted and placed in a stainless steel oxygen bomb at about 400 PSI of oxygen. The bomb is fired electrically and the ash removed with distilled water. The normal losses of phosphorus volatilized during ashing are avoided with this method. The sample is diluted to appropriate volume and analyzed by colorimetry in an Autotechnicon analyzer.

Tests of the accuracy of quantitative arc spectrography have been compared with analysis by neutron activation on sediment samples. The standard deviation of arc spectrography for aluminum and manganese is about twice that for neutron activation analysis. The two methods appear to be equally accurate for iron. Comparisons of accuracy between arc spectrography and neutron activation have been started on biological samples. Development of methods for concentrating metals from biological material for use in arc spectrographic analysis have been started using precipitation, liquid extraction and ion exchange.

MEASUREMENT OF CONCENTRATION FACTORS AND TURNOVER RATES IN MARINE ORGANISMS

The systematic study of the uptake of radionuclides by Artemia salina has been continued. Carrier-free radionuclides for all of the elements tested are not available and the effects of isotope-dilution upon uptake introduce significant errors into some of the tests.

Preliminary investigations have been completed on the uptake of Hg^{203} by the diatom Chaetoceros costatum. No difference in uptake was observed in non-dividing cells under light and dark conditions but dead cells accumulated twice as much Hg^{203} per cell as did living cells. This may be caused by increased surface adsorption in the dead cells, probably as a result of increased numbers of associated epiphyton.

Studies upon the effects of marine microbes in increasing the apparent uptake of Hg^{203} and other radionuclides in diatoms, marine benthic algae and the clam Donax are being continued.

MARINE ECOLOGY

Studies on the assemblages of foraminiferans off the west coast of Puerto Rico have been continued. Four species of reef foraminifers are present in the Amphistegina-Archaias assemblage off the west coast of the island and include Amphistegina gibbosa, Archaias angulatus, Asterigerina carinata and Rotorbinella rosea. The distribution of A. gibbosa is directly related to the position of submerged Pleistocene reefs off western Puerto Rico with relatively high percentages of the organism at two different levels representing submerged reefs and/or wave-cut terraces at 55 meters and submerged reefs at 85 meters depth. The dominance of this species in the relict reef fauna is an indication of temperatures colder than the present ones during the building of these reefs. The Amphistegina-benthonic foraminifera ratio is much higher if only glauconitized tests are considered, showing that the Amphistegina assemblage of the submerged reefs is older than the living assemblage.

Samples of foraminifera are being separated and prepared for trace element analysis. Comparisons will be made between recent and relict fauna.

Comparisons of the foraminiferan reef assemblages of Puerto Rico with those of Caribbean and Pacific Coasts of Panama and Colombia have been made. One of the principal factors causing differences of assemblages in the Caribbean appears to be that of local upwelling.

During the studies made on foraminiferan assemblages a new species and a new genus have been observed in the sediments off the island platform off the west coast of Puerto Rico. The new species, Reophax caribensis, is provided with a friable test composed of no well-cemented grains, generally laminar. It lives in shallow waters. The new genus, Glaucosammia, has been included in the family Lituolidae. The type species is Reophax trilateralis. The foraminifer is highly variable in shape and occurs on the outer submarine shelves of the Caribbean sea. Its friable test is composed of poorly cemented grains, mainly of glauconite or calcite or combinations of these with other dark minerals.

Total carbon, hydrogen and nitrogen content in the isopod Eurydice littoralis (Moore) and in the copepod Pontella mimocerami (González) has been determined. Results show that the copepod contain 41% C, 6% H and 11% N on a dry weight basis, and 30% C, 4% H and 6% N for the isopod.

The absolute amount of the three elements C, H, and N, in mg/g dry weight increases linearly with an increase in size (weight) of Pontella mimocerami; however, the ratio of the amount of C, H, and N to the organisms dry weight remains constant. The animal thus seems to be able to maintain a fixed per cent of the three basic elements carbon, hydrogen and nitrogen. Samples which had been ashed at 520°C showed amounts of ash ranging from 9% to 28% of the organism's dry weight. The ash did not contain any inorganic carbon.

Investigations continued on the distribution patterns of trace and major elements within components of biogeochemical systems. The frequency distributions of trace elements within sediment samples, rock types and homogeneous populations of marine organisms in all instances have always approximated a log-normal rather than a normal curve. Major elements appear to be normally distributed. When a pair of elements, whose biological uptake is similar, constitute trace elements in a tissue or organ (eg. calcium and strontium in muscle) their atom ratio frequencies are normal. When one of the elements is a major element in a tissue or organ (eg. calcium in bone or shell) and the other constitutes a trace element, the atom ratio frequencies are log-normal.

BIOLOGICAL PRODUCTIVITY AND TRANSFER BETWEEN TROPHIC LEVELS

The statement is often made that trace elements or radionuclides may be concentrated with passage through food webs in the marine environment. Marine food chains have been investigated during the past year for several elements including Sr, Mg, Cu, Fe, Zn, Cd, Ni, Pb, Mn and Co. The nine-armed starfish Luidia senegalensis eats the small clams Tellina punigea and Mulinia Portoricensis. Preliminary investigations suggest a slight increase in Sr, Mg and Cu in the predator over that in the prey. However, all other elements listed above were present in the starfish in lower amounts than in their food. the influence of sediments, accidentally ingested by the starfish, upon the levels of copper in these animals is being investigated.

In pelagic food chains including mixed plankton, herbivorous fish and carnivorous fish all elements examined thus far, with the possible exception of zinc, are discriminated against with increase in trophic level.

Studies have been made on biological productivity in Phosphorescent Bay on the South Coast of Puerto Rico. One of the principal organisms in the Bay is the luminescent flagellate Pyrodinium bahamense. Plankton samples are also being collected in the Bay at monthly intervals and analyzed for eleven elements. High concentrations of iron, manganese and aluminum have been found. These high values may indicate contamination of the phytoplankton with terrigenous detritus stirred up from the bottom although there is also the possibility that they indicate precipitation and coprecipitation of the elements from colloidal phases in runoff waters. Phytoplankton are known to concentrate aluminum by factors of 10^5 over the amounts in the water, mostly by surface adsorption.

In order to establish the mechanisms responsible for the high values of iron, manganese and aluminum in Pyrodinium a culturing room with light source and temperature control has been constructed for raising this dinoflagellate in culture. Carrier free radionuclides will be used to measure concentration factors and turnover rates under controlled conditions. It is anticipated that turnover of most nutrient structural and catalyst elements will not occur during the exponential growth phases.

BACKGROUND MEASUREMENTS IN PHYSICAL, CHEMICAL, AND GEOLOGICAL OCEANOGRAPHY

Large samples (40 liters) of sea water have been collected to depths of 1500 m in the Caribbean and Atlantic regions near Puerto Rico. Samples of plankton have been collected to 1000 m. Both the water and plankton samples are being analyzed for several trace elements to determine their distributions with depth. Vertical changes in amounts of elements in the water are expected if vertical biological transport is an important mechanism in carrying trace elements from the surface to deeper waters.

The transport mechanisms for scandium are of interest because Sc^{46} is produced in cooling waters of reactors using river water and is also produced in the use of nuclear explosives for excavation projects. Equilibrium calculations indicate that $Sc(OH)_2^+$ and ScF_3 are probably the most abundant species in solution under sea water conditions. Similar calculations indicate that the concentration of total scandium in the ocean is much too low to be controlled by equilibrium with solid scandium hydroxide. Studies reported thus far indicate that either adsorption onto hydrated iron oxide or onto phosphate minerals, or precipitation as scandium phosphate may be the controlling mechanisms for the concentration of scandium in sea water.

Two types of studies have been started for the purpose of tracing the sedimentary geochemistry of scandium. First, its vertical distribution in the ocean is being measured. The second phase involves the measurement of dissolved and particulate scandium around the outflow of the Añasco River to determine what happens to scandium during the interaction of fresh water with sea water. Results thus far show a disparity of concentrations between river and sea water which cannot be accounted for by dilution alone. Studies now underway include comparisons of changes in concentrations of scandium in soluble and particulate fractions with variations in salinity, pH, and in concentrations of iron and phosphate.

Background studies in sedimentary geology have been continued. Sediment samples collected on Mani Beach near the outflow of the Añasco River have been characterized by size analysis, trace element concentration and organic carbon. Other geological investigations have been carried out on the Cabo Rojo platform, just south of the outflow of the Guanajibo River. Underwater photography and

collection of sediments for physical and chemical analysis were done and studies of pollen content in the water and sediments were determined as a guide to sediment sources and water currents.

A cooperative project in mathematical modeling has been started with Dr. Aviva Gileadi, Department of Nuclear Engineering, UPR Mayaguez, in an effort to develop methods for better planning of field experiments, measurements and collections for use in defining the factors which control the use of the specific activity approach to predict hazards from marine contamination with radionuclides. One model has been developed for physical dilution of a plane source of radionuclides introduced into a marine area with an upper mixed layer overlaying a well defined pycnocline with a shear zone in between. A computer program has been written in which several variables may be altered, including: amount of radioactivity, depth of mixed layer, degree of shear and size of fallout pattern. The model is applicable only to radionuclides in solution.

Another model for vertical biological transport has been completed and a computer program written. Variables which may be altered include: depth of mixed layer, size of plankton population, biological half-life for the element, characteristics of the vertical migration, chemico-physical characteristics of the radionuclide.

DISTRIBUTION PATTERNS OF RARE EARTH

Studies on the amounts of "light" and "heavy" rare earths have been continued. Analyses of contaminants in Y^{88} used for yield determination, have been made. Preliminary tests on the use of Sc^{47} as a tracer for yield determinations have been started.

VISITING INVESTIGATORS

Several visiting scientists worked with the Marine Biology Program during 1968. Financial and/or logistical support were provided for: Dr. Edward R. Tompkins (U. S. Naval Radiological Defense Laboratory, San Francisco, California), who developed a method and is measuring the amounts of palladium in sea water; Dr. R. Gordon Pirie (Dept. of Geology, University of Wisconsin at Milwaukee) continued his studies of the sediments and clay minerals off western Puerto Rico; Dr. Daniel Habib, William Miller and Martine Dreyfus (Dept. of Geology, Queens College, City University of New York) began sediment and pollen studies off western Puerto Rico; Dr. Walter A. Glooschenko, an Oak Ridge Research Participant (Florida State University) began phytoplankton cultures and investigated the uptake of mercury by phytoplankton; Hugo José Jiménez, a technician from the Instituto Venezolano de Investigaciones Científicas worked on the development of analytical techniques for neutron activation and atomic absorption; Michael J. Canoy, a student at the University of North Carolina (Chapel Hill, N.C.) started his thesis research on the distribution patterns of DNA in marine ecosystems. Drs. D. Wolfe and F. A. Cross worked on neutron activation analysis in marine organisms.

Consultants visiting the Marine Biology Program during 1968 included Dr. Walter Slobodkin (State University of New York at Stony Brook), Dr. Bernard C. Patten (University of Georgia, Athens, Ga.) and Dr. Theodore J. Smayda (University of Rhode Island, Kingston).



- (1) Electronics technician Douglas Krom, far left, explains details of the Terrestrial Ecology project to a group of visiting scientists from the U.S. and Caribbean area who came to observe the irradiated site in El Verde
- (2) Bridge leading to the irradiated site in the Tropical Rain Forest at El Verde

TERRESTRIAL ECOLOGY PROGRAM I

THE RAIN FOREST PROJECT

The Rain Forest Project is an ecological research program focused upon an area of tropical montane rain forest on the side of El Yunque mountain in Eastern Puerto Rico. The original objectives of the program were: (1) to study the effects of gamma radiation on the tropical ecosystem; (2) to study cycling of stable and radioactive isotopes through the ecosystem (popularly known as "mineral" or "biogeochemical" cycling studies); (3) and to study the basic biological functions of this ecosystem, such as respiration, transpiration, and photosynthesis in order to better understand phenomena related to the first two objectives.

The project is now in its sixth year. The first objective has been accomplished; results will appear in a volume scheduled to be printed this year. The radiation experiment has been followed up via recovery studies, and basic biological studies, including diversity studies, have been continued, but the major effort during the past year has been the study of radioactive and stable isotope cycling.

CYCLING STUDIES

Over the past several years, there have been numerous tracer experiments to determine rate of isotope movement between ecosystem compartments, such as the movement between epiphyll and leaf, between soil and plant, and between litter and soil. Tracer studies this year included movement through canopy trees of several gamma-emitting radioisotopes, as well as tritiated water. Quantity of stable elements in all major compartments were determined, as well as the rate of movement of these elements between compartments. Nitrogen-fixing capability of epiphyllae on the leaves of trees was studied, and fallout work was continued.

Now that the isotope studies are well established and we have quantified balance and movement in certain parts of the ecosystem, the time has come to start to put all these studies together into an ecosystem model that will have predictive value for a large range of conditions which will occur, but for which it is impractical to conduct an experiment. Because of the complexity of the ecosystem, the model should then be transformed into a computer program. A computer could tell us, for example, the concentration of a given radioisotope in a given portion of the food chain at a given time following a given input of fallout into the system.

The first step in making the model is the framework (Fig.1) which ties together all the isotope studies completed, or currently under way. The arrows represent isotope movement into, through, and out of the tropical rain forest ecosystem. The boxes represent compartments where the isotope is held for varying lengths of time before being passed on to the next compartment.

Studies completed during the past several years relevant to this model

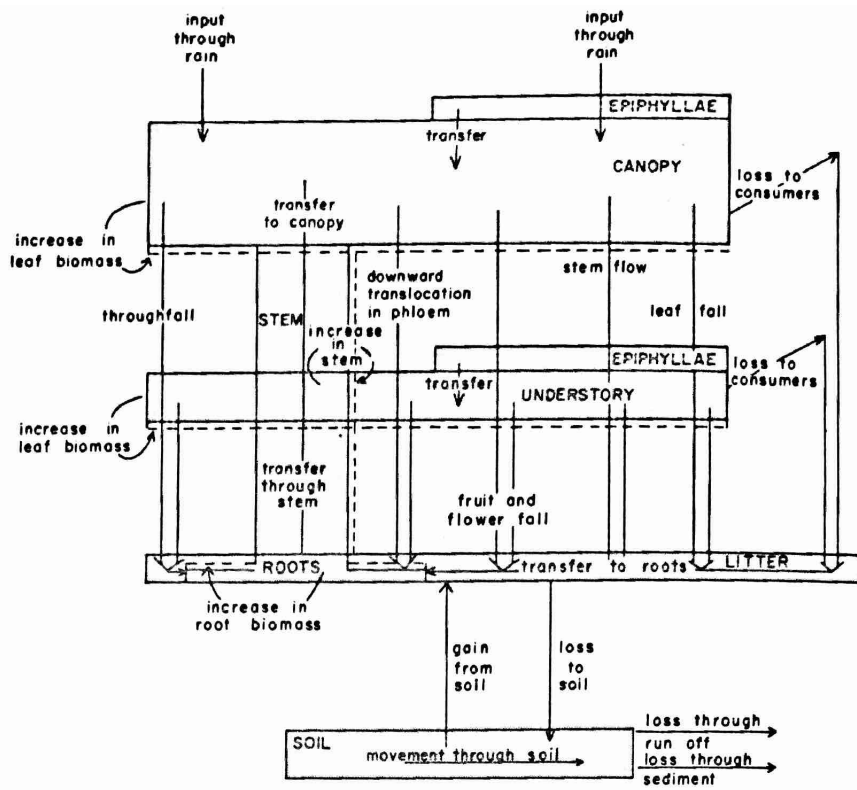


Fig. 1. Schematic diagram of the tropical rain forest, showing important storage compartments for isotopes (upper case letters), and transfer routes (lower case letters)

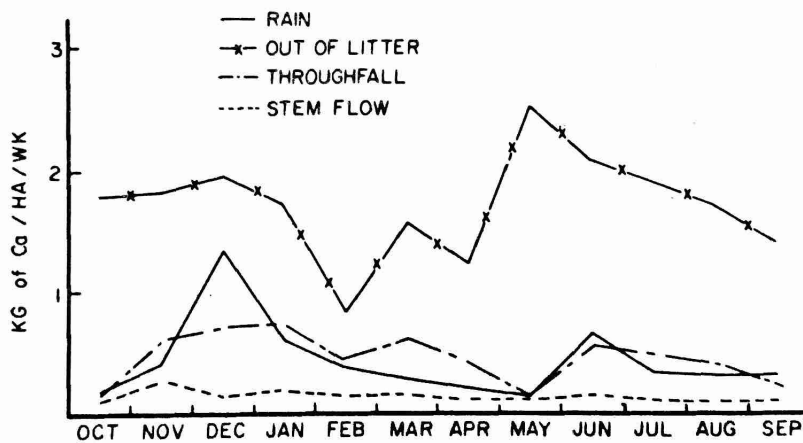


Fig. 2. Rate of calcium movement through four transfer routes, on a monthly basis

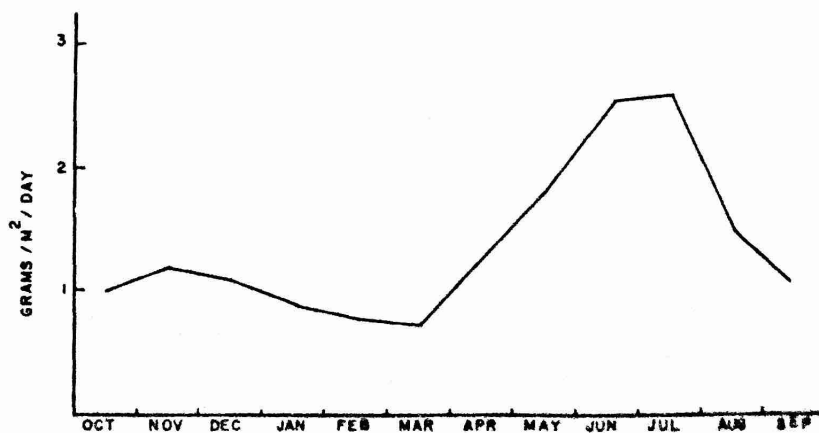


Fig. 3. Rate of leaf fall on a monthly basis

include: isotope movement from litter to soil; from epiphyll to leaf; and from mosses into snails; isotope uptake by understory plants; fallout input into the system; and fallout retention in the canopy. Current studies relevant to this model are discussed in the following paragraphs.

Fallout Distribution Within the Forest

To program the input of fallout into the system, we must know exactly how it enters the system once it is brought to the system by rain. Generally speaking, the rain carries it to the leaves and litter, but it is important to understand the factors that cause variations in the amount of fallout intercepted by leaves.

Table 1 shows that: site (location within a general area) has no influence on quantity of fallout present; species has no influence (except for the understory species Palicourea riparia not included in the table); location of leaves in the canopy or understory had no statistically significant difference in 1968 (although Kline found a definite difference in 1966, and the difference was still distinguishable in 1968); but presence or absence of epiphylls on the leaves had a marked effect on the quantity of fallout present. Judgement as to whether a factor is influential is based on how many tests showed differences, and what the error level was of these differences. For example, in the tests for differences caused by species, only 1 out of 12 tests showed a difference and that was at the 10% level, whereas 4 out of 6 tests for clean vs. epiphyll-covered leaves were significant, and these showed differences at the 1% error level, or less.

Movement of Stable Elements Through the Ecosystem

Rates of movement of a representative element, calcium, are graphed in Fig. 2. There is no apparent relation between amount of calcium input into the system via rainfall, and quantity of rainfall (compare Fig. 2 and Table 2). Most likely, input is more closely related to wind direction before and during rainstorms. Movement out of the litter and through the soil closely follows changes in leaf fall rate (compare Figs. 2 and 3). A graph of the difference between rainfall input and runoff loss (Fig. 4) shows that there is a net loss of calcium from the system during most of the year. Presumably this loss is made up by weathering of parent material.

Water Budget of the Forest

A knowledge of the water budget is essential for calculating the rates of isotope movement between compartments. A budget based on direct measurements is shown in Table 3.

A series of experiments using tritiated water has been initiated for two reasons: (1) Tagging the transpiration stream with a pulse of tritium is virtually the only method of studying the rate that water is pulled through the tree by transpiration (Fig. 5); (2) Movement of tritium through the ecosystem is in itself of interest, because tritium will be one of the principal products if thermonuclear devices are used to dig a new canal through Central America. Participating in the tritium studies are Dr. Jerry R. Kline, Argonne National Laboratory, Dr. John Koranda, and Mr. John Martin, Lawrence Radiation Laboratory.

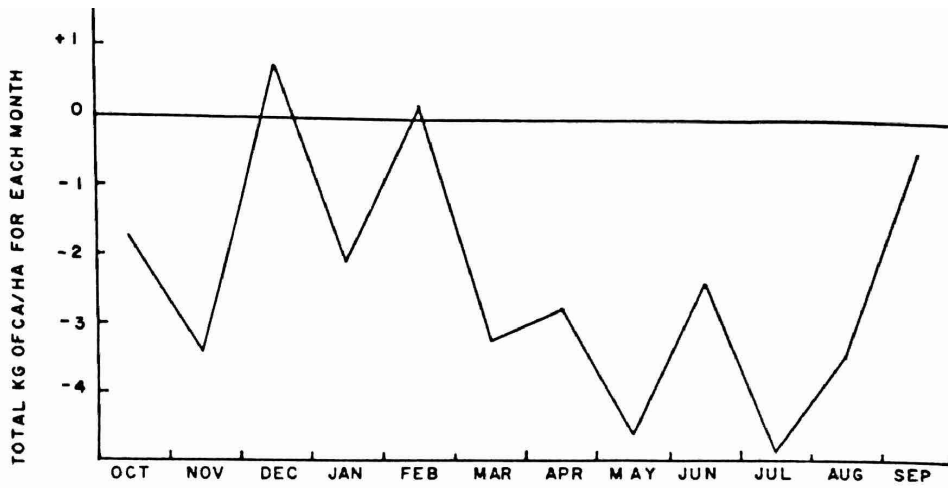


Fig. 4. Total amount of calcium input via rainfall per month minus total calcium loss via runoff

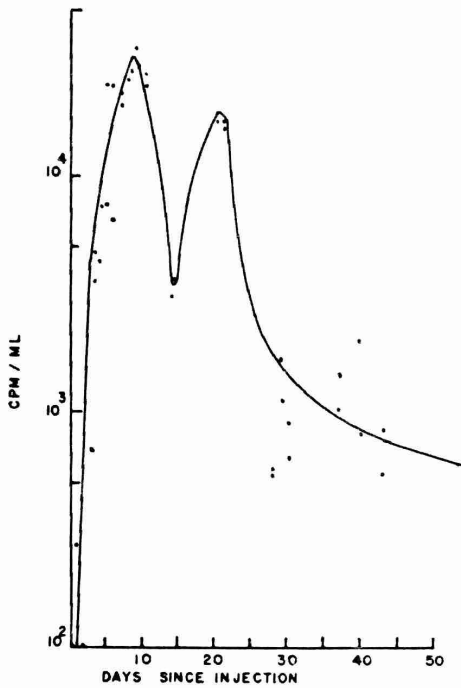


Fig. 5. Specific activity of tritium in leaves of *Dacryodes excelsa* as a function of days from date of injection of tritium into tree

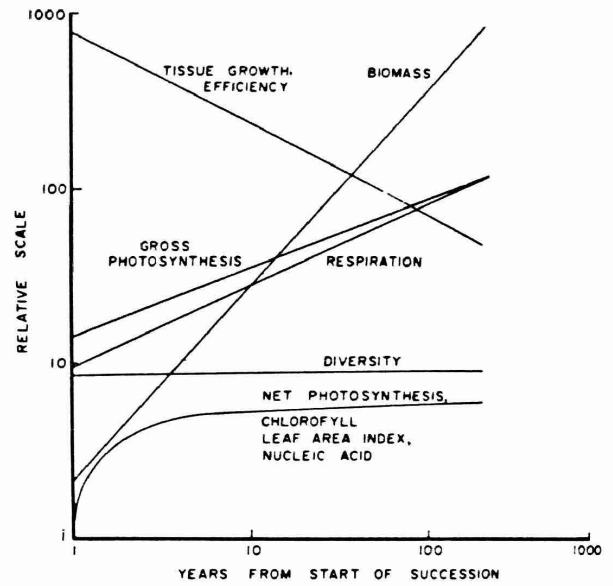


Fig. 6. General trends of ecosystem functions during secondary succession

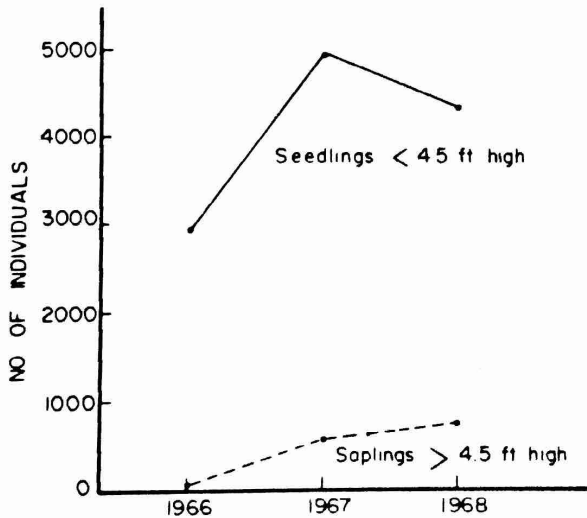


Fig. 7. Total population of tree species originating from seed after 1965 in the radiation area

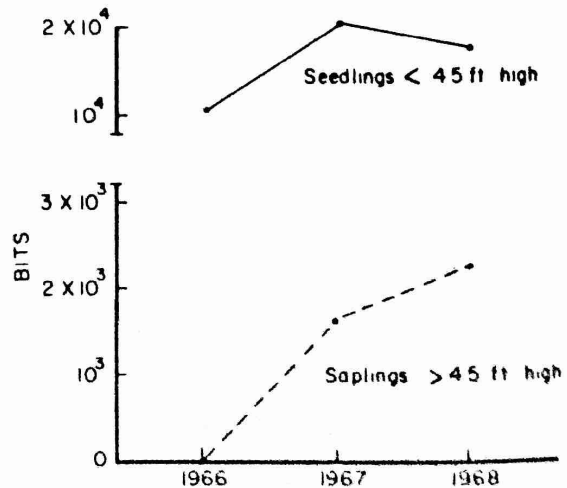


Fig. 8. Total information content in binary bits

Movement of Isotopes Through Canopy Trees

An important portion of Fig. 1 is the upward movement of isotopes through the stem of both understory and canopy trees. Kline (1967 Rain Forest Project Annual Report) showed very little uptake of isotopes applied to the litter surface by understory trees. To determine rate of isotope movement through canopy trees, and to determine if this rate is affected by transpiration rate, one canopy tree was injected with a pulse of ^{137}Cs , ^{54}Mn , and ^{85}Sr , simultaneously with a pulse of tritiated water. Preliminary results indicate that upward movement of ^{54}Mn , ^{85}Sr , and ^{137}Cs is very slow; ^{137}Cs actually shows a marked downward movement through the phloem from the injection point.

Stable Element Analysis of Ecosystem Compartments

Concentrations of stable calcium, potassium, manganese, magnesium, sodium, copper, iron, cobalt, strontium, and cesium are being determined for the leaves, stems, roots, and litter, and exchangeable elements in the soil. Concentrations, when multiplied times mass of each compartment, will give total elements in each compartment. Total quantity of elements in each compartment, when divided by loss rate for each element for each compartment, will give turnover time for the element in that compartment.

Nitrogen Fixation by Epiphyllae

Dr. Joe Edmisten and Mr. Michael Harrelson of the University of Georgia have determined unequivocally that certain algae and bacteria living on the leaves of rain forest trees have the ability to fix atmospheric nitrogen. The bacteria with this capability that were isolated are species of the genus Azotobacter; the isolated blue green algae are species of the genera Nostoc, Scytonema, Anabaena, and Calothrix.

Specific Activity Approach

Whether or not the specific activity approach is valid is one of the most important questions regarding the methodology of studies that predict radioisotope movement in ecosystems. The specific activity approach is based upon two premises: (1) that the measurement of the distribution pattern of biologically available stable elements in the organisms and their environments may be used to predict the approximate distribution patterns of introduced radioisotopes of the same elements and; (2) that if the specific activities (activity of radioisotope per gram of corresponding stable element) of the ecosystem are maintained below the allowable specific activities for those elements in the human body (or in human food), no individual can obtain more than the allowable amount from food derived from these sources. While there is little doubt that the second premise is valid for the tropical rain forest ecosystem, it is questionable whether the first premise will hold. The answer will not be forthcoming until the first approximation of the model is completed, but judging from the extremely slow rates at which isotopes are leached from leaves and litter, and are taken up by vegetation, it seems likely that most fallout isotopes will physically decompose to background levels before reaching the equilibrium of the stable isotopes in the ecosystem.

SECONDARY SUCCESSION

Jordan (1967 Rain Forest Project Annual Report) has stated that secondary succession following gamma radiation damage does not differ from succession following mechanical cutting, or following herbicide treatment, with the exception of the sprouting pattern. Sprouting following gamma radiation from a point source occurs principally from the base of stems that were shielded from radiation by rocks or soil.

This does not mean, however, that if large doses of radioactive debris are dumped on an area, this area will recover normally. The crucial difference is, after three months of irradiation by the gamma source, the radiation was stopped and there was no further disruptive energy input into the system; but if large doses of radioactive debris are dumped in an area, the disruptive energy may remain in the ecosystem for hundreds of years, depending, of course, on the half life of the isotopes involved. Therefore, the real key to understanding long term disruptive effects on the ecosystem is having a basic knowledge of how the ecosystem works.

While there is some basic knowledge of change in species composition during succession, there is very little information on change in ecosystem functions during succession, especially in the tropics. Because of the availability of facilities, and the impetus of the radiation recovery studies, a detailed study of secondary succession is now underway.

It is generally recognized that certain properties and functions of an ecosystem, such as biomass and gross photosynthesis, gradually increase during succession, and reach a maximum at the so called "climax" stage. Also generally recognized is that total respiration gradually approaches gross photosynthesis, and the two rates are equal at the climax stage. These trends have been found to hold for the tropical rain forest (Fig. 6) by comparative studies in the irradiated area 1, 2, and 3 years after radiation ceased, and in the recently undisturbed surrounding forest, which is in a much later stage of succession, equivalent to roughly 60 years.

However, the current successional studies indicate that some functions of the ecosystem approach the maximum just a few years after start of succession, others decrease during succession, and others remain steady throughout.

Net photosynthesis, leaf area index, total chlorophyll content, and possibly total nucleic acid content of the system increase very rapidly during the first few years, and reach nearly their maximum value within five to ten years after the start of succession (Fig. 6). Maximum rates, however, are probably not attained until climax.

Surprisingly, total diversity including trees and herbs in the irradiated area one year after radiation was equal to total diversity in the older undisturbed forest. This is probably due to a somewhat unique situation. In the successional area, much of the diversity is due to herbs, while in the forest, there are virtually none. In the successional area, there are also many tree seedlings, due probably to the proximity of the older forest. In most successional studies, such seed sources are not so near.

Total growth efficiency actually decreases during succession. During early stages, a larger proportion of the energy captured by the plants goes into manufacture of tissue than in later successional stages. What happens to this energy in the more mature forest has not been answered to everyone's satisfaction.

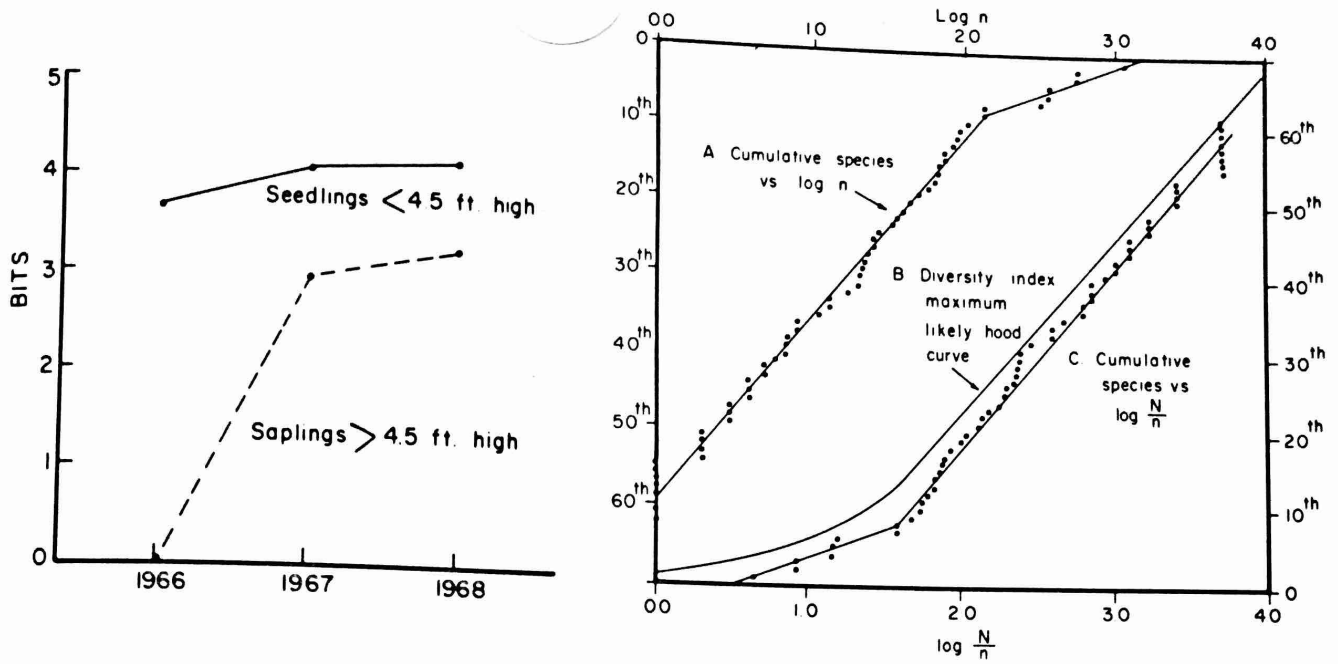


Fig. 9. Diversity (average information) in bits per individual

Fig. 10. Relationship between relative abundance curve (A) diversity index curve (B) and reciprocal concentration curve (C). The points are actual data from 1967 seedlings less than 4.5 feet high

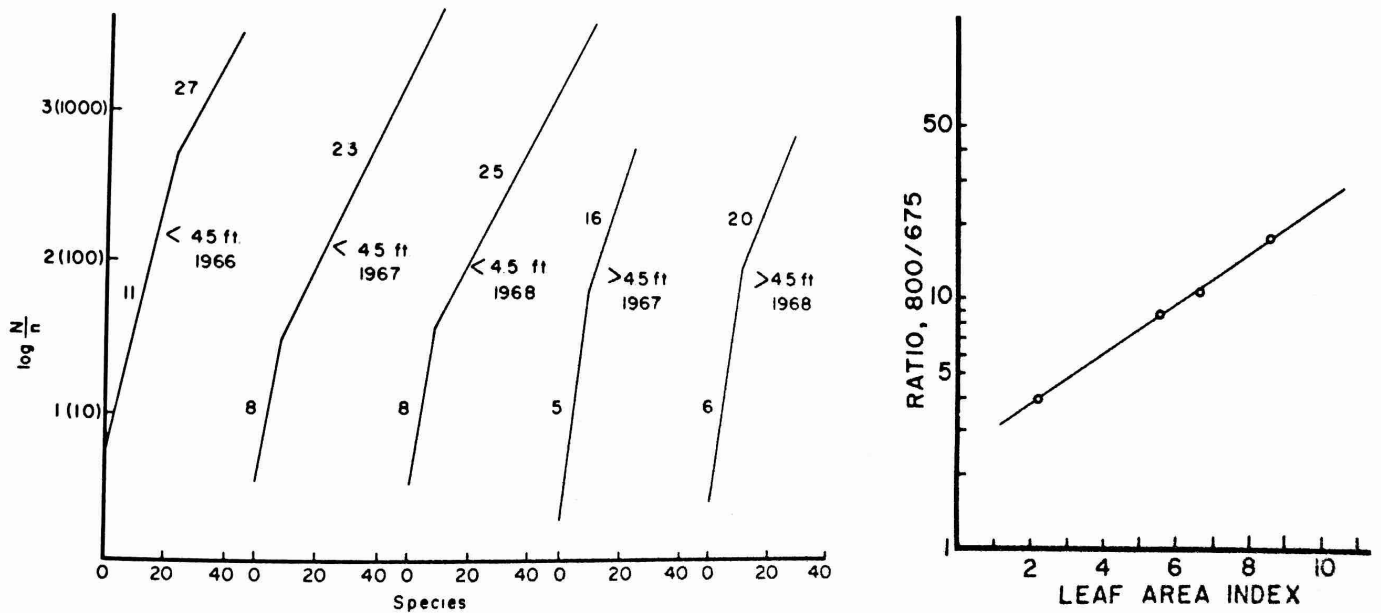


Fig. 11. Diversity structure in tree species of new vegetation after radiation. Slope is indicated in species per decade

Fig. 12. Relationship between ratio of light at 800 and 675 millimicrons on the forest floor, and leaf area index of the canopy

The successional study is also a productivity study, and thus will contribute directly to the overall mission of the International Biological Program, which is among other things, to determine the potential productivity of all the biomes of the world. Dr. Carl Jordan who is involved in the productivity portion of the successional study, is a member of the Tropical International Biological Program steering committee.

DEVELOPMENT OF TREE COMMUNITIES

Last year, detailed analyses of forest community structures were being made, both in the radiation recovery area and in the surrounding forest. A new technique for portraying community diversity has been developed, utilizing the large quantities of data collected yearly in the radiation recovery area, and drawn also from insect diversity studies which span several years. Recent techniques of diversity study, employing information theory equations, have also been applied to this data.

Results indicate three basic types of mature or steady-state forest at this elevation, related to drainage patterns in the soil, in addition to a continuum of successional states leading up to the mature condition. The changes in community structure associated with radiation damage and subsequent recovery have been complex, mostly due to overlapping effects on the quantity of light reaching the ground as radiation damaged trees slowly lost their foliage and the fast growing secondary trees began to produce shade.

The first effect of increased light reaching the ground was germination of large numbers of seedlings of sun-adapted successional trees, herbs and vines. Trees are defined as plants having woody stems, capable of standing alone and reaching heights of two meters or more. Seedlings measure less than 4.5 feet high, while saplings are young trees over this height. Figure 7 illustrates population changes in these categories for the first three years of regrowth following the irradiation in 1965 for 676 square meters that are censused annually. The number of tree species was 31 in 1966, increasing to 62 in 1967 and decreasing slightly to 59 in 1967.

Diversity is the relationship of numbers of species to numbers of individuals of a population. A simple ratio is a poor expression of diversity because the ratio of individual numbers for many species to that of the commonest species always spans many orders of magnitude and may approach infinity if a large enough sample is taken. Diversity of a sample is adequately expressed by using the equation developed to measure the symbolic information content of a message composed of different symbols. Each species is treated as a different symbol and the individuals of the sample comprise the "message." Total information content is $\log_{10} N! - \log_{10} n!$ where N is total individuals and n the number of individuals of each species taken in turn. Diversity is then the average information content of each individual. Figure 8 is the binary information content of the samples of new tree species, and figure 9 the sample diversity of each.

Because new species are added as a sample is enlarged and there are no clear theoretical limits to this in biological systems, sample diversity cannot be generalized to population diversity, which could be more or less diverse than the sample, depending on the rate at which new species are encountered as the sample is enlarged. A method of examining this rate of increase has been developed: the plotting of $\log N/n$ against cumulative species. Figure 10 illustrates this method and shows that the curve of $\log N/n$ closely duplicates the classical diversity index curve with respect to rate. It utilizes more

information, however, and gives better resolution of detail. In all vegetation studied the rate has been found to be a more or less linear function of $\log n$ with a single change of rate in passing from abundant to less abundant species. Deliberate or accidental inclusion of more than one category of plants such as trees and herbs will exhibit two break points, one for each, while sampling from more than one habitat type usually causes the break to appear much farther out on the curve. Figure 11 is the $\log N/n$ versus cumulative species curves for the radiation recovery area samples. The 1966 seedling curve suggests that two habitats, one sunny and one shady, were sampled.

The community development pattern that has emerged from these studies is an initial phase of low diversity caused by more rapid increase in individuals than in species, a second phase of increasing diversity as the number of species increases, and a prolonged third phase during which diversity continues to increase slowly without increase in number of species as the more abundant species are thinned more rapidly by competition and shading than are the less abundant ones.

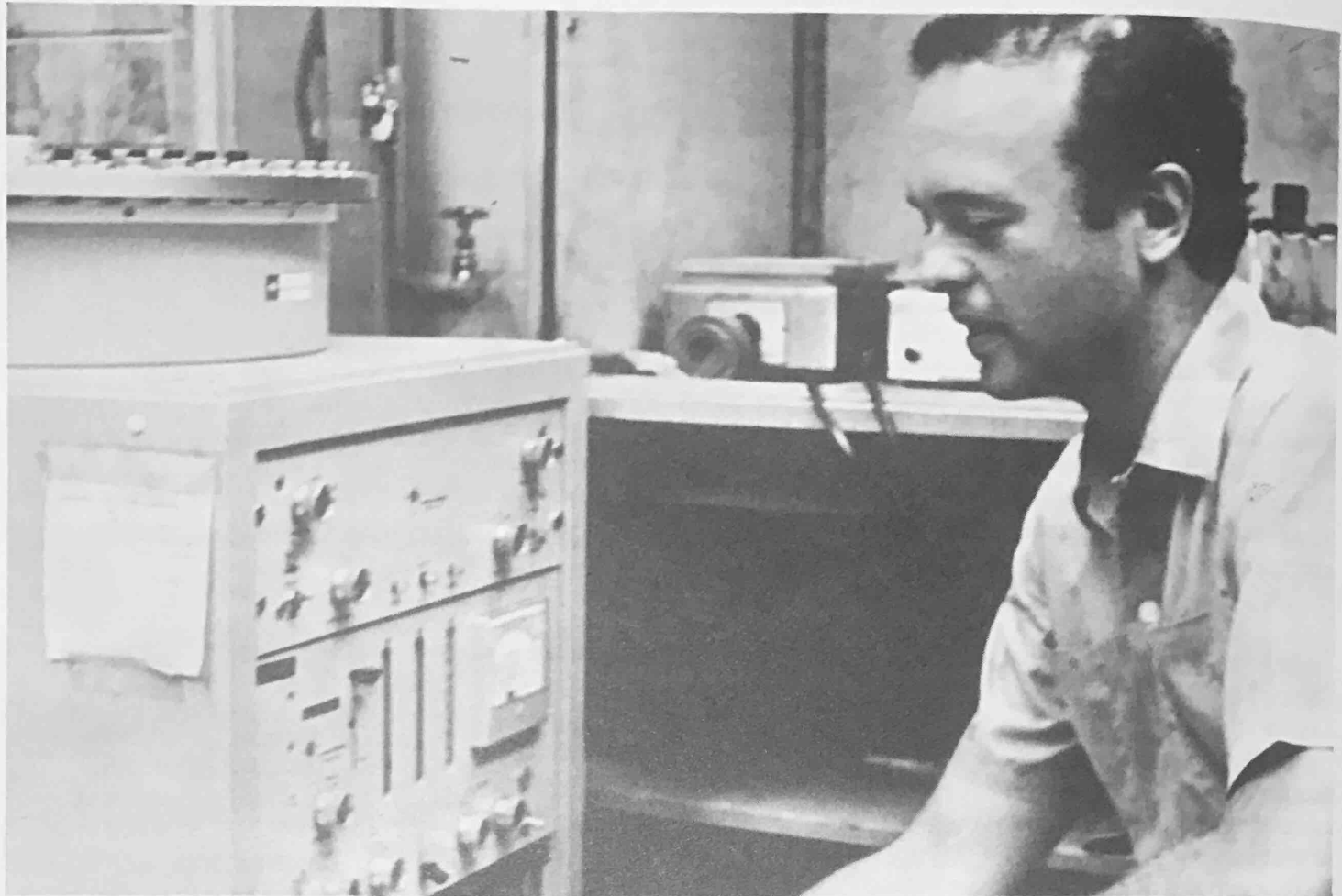
ANIMAL ECOLOGY

During the summer of 1968, Dr. Elizabeth McMahan of the University of North Carolina returned to continue her long term studies of termites in the irradiated and control areas. She found an increase in abandoned nests in the irradiated area since 1967, while there was no increase in the control area. This may be due to sterilization, but not to killing of the termites in the irradiated area during radiation. She also found new nests being established in the successional area.

The staff continued to study isotope tracers, insect diversity, and amphibian ecology. Isotope studies were enlarged to include uptake and bio-elimination of tritium in the form of tritiated water, HTO , applied as a spray to the forest floor. Tritium was absorbed by direct contact and respiration by insects, snails, frogs and lizards. No uptake was exhibited by insects, frogs or lizards captured in the area subsequent to 36 hours after treatment, while snails continued to show uptake as long as 72 hours after treatment when collected from the contaminated litter surface. No animals showed evidence of secondary uptake from trees that had taken up tritium. A method for live testing snails consisted of teasing them back into their shells, at which time they released from 1 to 4 ml. of urine. Urine samples were found to exhibit approximately the same count rates as tissue fluids obtained by dissection. Biological half life of tritium in snails was very short, less than 24 hours.

Tracer and bioelimination studies of zinc 65 in a natural population of the snail Caracolus caracolla moved into the second year, with resolution of some of the mysteries of the first year. Area of home range in this snail was found to be a function of age, increasing until the second year after sexual maturity and decreasing after that. Adult size, previously demonstrated to be independent of home range area, is likewise independent of age, shell diameter ceasing to grow at maturity. Present estimate of life span in this species is up to 18 years, with sexual maturity not developing until 8 years of age. Growth in the past two years places confidence limits of plus or minus two years on those estimates.

Insect diversity studies involved research on the methods of obtaining and expressing diversity measurements as well as the slow, continuing job of separating and identifying species of some of the poorly known groups. In some groups the known fauna of the island has been more than quadrupled in this



- (1) Dr. Jordan utilizes the gamma analyzer in tracer studies at the El Verde laboratory
- (2) Research assistant José Antonio Colón doing atomic absorption spectrophotometry in the Río Piedras laboratory



study alone. Comparisons are being made between diversity measurements obtained with various trapping methods such as sticky traps, pitfall traps, light traps and Malaise (flight) traps. Attractant traps avoid the distorting effects of irregular natural concentrations or foci of distribution by imposing their own focus on all species, and by comparison show the natural concentrations to produce a curvature of the usual linear relationship between number of species and log number of individuals.

Interest in amphibian ecology this year has been focused on the possible function of the vocal call as a population spacing device. Marked antagonistic behavior by males has been observed in response to artificially reproduced calls. Experiments are now in progress to determine if the speaker of a tape recorder playing a loop of normal call at the natural repetition rate will maintain an area free of infringement by males.

WEATHER STATION

A fully automated weather station is now operating. Temperature is being measured at four levels in the forest. Also being measured is solar radiation, rainfall, wind direction, and wind speed. The information from the sensors is carried to the instrument shack by cable. Once an hour, a scanner scans the output from all the sensors and transmits this information to a data logger tape. A computer program is now being written to get periodic averages directly from the tape, as well as other information such as what proportion of the time a temperature inversion exists in the forest, and what time during the day the wind usually comes up.

LEAF AREA INDEX

After several years of effort, a good correlation has been found between leaf area index and the ratio between infra-red light on the forest floor (Fig. 7). Leaf area index is important because it is a measure of biomass of leaves, a measure which is prerequisite to modeling biogeochemical cycles since leaves are an important ecosystem compartment (Fig. 1). The theory behind the correlation is, the forest canopy is relatively transparent to infra-red radiation while it absorbs red radiation proportionately to the amount of chlorophyll, and thus biomass, in the canopy. Thus with a meter that reads light at wave bands centered at 675 and 800 millimicrons, a man on the forest floor can measure leaf area index without having to read above the canopy as in the optical density method of measuring leaf area index. A paper entitled "Derivation of leaf area index from quality of light on forest floor" is currently being prepared for publication.

EL VERDE FIELD STATION

Maintenance and upkeep of the El Verde field station and associated roads and trails continue to constitute an important effort of the field crew.

Table 1

Results of analysis of variance to determine significance of differences in fallout. Blanks indicate a difference at greater than 10% error level (no significant difference).

Test for Difference in:	Condition	Significant differences at indicated error level		
		<u>¹³⁷Cs</u>	<u>¹⁴⁴Ce</u>	<u>⁹⁵Zr</u>
Species	Understory, clean	-	-	-
Species	Understory plus epiphylls	-	-	-
Species	Canopy, clean	10%	-	-
Species	Canopy plus epiphylls	-	-	-
Site	Understory, clean	-	-	5%
Site	Understory plus epiphylls	-	5%	-
Site	Canopy, clean	-	-	-
Site	Canopy plus epiphylls	-	-	-
Clean-epiphyll	Canopy	1%	-	-
Clean-epiphyll	Understory	1%	.05%	.05%
Canopy-understory	Clean	-	10%	-
Canopy-understory	Plus epiphylls	-	-	-

Table 2

Rate of water input into ecosystem via rainfall

<u>Month</u>	<u>Average rate of water input, m³/ha/week</u>
October, 1967	385
November, 1967	665
December, 1967	394
January, 1968	512
February, 1968	201
March, 1968	525
April, 1968	310
May, 1968	1143
June, 1968	905
July, 1968	954
August, 1968	883
September, 1968	425

Table 3

Water budget for the rain forest at El Verde.
Figures are based on yearly totals.

<u>Flux</u>	<u>Percent of total rainfall</u>
Throughfall	69.5
Stem flow	17.9
Evaporation from leaves	12.4
Transpiration	20.9
Run off and deep drainage	66.5



Dr. Pérez Rios and a student from the University of Cordoba, Argentina, prepare a sample to be radiated in the 5000 curie gamma radiation source at the "Atoms in Action" laboratory

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 in Latin America. The Oak Ridge Associated Universities is responsible for the Exhibit Training Programs and the USAEC operates a Technical Library as part of the Exhibit. 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 demonstrations, 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 April and May, the exhibit visited Caracas, Venezuela; in October it visited Córdoba, Argentina.

RESEARCH ACTIVITIES IN CARACAS, VENEZUELA

A preliminary visit to Caracas indicated that the basic interest of the Venezuelan Institute for Scientific Investigation (IVIC) was the use of radiation in agricultural applications. A food preservation by irradiation laboratory was to be established and, in coordination with IVIC, projects were designed to be initiated during the Exhibit.

The Science Faculty of the National University of Caracas made use for the first time of a gamma radiation source for its research work.

In general the gamma energy source was used in genetic, food preservation, microbiology, entomology, and chemistry projects.

The Exhibit received special cooperation from the Central University of Venezuela, IVIC and from the Center of Agronomical Investigations at the Ministry of Agriculture.

Seven scientists and two technicians from the Puerto Rico Nuclear Center collaborated in this program.

LECTURES IN CARACAS

In conjunction with the research program, PRNC personnel gave the following lectures during the Atoms in Action Exhibit which were attended by 755 persons:

- Dr. Owen H. Wheeler - Associate Director, PRNC, Mayaguez Campus.
(a) Radiolysis of Amino Acids and Peptides - Faculty of Pharmacy, University of Los Andes, Mérida, Venezuela; (b) Excited Atoms Reactives - Institute of Science, University of Los Andes, Mérida, Venezuela; (c) Peaceful Uses of Nuclear Explosives - Use of Radioisotopes and Radiation in Agriculture -



- (1) President Raul Leoni of Venezuela cuts the inaugural ribbon at the AEC "Atoms in Action" Exhibit held in Caracas in 1968. Looking on is USAEC Commissioner Gerald F. Tape
- (2) Dr. Aldo Lanaro of PRNC, together with Dr. Efrain Otero, works in the "Atoms in Action" Exhibit laboratory in Cordoba, Argentina, held in October 1968. They are preparing a radioactive solution to be used by doctors participating in the Medical Applications Course



Nuclear Plants in the United States - Congress of Chemical Engineers.

Dr. David W. Walker - Associate Scientist, PRNC. (a) Nuclear Energy Research and Specific Developments in Entomological Research - University of Los Andes, Mérida, Venezuela; (b) Agricultural Applications of Nuclear Energy - Faculty of Agronomy, UCV; (c) Project for Eradicating the Sugarcane Borer - Central University of Venezuela.

Dr. Alec Grimison - Associate Scientist, PRNC. (a) Radiation Effects on Solids - IVIC; (b) Use of Radiation and Radiation Sources in Research - Science Faculty, Central University of Venezuela.

Dr. Robert A. Luse - Associate Scientist, PRNC. (a) Application of Nuclear Energy in Agriculture - IVIC.

Dr. Ramiro Martínez Silva - Associate Scientist, PRNC. (a) Radiation Effects in Trypanosoma Cruzi - Tropical Medicine Institute, Central University of Venezuela.

Mr. Hector Barceló - Chief Scientist, PRNC. (a) Non-Destructive Testing and Comparative Studies in Nuclear and Fossil Fuels - Metallurgic Department and Chemistry Department, UCV.

Mr. Juan Silva - Associate Scientist, PRNC. (a) Nuclear Reactor Instrumentation - Exhibit; (b) Uses of Radiation Sources - Exhibit; (c) Radioisotopes in Research - Exhibit.

PRNC personnel gave 17 conferences as well as various seminars and round table discussions at the University of Los Andes, Mérida, Venezuela.

RESEARCH ACTIVITIES IN CARACAS

Food Preservation

IVIC carried out the following projects in food preservation, initiated by Dr. Luis M. Revetti from the Chemistry Department at IVIC.

Studies in Potato Preservation: (*Solanum tuberosum*). Samples from a recent crop were irradiated in doses varying from 5000 to 15000 rads (steps of 1000 rads). Measurements in ascorbic acid were performed in the laboratory. Successive analysis of free sugar starch and ascorbic acid will be continued by IVIC to make comparative studies in relation to non-irradiated samples.

Studies in Yuca Preservation (*Allium cepa*). Samples from a two week crop were irradiated in doses ranging from 3 to 12 rads. Laboratory determinations will be made later on by IVIC.

Studies in Yuca Preservation (*Manihot aipi*). Twenty four hour crop samples were irradiated. Since it is the first time work on this product is being done, doses ranging from 10 to 100 krads were used. In this first phase it was observed that better preservation was obtained by irradiating the products with doses of 80 krads.

The experience was repeated with doses ranging from 60 krads and 100 krads. Satisfactory results were obtained and will be published as soon as the experiments end. Qualitative tests were made to determine the presence of iron, copper, tin and ascorbic acid.

Studies in Paprika (*Capsicum frutescens*). Two varieties were studied: The Jalapeña and the Serrana. Doses ranging from 280 to 390 krads were applied. Laboratory studies will be made subsequently by IVIC.

Genetics

Studies in black beans (*Caraota*). Genetics studies of radiation effects in this typical Venezuelan product were initiated by the Center of Agronomical Investigation in Maracay. Dr. Pedro Obregón was in charge.

Studies in Corn (*Zea mays*). Genetics studies of 3 varieties were initiated by the Center of Agronomical Research with Dr. Obregón in charge.

Studies in Topido (*Solanum Topido*). This program was initiated in collaboration with UCV Experimental Biology School; Dr. Luis Torres de Martí was in charge.

Microbiology

Irradiation of fungus cultivation. Dr. Eddy Varsausky from the Department of Microbiology of the School of Biology began studies in the cultivation of 3 varieties of penicillium and 3 aspergillus in order to observe the differences in response to the same doses with different doses per unit of time.

Previous studies had been made applying a dose of 5 krads/hr. No satisfactory results were obtained, so the Atoms in Action gamma facility was used to irradiate them with doses of 170 krads/hr.

Entomology

Studies in the Ceratitis Capitata (Mediterranean Fruit Fly). The Center of Agronomical Research initiated studies irradiating samples of the fly pupae. Studies for the sterilization of this insect were later made with the assistance of a specialist from PRNC.

Studies in Anastrepha serpentina. Studies with this insect were initiated by the Center of Agronomical Research with the purpose of expanding them to pilot scale by liberating sterilized insects on the Island of Margarita, 200 kms. off Venezuela's coast. The Center for Agronomical Research for this project will visit PRNC installations.

Radiochemistry

Preparation of Molybdenum - 99. The Department of Nuclear Chemistry at IVIC initiated a project in the preparation of Molybdenum-99 in collaboration with Dr. Owen H. Wheeler. The IVIC reactor was used, as well as our gamma facility, since part of the samples were first subject to the effects of the gamma radiation and then introduced in the reactor. This project is still underway.

Chemistry

Radiation Effects in Petroleum Derivatives. Dr. Sergio Flores is in charge of this project, which consists of studying the effects of gamma radiation in crude petroleum fractions. The effect of oxygen was also studied. Analysis of the irradiated samples is being made by the Department of Natural Products of UCV's Faculty of Sciences.

RESEARCH ACTIVITIES IN ARGENTINA

The same procedure used to establish the PRNC program in Venezuela was followed in Argentina.

The program was given in the National University of Córdoba, and at the Catholic University.

The Chemistry Institute of the National University initiated works in Biology and Biochemistry using radioactive isotopes, and works in Physics and Chemistry in the Faculty of Engineering of the Catholic University.

Five scientists from the PRNC participated in the Exhibit.

Conferences in Córdoba

In conjunction with the research program the PRNC personnel offered 22 lectures, with 621 attending:

Mr. Juan Silva Parra - Director, Research Program Atoms in Action Exhibits. Power Reactor and Neutron Delay and Its Influence in Reactor Control, at the Faculty of Engineering, Catholic University of Córdoba; Reactor Instrumentation, at the Exhibit.

Dr. José A. Castrillón - Associate Scientist I, Radioisotope Applications Division. Liquid Scintillation Counting, Use of Radioisotopes in Organic Chemistry I and II and Counting Techniques, at the Chemistry Institute of the National University of Córdoba.

Dr. Aldo E. Lanaro - Associate Scientist II. Clinical Radioisotopes Applications Division. Current Research in Nuclear Medicine, Introduction to Nuclear Medicine, Uses of Radioisotopes for Studying Kidney Function, Organs and Tumors, at the Faculty of Medicine, Catholic University of Córdoba; Liver Scanning and Spleen, in the Exhibit Conference Room.

Dr. Julio Gonzalo - Associate Scientist II. Nuclear Science and Technology Division. Ferroelectricity, Neutron Diffraction, at the Faculty of Engineering, Catholic University; Ferroelectricity and Neutron Diffraction, at the Institute of Mathematics, Astronomy and Physics, National University of Córdoba.

Dr. Jorge Chiriboga - Assistant Director for Scientific Programs. Studies of the Relationship of a Parasite to its Host Using Radioisotopes, Use of Radioisotopes in Metabolism Studies, at the Chemistry Institute, National University of Córdoba.

RESEARCH ACTIVITIES

The following research projects were carried out during the Atoms in Action Exhibit in Córdoba:

Chemistry

Preparation of tritium-labeled Esphingosine and Acyl Esphingosine. Esphingosine and Acyl-Esphingosine stimulate the incorporation of UDP glucose and cerebroide. It is not certain whether these compounds act as catalysts or precursors in this stimulation. The tritium label will enable the investigator, Dr. Curtino of the Department of Biological Chemistry of the Institute of

Chemical Sciences, to answer this question. This investigation was started with the help of Dr. José P. A. Castrillón of the PRNC Physical Sciences Division and is now continuing.

Bio-chemistry

Study of the action mechanism of Phytohemaglutinine (PHA) using radio-isotopes. This study is designed to provide information on the action mechanism of PHA in a variety of Phaseolus vulgaris cultivated in Argentina. The investigation carried out by Prof. Beatriz Pacheco de Rupil will fulfill part of the requirements for a doctoral degree. Iron-⁵⁹ and ¹⁴C- labeled thymidine and uridine are used. Work now continues at the Institute of Chemical Sciences.

Study of the halogenation mechanism of phenyl-acetaldehyde with n-halomides. The injection of linoleic acid into animals deficient in topopherol increases ³²P incorporation into the messenger RNA of the liver. This increase may be the result of a change in phosphate pools, a change in phosphate permeability, or it may reflect a stimulation of RNA synthesis. It is possible to study this problem by using ³²P and tritium labeled orotic acid or iodine.

Dr. Señeriz of the Department of Biological Chemistry of the Institute of Chemical Sciences is carrying out this study. Dr. Jorge Chiriboga of the PRNC Directors' Office assisted Dr. Señeriz during the Exhibit.

Pharmacology

Study of learning mechanism using radioisotopes. This project in the field of Experimental Psychology was started by Dr. Osinger of the Department of Experimental Psychology of the Institute of Chemical Sciences to provide experimental information for a doctoral dissertation for one of his graduate students. Briefly, the project consists of studying learning mechanisms in laboratory animals and making observations by using labeled compounds.

Physics

Radiation effects of ferroelectric materials. The Department of Electrical Engineering of the Catholic University in Córdoba started a research project utilizing triglycine sulphate (TGS) as the ferroelectric material. Variations in critical temperature were studied by irradiating TGS crystals with different doses above and below the critical temperature. The experimental part of this project was completed during the Exhibit by two students who will use the data to prepare a thesis. Dr. Julio Gonzalo of the PRNC, Nuclear Sciences Division assisted with this project.

During the Exhibit a Technical Program was also carried out and is described below.

Dosimetric Study of the gamma facility. Two students from the Engineering College of the Catholic University carried out a detailed dosimetric study of the gamma facility, plotting isodose curves for different regions around the source and calculating actual activity, using thermoluminescent techniques. The experimental data was accumulated and the report will be submitted by the students as part of their degree requirements.

Use of isotopes in hydraulics. Two students from the Catholic University carried out a series of experiments with hydraulic systems utilizing radio-isotopes. Residence time measurements flow charts, and other data accumulated by the students will be used to prepare a thesis by the students.

Neutron-physics experiments. Six students from the Institute of Mathematics, Astronomy and Physics and two from the Engineering College of the Catholic University conducted four experiments with the six curies neutron source of the Exhibit. Experiments included a study of resonance integrals, neutron diffusion, neutron flux distribution and Fermi age.

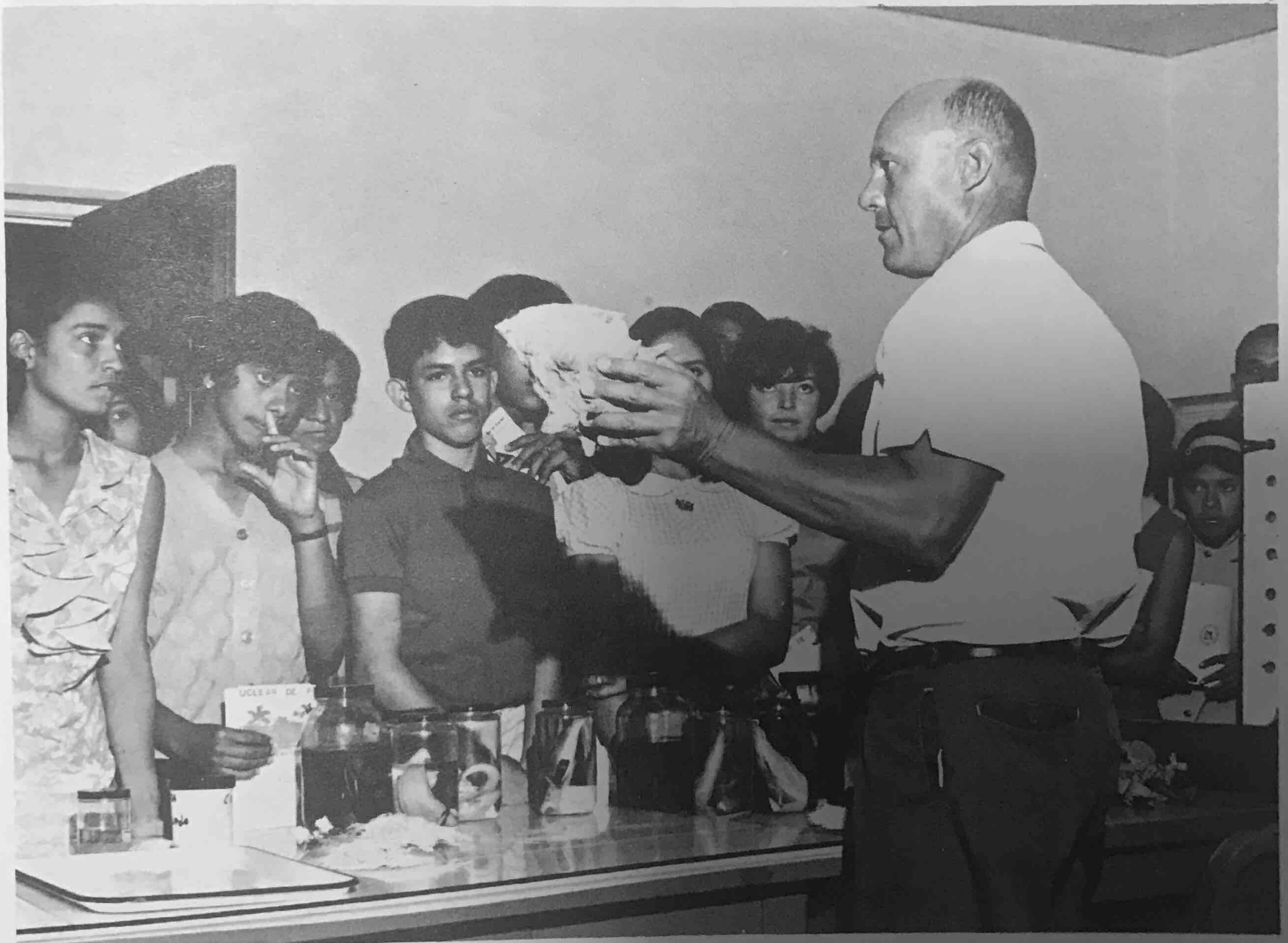
MANUSCRIPTS COMPLETED BY PARTICIPANTS
IN EXHIBIT RESEARCH PROGRAM

"Prolongación de la Vida Util de la Naranja Mediante Radiaciones Gamma",
by Mr. Bolivar Izurieta and Mr. Marcelo Coronel

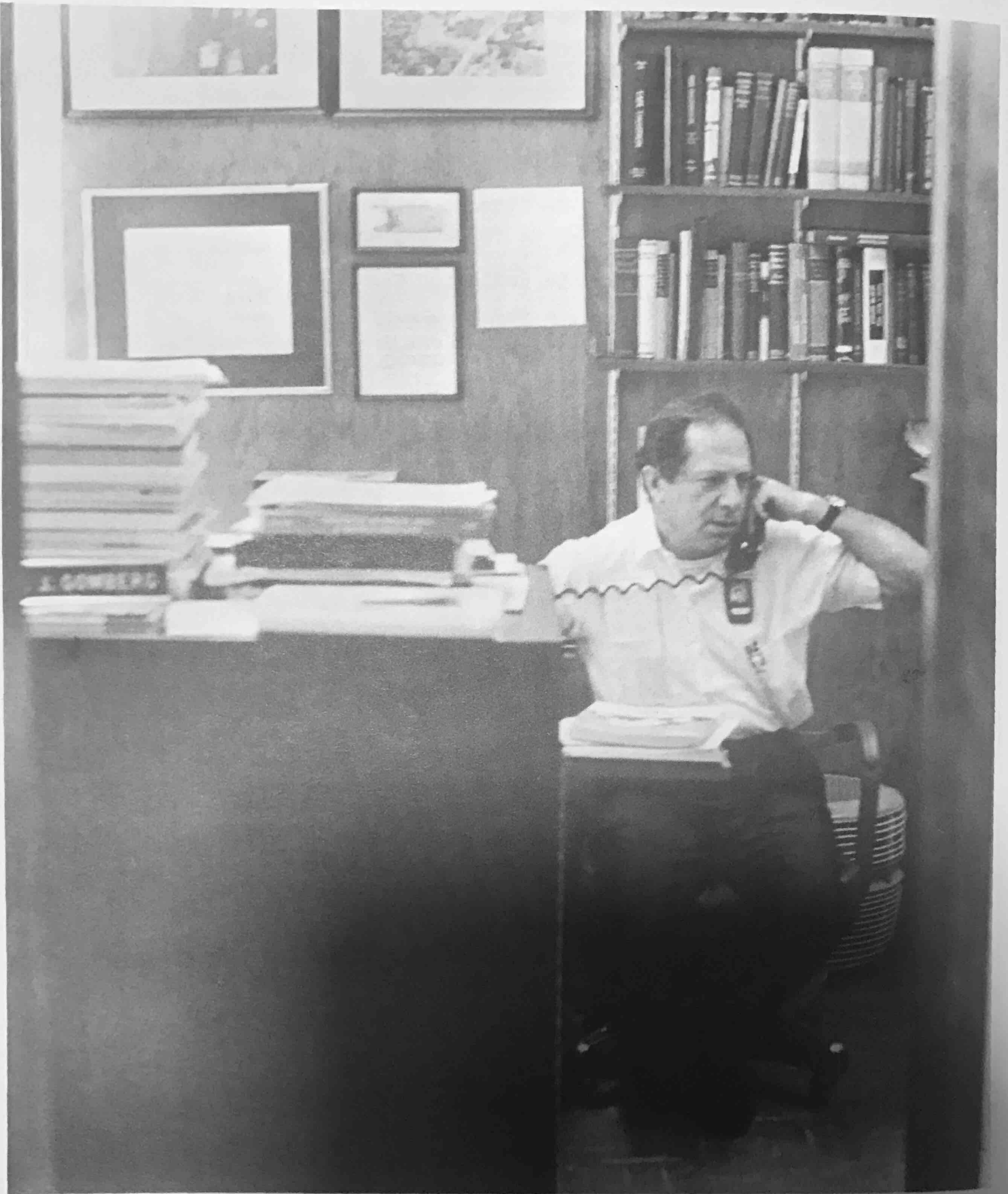
"Cinética de Intercambio Isotópico de Cloruro de Iónico con Cloruro Cicloalquilo",
by López, M. E., Wheeler, O. H. and Solé, P.

"Depolimerización de Pectina Mediante Radiación Gamma",
by Romo S., Luis A.

"Efecto de la Radiación Gamma Sobre la Estabilidad del Acido Ascórbico",
by Lomo J., Luis A.



Mr. Silva Parra shows a student from Catholic University of Cordoba the equipment he will be using in thermoluminescence experiments during the "Atoms in Action" exhibit



PRNC Director Henry J. Gomberg in his Río Piedras office

OFFICE OF THE DIRECTOR

The Director's Office, in addition to its tasks of daily administration, is concerned with planning and coordination of programs: it is responsible for support to ongoing operations and for initiating, or assisting in the creation of, new training and research.

The office also serves as the center for internal and external communication, including public information, management of meetings and conferences, and serving the needs of students and trainees from abroad. Individual members of the staff also participate in teaching and research activities of several PRNC divisions.

Significant progress was made during 1968 in serving the objectives of the Director's Office within PRNC. Particularly important was the establishment of new scholarship assistance programs for students and trainees from Latin America. The University of Puerto Rico has granted \$10,000 for PRNC-administered scholarships and the OAS--under the Centers of Excellence program--has granted 10 fellowships for study at PRNC in the last three months of FY 1969. Aid to Latin American students is essential if PRNC is to continue to play a meaningful role as a training center for Latin American scientists. Travel costs from most Latin American republics to Puerto Rico are quite high. The cost of living in Puerto Rico is higher than any other part of Latin America. This often makes it a hardship for a Latin American student to study here unless some supplementary funds are available to cushion these costs.

Latin American interest in PRNC is growing only because of the quality of the center's training and research programs. Maintenance and improvement of quality requires adequate facilities in which to carry out programs, and here, too, significant progress is being made. In Rio Piedras construction began on a new wing of the Bio-Medical Building, to be completed by early 1970. At a cost of about \$1 million, the new building provides 24,700 square feet of additional laboratory and office space. Facilities in temporary structures will be rehoused, adequate service areas will reduce the crowds of waiting patients in our hallways, and files and other gear now kept in passageways will return to office and laboratory areas. The present building will be renovated as functions in particular areas change, and space has been set aside for a staff reading room. There is also a small meeting and staff service area.

Our construction activity is part of a very extensive program in progress at the Medical Center, which will bring the UPR Medical School to Rio Piedras. The Director's Office, with assistance from a PRNC-UPR Medical School committee led by Associate Dean Dr. Conrado Asenjo, is studying the changing nature and needs of the interaction between PRNC, the Medical School and the community. Out of these considerations are emerging actions to increase Medical School and Medical Center responsibility for training and service, using the established techniques in radiation therapy and nuclear medicine; this frees PRNC to do more work of an experimental and research nature. Plans include greater interaction with the basic medical science activities, particularly as these groups move to the Medical Center, and a substantial increase in radiation biophysics activity.



The composite panorama photo on these pages and the next shows a rearview of the PRNC installation in Puerto Rico Medical Center, Río Piedras, with the new wing of the building dominating the central part of the photo. The low one-

The UPR Río Piedras campus is also expanding. PRNC has been invited to participate in the planning of UPR's new \$12 million sciences building to insure that adequate provision is made for the nuclear sciences.

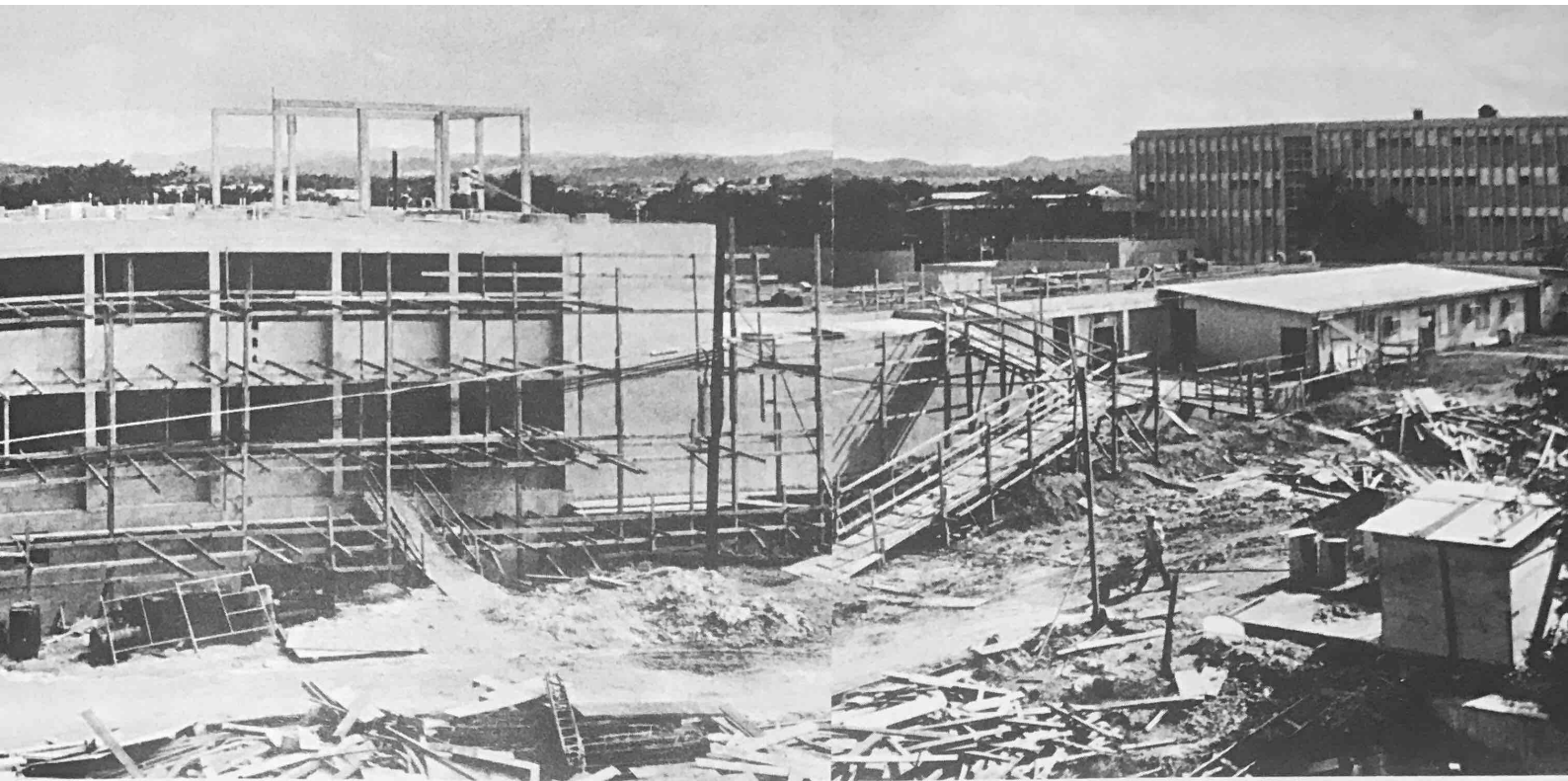
In PRNC's education and training programs, as well as in its research activities, continued emphasis is being given to explore new areas, and achieve new levels of sophistication.

In Nuclear Engineering we are striving to increase our work in reactor technology, radiation phenomena, direction conversion and participation in studies such as desalination. Our new Triga pulsed reactor opens new areas of research and study, as does the new Texas Nuclear Accelerator installation. This new equipment will also stimulate student research in radiation chemistry and physics.

In Agricultural Bio-Science, where good use has developed for our gamma source, we now foresee efforts to develop work related to the reactor, in the direction of radiation biology and applications thereof.

In Radioecology, PRNC's newest division, our training efforts are now being programmed. Interest in this area, and the level of training requests, are higher than in any other field and we can expect considerable activity in the next few years.

Our reactor operation and technical service areas have also become the source of training activity requests, particularly from Latin America. These include reactor operation maintenance and safety, electronics servicing and glass blowing.



story building at extreme left is the animal house. A considerable part of the existing PRNC building is concealed by the new structure, but part of it is visible far to the right of center

In research, the following developments are representative of the new directions in which PRNC is heading. In view of the proposed use of nuclear explosives for peaceful purposes, as well as the development of reactors for power and desalination in tropical and sub-tropical areas, PRNC's Radioecology Division has begun a new program to determine the feasibility of a field investigation on the effects of short-term high-intensity gamma radiation, elevated temperatures, or increased salinity upon a tropical coral reef or mangrove grove. Radiation effects upon such areas have not yet been measured. By late 1970 or early 1971, our new 93-foot research vessel should be in operation. This will increase the efficiency of radioecological field work and provide sea-going capability for surveys and special research problems.

Our schistosomiasis program has also branched out into a study of fascioliasis because of its great importance in the dairy, beef, mutton and wool industries in Latin America.

In PRNC's program to adapt crops to tropical environmental stresses by mutation breeding, preliminary testing for regional adaptability of chickpeas and a group of soybean varieties and selections from high protein crosses has been made. In the 1968-69 winter season the first generation of the gamma-ray treated soybeans was being grown in isolated plots under supplemental lighting. This group of material is expected to provide the first massive source for selection for desirable mutant types beginning in summer 1969 and extending to later years.

Our presence in the area of radiation preservation of foods, is imperative since a number of Latin American countries are anxious and prepared to begin or intensify their investigations.

TRAVEL

Officials from the Director's Office participated in several meetings, conferences and trips within Puerto Rico and in the United States and Latin America.

Perhaps the most significant were two visits to the lowlands of Colombia to explore possible areas of agriculture-oriented research, undertaken in April and July. Dr. Gomberg traveled together with Mr. Héctor Barceló, Assistant Director for Operations, and Dr. Robert A. Luse, head of the Agricultural Bio-Sciences Division. The purpose was to conduct an on-site survey of the very humid, low-land area lying directly east of the Andes, called the Llanos Orientales. The area, almost uninhabited, has good climate conditions and river transport facilities, which make it attractive as a potential agriculture-dairy production center of vast proportions. However poor soil conditions have discouraged any large-scale ventures and it appears that only some technological breakthrough in soil fertilization can permit the area to realize its potential. Colombian government agencies have displayed interest in the area, through its Institute of Nuclear Affairs, and Institute of Agriculture and Animal Husbandry. We have discussed with USAID officials in Bogotá, the Colombian capital, the possibility of PRNC's participation in research programs aimed at seeking ways to make Los Llanos a productive agricultural area. The subject was also discussed with officials of the Rockefeller Foundation. During the two survey trips, the PRNC officials, accompanied by members of the Colombian government, traveled by plane, boat, jeep and truck through the extensive--and quite primitive--area.

Of particular interest was a visit to Hacienda El Piñal, far into the interior, where Colombian agronomists are experimenting with corn, soya, peanuts and sorghum.

In October, Dr. Gomberg made a multi-nation program planning tour of Latin America. He met with atomic energy commission and university officials in Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay.

INTERNATIONAL COOPERATIVE RESEARCH

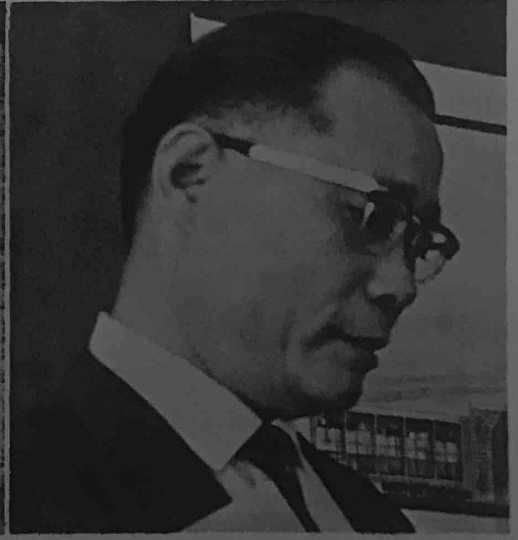
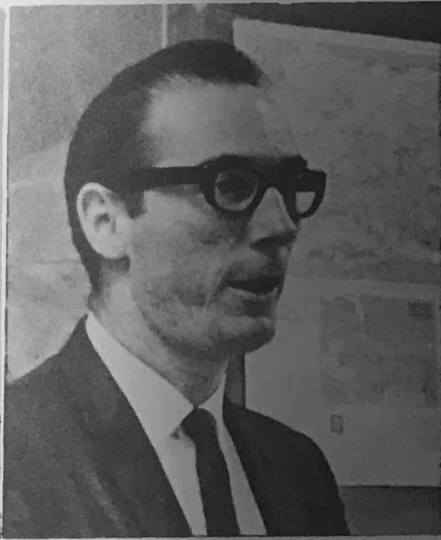
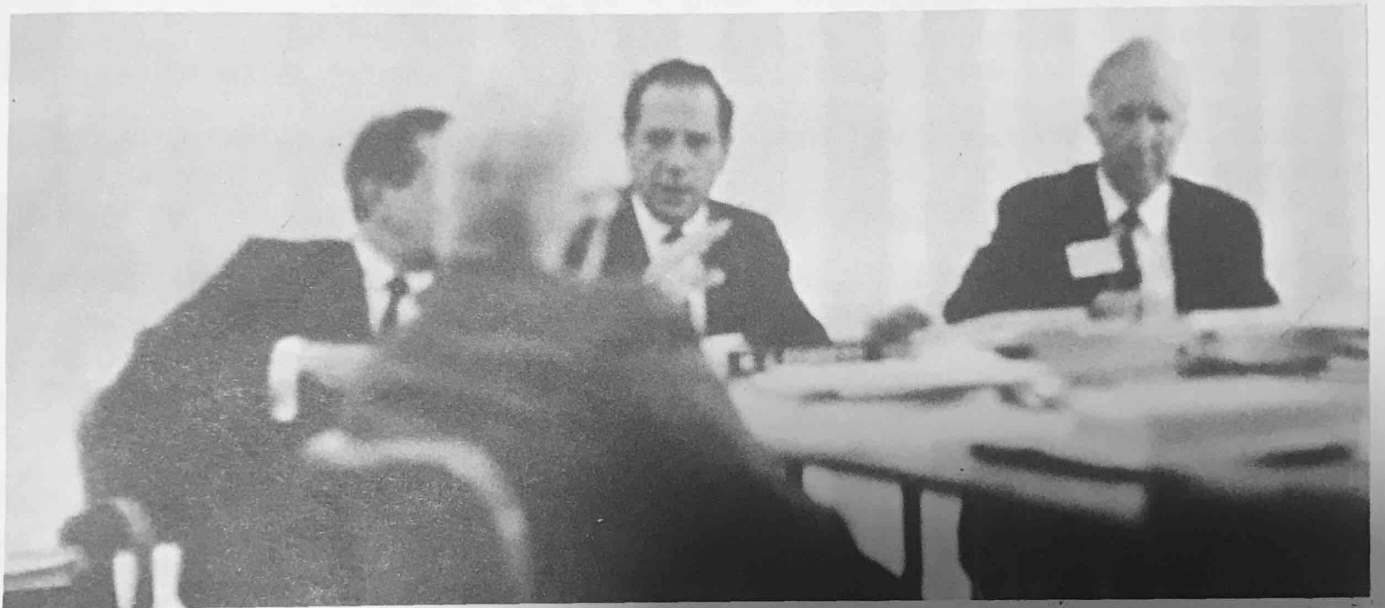
PRNC participated in two formal international cooperation projects during 1968.

Through its Agricultural Bio-Sciences Division, PRNC will exchange with IAEA information on research dealing with the effect of neutron irradiation on seeds. Anticipated future research on genetic and physiological effects of fast neutrons will be coordinated with the international IAEA program in genetics and mutation breeding.

In a program receiving financial assistance from US AID, ICAITI(The Central American Institute of Research and Industrial Technology) in Guatemala City will send staff to PRNC for training in food irradiation preservation. PRNC will make follow-up visits to Guatemala to assist in setting up food irradiation research programs. Dr. Koo of PRNC visited Guatemala to coordinate final planning for this program.

MEETINGS

Early in May, PRNC participated in a meeting in San Juan sponsored by the US AEC Division of Technical Information to discuss the future direction and program of Atoms in Action Exhibits in Latin America. Participants were from the U.S., Puerto Rico, Guatemala, Argentina, Brazil, Ecuador and Colombia.



Various scenes from the PRNC Advisory Committee held in Mayaguez during 1968. In top photo at far right is Dr. Frederick Seitz; in foreground, back to camera, Dr. James G. Horsfall. In next photo, Dr. Gomberg(center, rear) explains a point to the Committee. Far left, partially visible is UPR President Jaime Benítez. Far right is Dr. Paul B. Pearson, Committee Chairman. Foreground, back to camera is Dr. Juan A. del Regato. In photos below, various PRNC staff members make their reports to the committee. From left to right: Dr. Lee, Dr. Sasscer, Dr. Koo

In August, PRNC scientists took part in a Special Seminar on Food Production and Economic Development organized by UPR in Mayaguez, and attended by food science and nutrition specialists from Latin America, Haiti, Trinidad, Jamaica, England and U.S.

In February and September, the PRNC Advisory Committee met for its two-day semi-annual review of the Center's activities.

VISITORS

Numerous visitors from different parts of the world came to inspect PRNC's installations in Río Piedras and Mayaguez during the year.

PRNC's increasing role as an international center was characterized during the year by several visits from European scientists. Dr. Milan Osredkar of Yugoslavia, a consultant for IAEA in Jamaica, consulted in our Mayaguez installation in February on the use of nuclear techniques in agriculture and the development of nuclear power in Puerto Rico. In March, Dr. J. Joseph, director of the IAEA Laboratory of Marine Radioactivity, Oceanographic Museum, Monaco-Ville in Monaco, observed our Marine Biology and Terrestrial Ecology programs. In April, Dr. Walter Seelentag, Chief Medical Officer, Radiation Health, World Health Organization, Geneva, and Dr. H. Eisenlohr, Dosimetry Section, Division of Life Sciences, IAEA, Vienna, discussed a possible training course in medical radiation physics for South American participants. And in June, Dr. H. Altmann of IAEA Vienna visited our Mayaguez laboratory.

We were particularly gratified to learn of a letter written by William C. Johnson, M.D. of St. Mary's Long Beach Hospital, Long Beach, California, who spent a month here during the summer of 1968. His visit resulted in the following article being included in the September 5, 1968 Congressional Record:

HON. CRAIG HOSMER
of California
in the House of Representatives
Thursday, September 5, 1968

MR. HOSMER. Mr. Speaker, I was pleased to receive from an expert qualified to render expert judgment in matters pertaining to nuclear medicine the following communication:

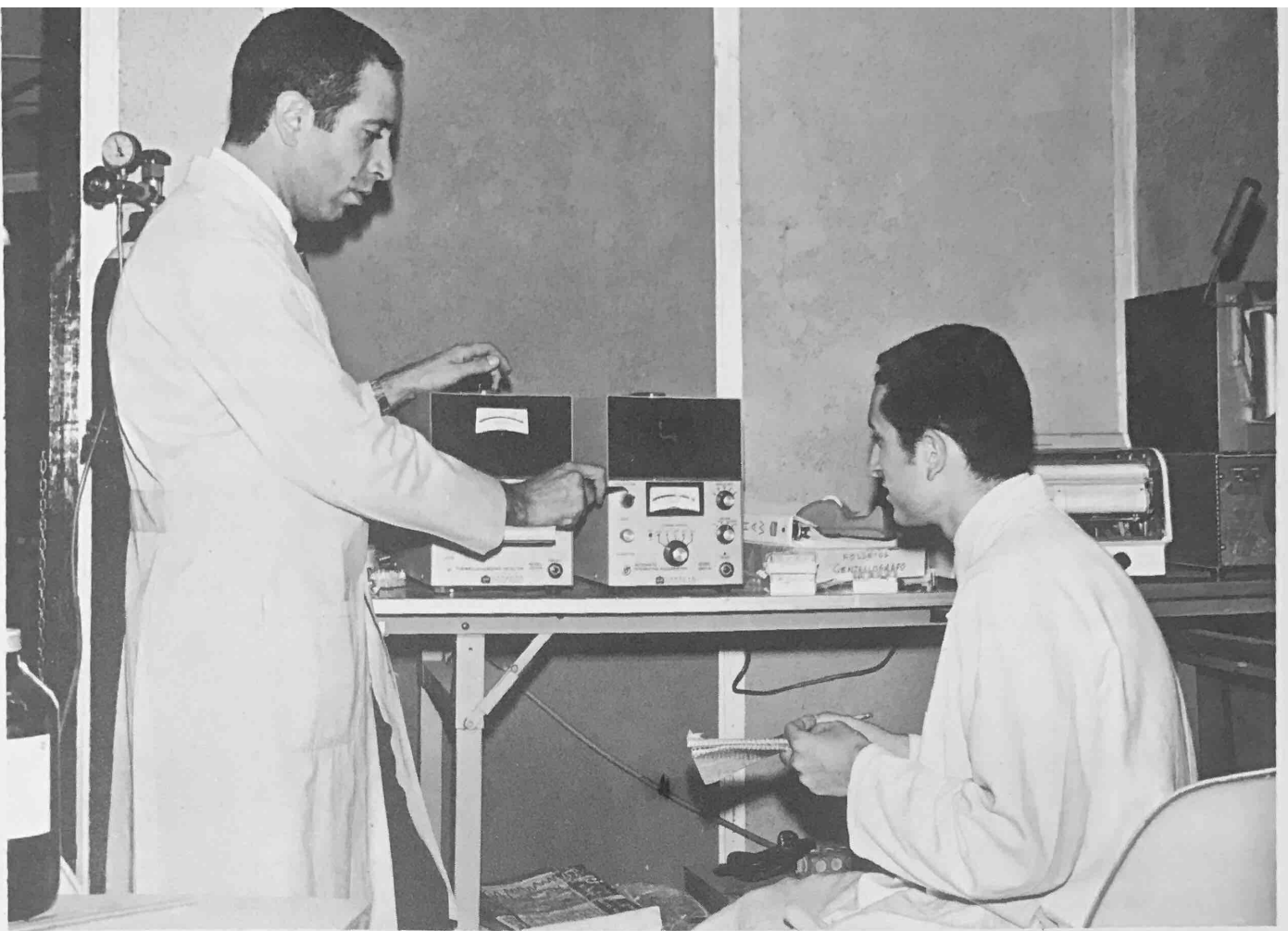
St. Mary's Long Beach Hospital
Long Beach, California
August 26, 1958

Hon. Craig Hosmer
Member of Congress, House Office Building
Washington, D.C.

Dear Mr. Hosmer:

Thank you for your letter of June 26th, 1968, prior to my visit to Washington. I am sorry that the shortness of our stay there precluded my visiting you in your office and I hope that I shall have the opportunity to do this on another occasion.

Following my visit to Washington, I spent a month in San Juan, Puerto Rico, visiting at the Puerto Rico Nuclear Center there.



In this scene from the PRNC Open House at its Mayaguez facility local students are shown marine specimens by Mr. Stephen Walsh, Deputy Director of the Radioecology Division. (Right) Photos taken during visit by Dr. Gomberg and other PRNC staff members to Los Llanos area of Colombia. At top, the group prepares to travel along the Meta River to visit El Piñal experimental farm together with Colombian government officials. Standing in the front of the small boat is Dr. Luse of PRNC. In bottom photo, Dr. Luse and Mr. Barcelo stand at far right aboard the truck which continues the trip to El Piñal. At far left in picture is Captain Ramírez, director of Colombia's Institute of Nuclear Affairs



As you probably are aware, this is a medical unit operated by the University of Puerto Rico for the United States Atomic Energy Commission. My interest was primarily in the clinical radiotherapy, nuclear medicine and radiobiology section. The group of very excellent clinicians headed by Dr. Víctor Marcial have over the past ten years proven that first class clinical cancer treatment can be very beneficial in a developing country, particularly one with a high cancer risk population. Not only do they do a superb job of diagnosis and treatment of cancer, but they have also been active in training physician-radiotherapist medical students and x-ray therapy technicians in their several programs. Their level of patient care and medical personnel training would be considered excellent by any standards in the continental United States.

The Atomic Energy Commission and the varying Congressional committees interested in atomic-related programs (of which I believe you are a member) should be highly commended for their farsightedness in this vital part of medical care in the Commonwealth of Puerto Rico. I was impressed at the effectiveness of this use of United States aid money spent in an underdeveloped country.

Sincerely yours,

William C. Johnson, M.D.

STAFF

Reverend Dr. Ignacio Cantarell, Associate Scientist in the Nuclear Science Division, was named Citizen of the Year for the city of Mayaguez for 1967 by the Junio Chamber of Commerce. The award was given for Dr. Cantarell's intellectual and scientific contribution to the community through the creation of the Institute of Modern Science in Mayaguez. The Institute offers a graduate program in nuclear science, leading to an M.S. degree, in which 10 students are enrolled.

Mr. Kal Wagenheim, part-time correspondent for the N.Y. Times in Puerto Rico, joined PRNC on July 1 with the title of Editor. His duties include the editing, design and production of the annual report and other PRNC publications, as well as assisting Latin American PRNC staff members in Spanish-to-English translation of their scientific material.

APPENDIX

PUERTO RICO NUCLEAR CENTER DIRECTORY

Advisory Committee

Chairman: Dr. Paul B. Pearson
President, The Nutrition Foundation
New York, New York

Dr. John A. D. Cooper
Dean of Sciences
Northwestern University

Dr. W. O. Baker, Vice President,
Research
Bell Telephone Laboratories
Murray Hill, New Jersey

Dr. Michael Ference, Jr.,
Vice President
Scientific Research Staff
Ford Motor Company

Dr. John C. Bugher
USAEC General Advisory Committee
Washington, D.C.

Dr. James G. Horsfall, President
The Connecticut Agricultural
Experimental Station

Dr. Juan A. del Regato
The Penrose Cancer Hospital
Colorado Springs, Colorado

Dr. Frederick Seitz, President
National Academy of Sciences
Washington, D.C.

Scientific and Administrative Staff

Office of the Director

Henry J. Gomberg, Director, Ph.D., U. of Michigan (Physics)
Amador Cobas, Deputy Director, Ph.D., Columbia U. (Physics)
Owen H. Wheeler, Associate Director, Mayaguez, D.Sc., U. of London (Chemistry)
Victor A. Marcial, Associate Director for Medical Programs, M.D., Harvard, U.
(Radiotherapy)
Jorge Chiriboga, Assistant Director for Scientific Programs, M.D.,
University of San Marcos, Perú (Biochemistry)
Héctor M. Barceló, Assistant Director for Operations, M.S.,
University of Puerto Rico (Nuclear Technology)
Marie Barton, Executive Assistant to the Director
Frederick Rushford, Technical Assistant to the Director
Kal Wagenheim, Editor
*John D. Weaver, Chief Scientist I.

*Terminated before Dec. 31, 1968.

Nuclear Science Division

Julio A. Gonzalo González, Head, Chief Scientist I., Ph.D.,
U. of Madrid, (Physics)
Florencio Vázquez Martínez, Chief Scientist I., Ph.D.,
U. of Madrid, (Electrical Engineering)
Rev. Ignacio Cantarell, Associate Scientist II, Ph.D.,
U. of Santiago de Compostela, Spain (Nuclear Physics)
Rupert A. Lee, Associate Scientist II, Ph.D., U. of London (Chemistry)
Baltasar Cruz Vidal, Associate Scientist II, Ph.D., Harvard U. (Physics)
Josefa Elisa Trabal, Research Associate I, B.S., U. of Puerto Rico (Chemistry)
*Luis C. Niño Villamarín, Research Associate I, M.S.,
U. of Puerto Rico (Chemistry)
José M. Rivera, Research Associate I, M.S., U. of Puerto Rico (Physics)

Neutron Diffraction Program

Mortimer I. Kay, Head, Chief Scientist II, Ph.D., U. of Connecticut (Chemistry)
Robert Kleinberg, Chief Scientist I, Ph.D., Michigan State U. (Physics)
*Seymour F. Kaplan, Associate Scientist II, Ph.D., U. of New Mexico (Geology)
Braulio F. Mercado, Research Associate II, B.S.
U. of Puerto Rico, Mayaguez (Electrical Engineering)

Hot-Atom Chemistry Project

Owen H. Wheeler, Head (See also office of the Director)
Josefa Elisa Trabal, Research Associate I, (See also Nuclear Sciences Division)
María Luisa McClín, Research Associate I, M.S., U. of Puerto Rico (Chemistry)

Nuclear Engineering Division

Donald S. Sasscer, Head, Chief Scientist I, Ph.D.
Iowa State U. (Nuclear Engineering)
Aviva E. Gileadi, Chief Scientist I, Ph.D., U. of Budapest (Reactor Analysis)
*Phillip W. Osborne, Chief Scientist I, Ph.D., U. of California (Metallurgy)
Eddie Ortiz Muñoz, Chief Scientist I, Ph.D., Texas A & M College (Physics)
José L. García de Quevedo, Chief Scientist I, Ph.D., Duke U. (Physics)
Knud E. Pedersen, Associate Scientist II, Ph.D.,
Iowa State U. (Nuclear Engineering)
**Kenneth Soderstrom, Research Associate III, M.S.E.,
U. of Florida (Mechanical Engineering)
Fausto Muñoz Ribadeneira, Associate Scientist I, M.S.,
U. of Puerto Rico (Nuclear Engineering)
Heriberto Plaza Rosado, Associate Scientist I, Ph.D.,
Texas A & M (Nuclear Engineering)
Antonio Rivera Cordero, Research Associate II, M.S.,
U. of Puerto Rico, Mayaguez (Nuclear Engineering)
Erick Méndez Veray, Research Associate I, M.S.,
U. of Puerto Rico (Nuclear Engineering; Metallurgy)

* Terminated before Dec. 31, 1968
** On leave as of Nov. 30, 1968

Physical Sciences Division

Amador Cobas, Head (See also Office of the Director)

*H. Harry Szmant, Head, Chief Scientist II, Ph.D., Purdue U. (Organic Chemistry)

Alec Grimison, Chief Scientist I, Ph.D., U. of London (Chemistry)

José P. A. Castrillón, Associate Scientist II, Ph.D.,

U. of Buenos Aires (Radioisotopes in Organic Chemistry)

George A. Simpson, Associate Scientist I, Ph.D., Notre Dame U. (Chemistry)

*Mariel M. Muir, Associate Scientist I, Ph.D., Tübingen U. (Organic Chemistry)

*Gerardo Molina Vega, Research Associate II, M.S.,

U. of Pennsylvania (Chemistry)

Manfred Eberhardt, Associate Scientist I, Ph.D., U. of Lubingen (Chemistry)

Rosa Santana de Tirado, Research Associate II, M.S.

U. of Puerto Rico (Chemistry)

Radiation Chemistry

Alec Grimison, Head (See also Physical Sciences Division)

George A. Simpson, Associate Scientist I, (See also Physical Sciences Division)

Solid State Physics Program

Amador Cobas, Head (See also Office of the Director)

Shmuel Zvi Weisz, Chief Scientist I, Ph.D., Hebrew U. of Israel (Physics)

Jacob Yehuda Levinson, Associate Scientist I, Ph.D., The Hebrew U. (Physics)

Clinical Radioisotope Applications Division

Sergio Irizarry, Head, Chief Scientist II, M.D.,

U. of Buffalo (Internal Medicine)

*José Oscar Morales, Chief Scientist II, M.D., U. of Maryland (Endocrinology)

Aldo Ernesto Lanaro, Chief Scientist I, M.D.,

U. of Buenos Aires (Nuclear Medicine and Endocrinology)

*Pedro Juan Santiago, Associate Scientist I, M.D., U. of Puerto Rico (Pediatrics)

*Norma Gándara Cruz, Res. Tech. in Clinical Applications of Radioisotopes

Leila Crespo de García, Res. Tech. in Clinical Applications of Radioisotopes

Adriana Rodríguez de Calderón, Res. Tech. in Clinical Applications of Radioisotopes

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Zenaida Frías Monserrate, Research Associate II, M.P.H.,

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Agricultural Bio-Sciences Division

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Francis K. S. Koo, Acting Head, Chief Scientist I, Ph.D.,
U. of Minnesota (Radiation Genetics)
José A. Ferrer Monge, Chief Scientist I, Ph.D., Louisiana State U. (Biology)
David Walker, Chief Scientist I, Ph.D., Washington State U. (Entomology)
*Shrinivas H. Kamath, Associate Scientist II, Ph.D.,
U. of Bombay, India (Biochemistry)
Horace D. Graham, Chief Scientist I, U. of Illinois (Food Science)
Shreekant N. Deshpande, Associate Scientist I, Ph.D.,
Purdue U. (Food Technology, Biochemistry)
José Cuevas Ruiz, Research Associate II, M.S., U. of Puerto Rico (Biology)
Angélica Muñiz Oliver, Research Associate I, M.S., U. of Puerto Rico (Biology)
Edith Robles de Irizarry, Research Associate I, M.S.,
U. of Puerto Rico (Genetics)

Sugarcane Borer Project

David W. Walker, Head (See also Agricultural Bio-Sciences Division)
Victoriano Quintana, Research Assistant I

Radioecology Division

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U. of Washington (Marine Biology)
Stephen Walsh, Deputy Director

Marine Biology

Frank G. Lowman, Head, (See also Radioecology Division)
*Donald K. Phelps, Chief Scientist I, Ph.D., U. of Rhode Island (Marine Ecology)
Robert Y. Ting, Associate Scientist I, Ph.D.,
U. of Washington (Fisheries Biology)
Steven S. Barnes, Associate Scientist I, Ph.D., U. of California (Chemistry)
John H. Martin, Associate Scientist I, Ph.D.,
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Stephen H. Walsh, (See also Radioecology Division)
Henry L. Besselievre, Associate Scientist I, B.S., U. of Puerto Rico (Physics)
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George A. Sieglie, Research Associate II, M.S., U. de la Habana (Geology)
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Russel W. Davis, Research Associate I, B.A., Inter American U. (Chemistry)
Rosa Julia Santiago, Research Associate I, M.S.,
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Terrestrial Ecology Program I - The Rain Forest Project

*Jerry R. Kline, Head, Chief Scientist I, Ph.D., U. of Minnesota (Soil Science)
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*Terminated before Dec. 31 1968

**On leave as of July 31, 1968

Administration and Services

Luis E. Boothby, General Administrative Officer
Ramón Muñiz, Jr., Administrative Officer II
*Juan López Rodríguez, Administrative Officer I
Nelida Banuchi de Gomez, Administrative Officer I
Pedro Velez Mendoza, Administrative Officer I

Technical Services

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Rio Piedras:

Julio Gagot Mangual, Research Associate II
Guillermo Torres Carmona, Building and Grounds Maintenance Supervisor
*William A. Moore, Electronics Tech. Supervisor

Mayaguez:

*Alfredo Carmona Trutten, Electronic Technician Supervisor
Nelson Quiñones, Technical Supervisor
Víctor Lequerique, Scientific Glassblower
Vicente C. Santiago Batis, Research Associate II
Jean M. Dietsch, Reproduction Section Supervisor
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Carmen Rivera de Campos, Research Associate I, B.S.,
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Mirta Toro González, Research Associate I, M.S.,
U. of Puerto Rico (Microbiology)
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*Sara Jean de Jesús, Med. Res. Technician II in Virology and Tissue culture
*Irma Elena Torres de Vázquez, Med. Res. Technician II in Virology and Tissue culture

Schistosoma mansoni Project

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Virus Program

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Reactor Division

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Hiram Ojeda, Nuclear Reactor Operator
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Sigfredo Torres, Nuclear Reactor Operator II
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Health Physics Division

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Efigenio Rivera, Associate in Health Physics
Michael Gileadi, Health Physics Assistant II
*Miriam H. Vega Soderstrom, Health Physics Assistant II

"Atoms in Action" Exhibit Project

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PAPERS PRESENTED

Berrios-Durán, L. A. - See Ritchie, L. S.

Castellanos, J. - See Weisz, S. Z.

1. Chiriboga, J., Colón, J. I., and Martínez Silva, R., Effects of Irradiation of the Definitive Host in Relation to Its Resistance Against Schistosoma mansoni, presented (by J.C.) at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.

Chiriboga, J. - See Knight, W. B.

Chiriboga, J. - See Martínez Silva, R.

Cobas, A. - See Weisz, S. Z.

2. Colón, J. I., The Viral Etiology of Infantile Diarrheas in Puerto Rico, presented at 2nd Central M. Congr. Microbiol., Panamá, Dec. 1968.

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Colón, J. I. - See Martínez Silva, R.

Correa, A. - See Martínez Silva, R.

Croker, W. L. - See Ritchie, L. S.

3. Cromer, D. T. (LASL) and Kay, M. I., Thermal Motion in Sodium Alum $\text{NaAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, presented (by D. T. C.) at the Am. Cryst. Assoc. Mtg., Tucson, Arizona, Feb. 1968 (Abstract published).
4. Cruz Vidal, B., F-Center Formation at 78°K in KBr During Exposure to Monochromatic X-Ray Energies Around the Bromine K-Edge, presented at the Am. Phys. Soc. Mtg., Berkeley, March 1968.
5. Cruz Vidal, B., Gomberg, H. J., and Díaz, F., F-Center Formation at 78°K in KBr and in RbBr During Exposure to Monochromatic X-Ray Energies Around Bromine K Edge, presented (by B. C. V.) at 1968 Intern. Symp. on Color Centers in Alkali Halides, Rome, Italy, Sept. 1968.
6. Cuevas-Ruiz, J., Luse, R. A., and Graham, H. D., Preservation of Tropical Foodstuffs by Irradiation, presented (by J. C. R.) at 6th Ann. Conf. Caribbean Food Crops Soc., Trinidad, July 1968.

Diaz, F. - See Cruz Vidal, B.

7. Eberhardt, M. K., Steric Effect in the Radiolysis of Cis-and-Trans-1, 2-Dimethylcyclohexane, presented at the Am. Chem. Soc. Mtg., San Francisco, April 1968.

Frick, L. P. - Ritchie, L. S.

8. Gomberg, H. J., Science and Technology in the Caribbean, presented at Caribbean Universities Conf., San Juan, Nov. 1968.
9. Gomberg, H. J., Scientific and Industrial Potential in Latin America, presented at the 7th Ann. Mtg. Southern Interstate Nucl. Board, Hot Springs, Arkansas, Apr. 1968.

Gomberg, H. J. - See Cruz Vidal, B.

Gomberg, H. J. - See Paraskevoudakis, P.

Graham, H. D. - See Cuevas, J.

10. Gomberg, H. J. and Rushford, F. E., Industrial Benefits From Use of Atomic Energy Information, presented (by H. J. G.) at the Information Technol. Conf., San Juan, June 1968.
11. Gonzalo, J. A., Free Energy Expansion Coefficients from Double Hysteresis Loops, presented at the Solid State Phys. Conf., Manchester, Jan. 1968.
12. Grimison, A., The Radiation Chemistry of Organic Glasses, presented at the 2nd Inter. Am. Radiochem. Cong., Mexico, April 1968.
13. Irizarry, S., The Contribution of Lung Scanning to the Diagnosis of Pulmonary Embolism, presented at the Quarterly Mtg. P. R. Chapter, Health Phys. Soc., Rincón, Dec. 1968.

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14. Julián, D. A. and Wheeler, O. H., Radiolysis of Peptides, presented (by D. J.) at the Junior Tech. Mtg., P. R. Chapter, Am. Chem. Soc., Mayaguez, Aug. 1968.

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15. Knight, W. B., Liard, F., Ritchie, L. S., Pellegrino, J., and Chiriboga, J. Labeling of Biomphalaria glabrata with Radioisotopes, presented (by J. C.) at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.
16. Knight, W. B., Liard, F., Ritchie, L. S., Pellegrino, J., and Chiriboga, J. Labeling of Cercariae of Schistosoma mansoni with Radioisotopes, presented (by J. C.) at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.

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17. Koo, F. K. S., Puerto Rico Nuclear Center Participation in Food Irradiation Program Development in Latin America, presented at the Mtg. of the Working Group to Study the Problem of Food Preservation by Irradiation, Montevideo, Dec. 1968.
18. Lanaro, A. E., Teaching of the Medical Applications of Radioisotopes in Puerto Rico and the Relation of This Program to Latin America, presented at the 2nd Latin Am. Congr. Biology Nucl. Medicine, Mar del Plata, Argentina, Nov. 1968.

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19. Luse, R. A., Radiation Inactivation of Enzymes Important in Fruit Ripening, presented at FAO/IAEA Panel Mtg., Vienna, Austria, Apr. 1968.

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21. Marcial, V. A., Radiotherapy of Cancer of the Uterine Cervix (in Spanish), presented at IX Colombian Radiological Review, Medellín, Colombia, Sept. 1968.

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23. Marcial, V. A., Therapy of Adenocarcinoma in the Cervix and Body of the Uterus (in Spanish), presented at IX Colombian Radiological Review, Medellín, Colombia, Sept. 1968.

24. Marcial, V. A., Therapy of Patients With Cancer of the Uterine Cervix Complicated With Pregnancy (in Spanish), presented at IX Colombian Radiological Review, Medellín, Colombia, Sept. 1968.

25. Marcial, V. A., Tomé, J. M., and Ubiñas, J., The Combination of External Irradiation and Curietherapy Used Pre-Operatively in Adenocarcinoma of the Endometrium, presented (by V. A. M.) at the Am. Radium Soc. Ann. Mtg., Miami Beach, Apr. 1968.

26. Martínez Silva, R., Correa, A., Colón, J. I., and Chiriboga, J., Cell Lines From a Murine Chondrosarcoma, presented (by J. I. C.) at the 19th Ann. Mtg. Tissue Culture Assoc., San Juan, June 1968.

27. Martínez Silva, R., López, V., Colón, J. I., and Chiriboga, J., Radiation Effect on the Susceptibility of Tissue Culture to Trypanosoma cruzi, presented (by R. M. S.) at the 19th Ann. Mtg. Tissue Culture Assoc., San Juan, June 1968.

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Morris, J. M. - See Ritchie, L. S.

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28. Paraskevoudakis, P. and Gomberg, H. J., Calorimeter Design, Calibration, and Measurements of the G-Values of the Fricke Dosimeter in the Energy Range 5-10 Kev of X-Rays, presented (by P. P.) at the 1st European Congr. Radiation Protect., Menton, France, Oct. 1968.

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29. Pérez Bracetty, F., Use of Gamma Ray Threshold Detector in Reactor Control, presented at the 7th Ann. Student Conf., Am. Nuclear Soc., Gainesville, Fla. Apr. 1968.

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30. Ritchie, L. S., Evaluation of Molluscicides, Their Formulations and Methods of Application, presented at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.
 31. Ritchie, L. S., Possibilities for Multimeasure Control of Schistosomiasis, presented at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.
 32. Ritchie, L. S. and Berríos Durán, L. A., Laboratory Infections of Schistosomes in the Snail Vectors, presented (by L. S. R.) at the Caribbean Comm. Bilharzia Res. Mtg., St., Lucia, British West Indies, Jan. 1968.
 33. Ritchie, L. S., Knight, W. B., Oliver González, J., Frick, L. P., Morris, J. M., and Croker, W. L., Schistosoma mansoni Infections in Cercopithecus sabaeus Monkeys, presented (by L. S. R.) at the Caribbean Comm. Bilharzia Res. Mtg., St. Lucia, British West Indies, Jan. 1968.
- Ritchie, L. S. - See Knight, W. B.
34. Rivera Cordero, A., Investigation of Gas Produced by the Nuclear Irradiation of Barytes Concrete Containing Boron, presented at the 7th Ann. Student Conf., Am. Nuclear Soc., Gainesville, Fla., Apr. 1968.
 35. Rivera Oyola, L., In-Pile Radiolysis of HCL Gas, presented at the 7th Ann. Student Conf., Am. Nuclear Soc., Gainesville, Fla., Apr. 1968.
 36. Rodríguez Olleros, A. and Irizarry, S., Functional Profile, Experimental Cirrhosis in Dogs Induced by Thioacetamide, presented (by A. R. O.) at the Intern. Soc. Study of Liver Diseases, Prague, Czechoslovakia, July 1968.
 37. Rodríguez Olleros, A. and Irizarry, S., A Study of Ultrastructure and Metabolic Alterations of Hepatic Injury Induced in Dogs by Thioacetamide, presented at the Joint Mtg. Am. Col. Gastroenterology and the Puerto Rican Asoc. Gastroenterology, School of Medicine, Nov. 1968.
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- Rushford, F. E. - See Gomberg, H. J.
39. Saca, M. and Lee, R. A., W Values Determination of Fluoroform (CHF_3), and Methyl Fluoride (CH_3F), presented (by M. S.) at the Junior Tech. Mtg., P.R. Chapter Am. Chem. Soc., San Juan, Aug. 1968.
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40. Tomé, J. M., Wilms' Tumor, presented at Ann. Mtg. P. R. Med. Assoc., San Juan, Nov. 1968.
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- Ubiñas, J. - See Marcial, V. A.
41. Vélez Delgado, G., Measurement of Temperature Distribution in the Water Pool of the PRNC Reactor, presented at the 7th Ann. Student Conf., Am. Nuclear Soc., Gainesville, Fla., Apr. 1968.

42. Walker, D. W. and Quintana-Muñiz, V., Inherited Sterility in the Sugarcane Borer Moth, presented (by D. W. W.) at the 1968 Mtg. Entomol. Soc. Am., Dec. 1968.

43. Weisz, S. Z., Castellanos, J., Cobas, A., and Simpson, G. A., Paramagnetic Radiation Induced in Anthracene and Deuterated Anthracene, presented (by A. C.) at the 4th Mol. Crystal Symp., Twente, Netherlands, July 1968.

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2. Hot-Atom Chemistry Program, Progress Summary Report No. 3, April 1967 to March 1968, PRNC 114.
3. Puerto Rico Nuclear Center Tenth Anniversary Symposium on Nuclear Energy and Latin American Development, PRNC-112, Oct. 1967.
4. Adam, W., Grimison, A., Hoffmann, R., and Zuazaga de Ortiz, C., The Extended Huckel Theory Applied to Hydrogen Bonding and Pyridine, J. Am. Chem. Soc. 90, 1509-13 (1968).
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Almodóvar, I. - See Kay, M. I.
Armstrong, D. A. - See Lee, R. A.
5. Barceló, H., Program of Instruction for PRNC Reactor Operators (Academic Phase), PRNC-17, June 1968.
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6. Bosch, A., Lanaro, A.E., Irizarry, S., and Palacios, M. M., Early Effects of Irradiation on the Normal Thyroid Gland, Rev. Interam. Radiología 3, 65-74, (1968).
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Castellanos, J. - See Simpson, G.
Castillo, M. - See Lanaro, A. E.
7. Chiriboga, J., Synthesis of Glucuronic Acid in Vivo and in Vitro After Whole Body Irradiation in Rats, Acta Physiol. Latino Am. 18, 31-41 (1968).
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Chiriboga, J. - See Liard, F.
Chiriboga, J. - See Umpierre, G. M.
Cobas, A. - See Simpson, G.
Cobas, A. - See Weisz, S. Z.
Colón, J. I. - See Liard, F.
Cromer, D. T. - See Kay, M. I.

- De León, E. - See Marcial, V. A.
- Drewry, G. E. - See Kline, J. R.
8. Eberhardt, M. K., Steric Effect in the Radiolysis of cis- and trans-1, 2-Dimethylcyclohexane, Phys. Chem. 72, 4509-12 (1968)
9. Facetti, J. F. and Vélez de Santiago, M., Rapid Electrophoretic Separation of Rhenium (VII), Anal. Chem. 40, 1726-7, (1968).
10. Facetti, J. F. and Vélez de Santiago, M., Search for One Hour ¹⁸⁶Re (in Spanish), Rev. Soc. Cient. 8, 4-7, (1967)
- Facetti, J. F. - See Ortiz, E.
- Frías, Z. - See Lanaro, A. E.
11. Gileadi, A. E. and Lee, C. F., A Study of Computational Methods Used to Determine Fuel Depletion in Nuclear Reactors, PRNC-117, March 1968
12. Gileadi, A. E. and López Carrasco, F., Determination of the Transfer Function and Certain Kinetics Parameters of the L-77 Reactor, PRNC-118,
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14. Gonzalo, J. A., A Set of Experimental Critical Exponents for Ferroelectric Triglycine Sulfate, Phys. Rev. Letters 21, 749-51 (1968).
- Gonzalo, J. A. - See Kay, M. I.
- Gonzalo, J. A. - See Niño, L. C.
15. Grimison, A. and Simpson, G. A., Matrix Isolation Studies of the Gamma-Radiolysis of Heterocyclic Molecules, Technical Progress Report, PRNC-116, April 1968.
16. Grimison, A. and Simpson, G. A., Spectrophotometric Identification of Gamma Radiolytic Intermediates in a New Halogenic Glassy Matrix, J. Phys. Chem. 72, 1776-9, (1968).
- Grimison, A. - See Adam, W.
- Hoffman, R. - See Adam, W.
- Irizarry, S. - See Bosch, A.
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- Jarnagin, R. C. - See Weisz, S. Z.
17. Jordan, C. F., A Simple, Tension-Free Lysimeter, Soil Sci. 105, 81-6 (1968).
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- Kaplan, S. F. - See Kay, M. I.
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19. Kay, M. I., Kaplan, S. F., Kleinberg, R., Gonzalo, J. A., and Cromer, D. T., Neutron Diffraction Program Progress Summary Report No. 6, PRNC-115, April 1968.
- Kleinberg, R. - See Kay, M. I.
20. Kline, J. R. (and staff), The Rain Forest Project Annual Report, Fiscal Year 1967, PRNC 103.
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23. Kong, L. and Luse, R. A., The Uptake of ^{65}Zn by Sugarcane, Taiwan Sugar Res. Quart., Fall 1967.
24. Lanaro, A. E., Castillo, M., Frías, Z., Irizarry, S., and Marcial Rojas, R., Thyroid Histopathology: The Correlation With the Scanning in Puerto Rico (in Spanish), Radiología 18, 89-94 (1968).
- Lanaro, A. E. - See Bosch A.
- Lee, C. F. - See Gileadi, A. E.
25. Lee, R. A. and Armstrong, D. A., Radiolysis of Gaseous Hydrogen Bromide: The Effects of Pressure, Bromine, and an Applied Electric Field, Intern. J. Appl. Radiation Isotopes 19, 585-91 (1968).
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- López Carrasco, F. - See Gileadi, A. E.
- Luse, R. A. - See Kong, L.
- McClin, M. L. - See Wheeler, O. H.
- Many, A. - See Weisz, S. Z.
- Marcial Rojas, R. - See Lanaro, A. E.
27. Marcial, V. A., Blanco, M. S., and De León, E., Persistent Tumor Cells in the Vaginal Smear During the First Year After Radiation Therapy of Carcinoma of the Uterine Cervix: Prognostic Significance, Am. J. Roentgenol., Radium Therapy Nucl. Med. 102, 170-5 (1968).

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- Martínez Silva, R. - See Liard, F.
- Muñoz Candelario, R. - See Muñoz-Ribadeneira, F.
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- Palacios, M. M. - See Bosch, A.
- Pellegrino, J. - See Knight, W. B.
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- Pinto, S. - See Ortiz, E.
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WEEKLY SEMINARS, RIO PIEDRAS

Drs. José A. del Castillo and Dr. Félix Córdova, UPR School of Medicine, The Relaxing Action of Fermamide in Muscle, January 19.

Professor A. G. Maddock, Cambridge University, Radiation Damage Due to Nuclear Transformation in Solids, February 9.

Dr. G. J. Dienes, Brookhaven National Laboratory, Theoretical Studies on the Kinetics of Cell Proliferation, February 23.

Dr. George Bemski, Instituto Venezolano de Investigaciones Científicas (IVIC), Paramagnetism in Hemoglobin, February 27.

Dr. Francis K. S. Koo, Human Chromosomes and Syndromes, March 1.

Dr. Víctor A. Marcial, Fractionation in Radiation Therapy of Carcinoma of the Uterine Cervix: Results of Prospective Study of 3 Vs. 5 Fractions per Week, March 8.

Dr. Efraín Toro-Goyco, UPR School of Medicine, Labeling of Protein With ^{131}I for Immunological Purposes, March 15.

Dr. Robert Kleinberg, Magnetic Structure Determination of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, March 29.

Dr. Allan Maccoll, University College, London, Mass Spectroscopy, April 10.

Dr. Julio I. Colón, UPR School of Medicine, Effect of Radiation on Virus Growth in Mice, Wild Rats, and Tissue Culture, April 19.

Dr. Rodman A. Sharp of Beckman Instruments, Inc., Recent Advances in Liquid Scintillation Counting, April 26.

Dr. Owen H. Wheeler, Radiolysis of Amino Acids and Peptides, May 3.

Dr. Anthony Kaney, University of Puerto Rico, Molecular Analysis of the Bobbed Mutant of Drosophila melanogaster, May 10.

Dr. Theodore Villafaña, Johns Hopkins University, Radiologic Image Evaluation, July 2.

Dr. Gabriel Chuchani, Instituto Venezolano de Investigaciones Científicas (IVIC), The Effects of Amine Groups in Aromatic Electrophilic Substitution, July 11.

Dr. Michael Barfield, University of Arizona, Theory of Nuclear Spin - Spin Coupling, July 18.

Dr. Baltazar Cruz, F Center Formation at 78°K in KBr and in RbBr During Exposure to Monochromatic X-Ray Energies Around the Bromine K Edge, August 2.

Dr. D. A. Armstrong, University of Calgary, Radiolysis of Aqueous Solutions of Sulfur Compounds, August 8.

Dr. Roger Pedersen, Yale University, Specific Gene Multiplicity: The Ribosomal Cistrons, August 30.

Dr. Raymond Brown, A System for Studying Immunological Phenomena, September 6.

Dr. Julio A. Gonzalo, Critical Behavior of Ferroelectrics, September 13.

Dr. Rodrigo Fierro, National Polytechnical School, Quito, Ecuador, Biology of the Andean Man, September 20.

Dr. Walter Stahl, UPR School of Medicine, Leishmaniasis cutanea--Ulceras de los Chicleros, September 27.

Dr. José P. A. Castrillón, Relación Entre Estructura Química y Extinción en el Centelleo en Fase Líquida (Relation Between Chemical Structure and Extinction in Liquid Phase Scintillation), October 4.

Dr. Carl F. Jordan, Tritium Movement in the Tropical Ecosystem, October 11.

Dr. Naftale Katz, National Institute of Endemic Rural Diseases, Belo Horizonte, Minas Gerais, Brazil, General Aspects of Schistosomiasis in Brazil, October 18.

Dr. Alfredo Bennun, University of Puerto Rico, The Relationship of ATPase to Structure and the Function of the Photophosphorylative Mechanism, October 25.

Dr. José Nine Curt, UPR School of Medicine, Health Conditions in Puerto Rico, November 1.

Dr. Ruheri Pérez Tamayo, Ellis Fishell State Cancer Hospital, Columbia, Missouri, The Use of Digital Computers in Radiation Therapy, November 15.

Dr. Víctor A. Marcial, Smoking and Health, November 22.

Dr. Fermín Sagardía, UPR School of Medicine, Comparative Studies on Glycogen Phosphorylase from Crab Muscles and Yeasts, December 13.

WEEKLY SEMINARS, MAYAGUEZ

Professor A. G. Maddock, Cambridge University, and Visiting Professor of Chemistry, State University of New York at Buffalo, Nuclear Techniques in the Study of Solids, February 6.

Dr. Jack Chernick, Brookhaven National Laboratory, Reactor Physics at the Brookhaven National Laboratory, Reactor Physics at the Brookhaven National Laboratory, February 16.

Dr. George Bemski, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Paramagnetism in Hemoglobin, February 26.

Dr. Robert Kleinberg, Magnetic Structure Determination of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, March 29.

Dr. Allan Maccoll, University College, London, Ion Molecule Reactions, April 8.

Dr. Milton Yatvin, University of Wisconsin, Madison, Regeneration Activity in Irradiated Cells With Particular Reference to Protein Nucleic Acids, April 11.

Dr. Robert Kleinberg, On the Use of Moderators to Reduce Fast Flux in Beam Tubes and General Problems to be Considered for Installation of a D₂O Tank at PRNC, April 29.

Dr. J. A. Swallow, Christie Cancer Institute, Manchester, England, Radiation Chemistry of Organic Compounds, April 30.

Dr. James A. Muir, University of Puerto Rico, GeSe_{0.75}Te_{0.25}--A New Semiconductor, May 13.

Mr. Mario Saca, W Value Determination of Fluoroform, May 27.

Dr. Gabriel Chuchani, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, The Effects of Amine Groups in Aromatic Electrophilic Substitution, July 10.

Professor D. R. Wiles, Carleton University, Ottawa, Canada, Recoil Reactions in Arene Metal Carbonyls, July 15.

Dr. H. A. Gersch, Oak Ridge Research Participant, Georgia Institute of Technology, Space Time Spin Correlations, July 29.

Dr. D. A. Armstrong, University of Calgary, Alberta, Canada, Radiolysis of Aqueous Cysteine Solutions, August 6.

Dr. Walter A. Glooshenko, Oak Ridge Research Participant, Thermal Pollution--A Problem to Aquatic Life, September 11.

Dr. Werner Fialla, University of Puerto Rico, Neutron Reflection, September 16.

Dr. Jack Chernick, Brookhaven National Laboratory, Reminiscences of a Brookhaven Scientist, October 21.

Dr. Flavio Padovani, Louisiana State University, Isolation Characterization and Partial Purification of the Sex Ratio Factor in Estigmene acrea (Drury), November 6.

TABLE SHOWING PRNC STUDENTS BY COUNTRY*
From FY-1958 through FY-1968

	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	TOTAL
Argentina	1	-	2	1	4	1	2	-	4	1	1	17
Bolivia	1	-	-	-	1	4	1	-	-	1	1	9
Brazil	-	-	-	-	-	-	-	-	-	1	1	2
Chile	1	1	2	2	-	-	1	1	2	4	4	18
Colombia	1	5	3	6	3	6	7	4	4	5	4	48
Costa Rica	-	2	-	-	-	-	1	-	-	2	1	6
Cuba	-	1	3	-	-	-	3	1	1	-	-	9
Dominican Republic	-	-	1	-	-	14	1	1	2	2	5	26
Ecuador	3	-	1	1	-	-	1	1	1	2	1	11
El Salvador	-	-	1	-	1	1	2	-	1	2	1	9
Formosa	-	-	-	-	-	-	-	1	3	2	6	12
Germany	-	-	-	-	-	-	-	-	1	1	-	2
Great Britain	-	-	-	-	1	-	1	-	1	1	1	5
Guatemala	-	-	-	-	-	1	2	-	-	-	2	6
Haiti	-	-	1	-	-	-	-	-	-	-	-	1
Hungary	-	-	-	-	-	-	-	-	-	-	-	1
India	1	-	-	-	1	-	-	-	3	4	-	9
Israel	-	-	-	-	-	-	-	-	-	-	1	1
Japan	-	-	-	-	-	-	1	-	-	-	-	1
Lebanon	-	-	-	-	-	-	-	-	-	1	1	2
Liberia	-	-	-	-	-	-	-	-	-	1	1	2
Mexico	-	5	1	1	2	1	3	2	-	1	2	18
Nicaragua	-	-	1	1	-	-	-	-	-	1	2	5
Panama	-	-	-	-	-	1	1	-	-	-	-	2
Paraguay	-	-	-	-	1	3	2	-	-	-	-	6
Peru	-	1	3	1	1	1	5	-	1	-	3	16
Philippine Islands	1	-	-	-	1	-	-	-	2	1	1	5
South Africa	-	-	-	-	1	-	-	-	-	-	-	1
Spain	-	1	3	3	3	2	1	-	2	-	2	17
Turkey	-	-	-	-	-	-	-	-	-	-	1	1
United Arab Republic	-	-	-	-	-	-	-	-	1	-	-	1
Uruguay	-	1	1	-	-	1	-	-	1	1	-	7
Venezuela	-	3	4	3	2	-	-	1	2	3	3	21
TOTAL NON-U.S. CITIZENS	9	20	27	21	21	36	35	13	32	37	46	297
TOTAL U. S. CITIZENS	50	52	71	74	101	161	176	198	141	199	167	1390
TOTAL STUDENTS	59	72	98	95	122	197	211	211	173	236	213	1687

* A student is counted once each Fiscal Year he is in training.

September 1968

STUDENT ENROLLMENT AT PRNC DURING FISCAL YEARS 1967 AND 1968

PROGRAMS AND COURSES	MONTHS	FY-1967		FY-1968	
		Stu- dents	Stu. Months	Stu- dents	Stu. Months
Radioisotope Techniques Course	1	21	21	21	21
Chemistry-Thesis Research	12	21	252	14	168
Chem 465- Radiochemistry	4	7	28	1	4
Clinical Applications of Radioisotopes	2	6	12	9	18
Advanced Course in Nuclear Medicine: Nephrology	1	2	2	-	-
Radiotherapy and Cancer Residency	6-12	1	12	2	18
Short-Term Radiotherapy Training	1	1	1	8	8
One Month Cancer Course	1	15	15	10	10
Radiological Physics Conferences	4	4	16	-	-
Tissue Culture and Radioisotopic Techniques at Cell and Subcellular Level	1-5	8	8	13	19
Special Training in Medical Sciences and Radiobiology	0.10-12	26	52.90	8	26
Thesis Research - Biology	12	-	-	4	48
Biol 372 - Nuclear Techniques in Biological Research	4	5	20	-	-
Biol 379 - Readings and Research in Radiobiology	4	2	8	-	-
Biochemistry 414 - Instrumental Techniques in Biological Research	4	-	-	6	24
Biol 335 - Cytogenetics	4	-	-	9	36
Radiation Chemistry of Food Irradiation	3-5	1	3	1	5
FMPH 231 - Fundamentals of Radiological Hygiene	4	9	36	10	40
Special Training in Library Techniques	6	1	6	-	-
Individual Courses, PRNC	4	-	-	9	36
RIO PIEDRAS TOTALS		130	492.50	125	481
Nuclear Engineering	8-12	15	120	10	120
Nuclear Sciences	8-12	8	64	8	96
Thesis Research - Agriculture and Biology	8-12	7	56	7	84
Health Physics	12	-	-	1	12
Special Training in Health Physics	3	-	-	1	3
Special Training in Glassblowing	5	-	-	1	5
Reactor Supervisor Training	12	1	12	-	-
Reactor Supervisor Training (Special Course)	2	8	16	-	-
Individual Courses, PRNC	1-12	49	196	57	221
MAYAGUEZ TOTALS		88	464	85	541
Oak Ridge Research Participation Program	1-12	4	21	4	12
Oak Ridge Graduate Fellowship Program	12	2	24	-	-
ORAU TOTALS		6	45	4	12
PRNC TOTALS					
UPR, Rio Piedras	1-12	61	396	-	-
UPR, Mayaguez	1-12	24	96	-	-
UPR TOTALS		85	492	-	-
GRAND TOTALS		309	1493.50	214	1034