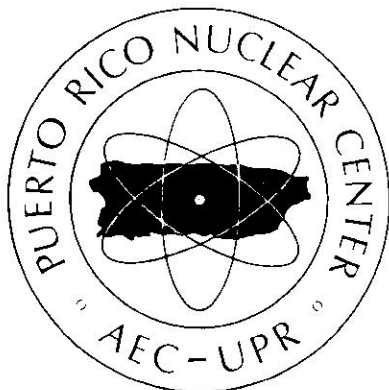


PUNO-213

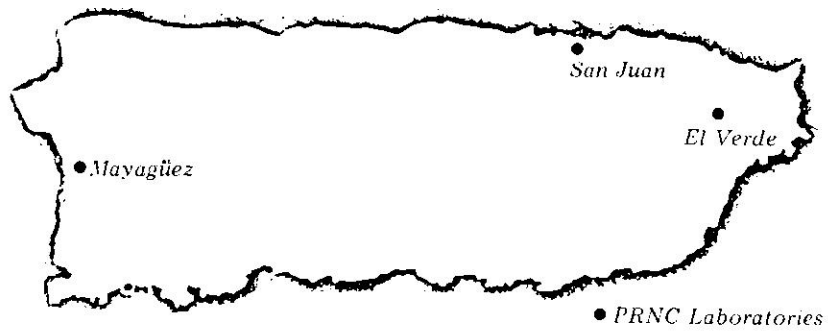
PUERTO RICO NUCLEAR CENTER

AEC BIO-MEDICAL PROGRAM DIRECTORS MEETING

SAN JUAN, PUERTO RICO
FEBRUARY 7-8, 1972



OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT
NO. AT (40-1)-1833 FOR U S ATOMIC ENERGY COMMISSION



AEC BIO-MEDICAL PROGRAM DIRECTORS MEETING

San Juan, Puerto Rico
February 7-8, 1972

Puerto Rico Nuclear Center
Bio-Medical Building - Rio Piedras

MONDAY, FEBRUARY 7

8:15 a.m.	Bus leaves hotel for meeting	
9:00 - 9:30 a.m.	Introductory Remarks	Dr. E. Roig
9:30 - 10:15 a.m.	Jobos Bay	Dr. M. Canoy
10:15 - 10:30 a.m.	Coffee Break	
10:30 - 11:15 a.m.	Temperature Studies in Tropical Marine Environment	Dr. S. Kolehmainen
11:15 - 11:30 a.m.	International Decade of Ocean Exploration	Dr. W. Forster
	Lunch	
1:00 - 2:00 p.m.	Marine Biology	Dr. F. G. Lowman
2:00 - 3:00 p.m.	Terrestrial Radioecology and International Biological Program	Dr. R. Clements
3:00 p.m.	Visit PRNC research vessel "PALUMBO"	

TUESDAY, FEBRUARY 8

8:15 a.m.	Bus leaves hotel for meeting	
9:00 - 9:15 a.m.	Introductory Remarks	Dr. V. Marcial
9:15 - 9:30 a.m.	Effect of Gamma Radiation on Interferon Production in Chick Embryos and Mice	Dr. J. I. Colón
	Effects of Gamma Radiation on Antibodies and on Activation of Cocksackie Virus in Immune Animals	Dr. J. I. Colón

TUESDAY, FEBRUARY 8 (continued)

9:30 - 9:40 a.m.	Effect of Gamma Radiation on Mice Immunized Against <u>T. Cruzi</u>	Mr. V. A. López
9:40 - 9:50 a.m.	Delayed Effects of Radiation Upon the <u>Schistosoma mansoni</u> Worm	Dr. R. A. Brown
9:50 - 10:00 a.m.	Bacteria in the "Curative" Effect of Hemolymph of <u>Biophalaria Glabrata</u> on <u>Schistosoma mansoni</u> -Infected Mice	Dr. R. A. Brown
10:00 - 10:10 a.m.	The Use of Slow Release Organotin in a Multilevel Control of Schistosomiasis	Dr. L. Ritchie
10:10 - 10:20 a.m.	Poly IC Stabilized with Different Metals Structural and Functional Differences	Dr. J. Chiriboga
10:20 - 10:40 a.m.	Coffee Break	
10:40 - 10:50 a.m.	<u>Fasciola hepatica</u> - General Statement	Dr. J. Chiriboga
10:50 - 11:00 a.m.	An Epidemiologic Observation on Fascioliasis in Puerto Rico	Dr. L. Ritchie
11:00 - 11:10 a.m.	On the Immunogenic Effect in Rats of Triple Vaccination with 2,500 R Gamma-Irradiated <u>F. hepatica Metacercariae</u>	Dr. D. de León
11:10 - 11:45 a.m.	Sugarcane Borer	Dr. D. Walker
11:45 - 12:15 p.m.	Heterocyclic Molecules	Dr. A. Grimison
	Lunch	
AFTERNOON	Dr. John R. Totter's meeting	

ENVIRONMENTAL STUDIES FOR THE AGUIRRE POWER COMPLEX

Michael J. Canoy

In April 1970, the Radioecology Division of PRNC was approached by Puerto Rico Water Resources Authority to undertake a cooperative study to provide an environmental baseline and monitor the Jobos Bay area in southeast Puerto Rico. A large power complex, including 4 nuclear plants, was planned for the site. The study proposed represented a positive benefit to both PRWRA and the AEC.

A history will be given for the investigation. This will be followed by a description of the area and a summary discussion of the data thus far obtained. This discussion will be illustrated by 35 mm color slides.

This duty has shown the depth and complexity of the tropical environment in relation to power generation. Common features of environmental research in siting and licensing power facilities will be discussed. The continuing program will be described. The conclusions pertinent to other research elsewhere are outlined and "spin-off" of two other projects will be mentioned.

THERMAL STUDIES IN TROPICAL MARINE ECOSYSTEMS

Seppo Kolehmainen

During the ecological studies in Jobos Bay it became apparent that there was an urgent need for data about the effects of elevated water temperatures upon tropical marine organisms and ecosystems. A research proposal on this subject has been solicited by the Puerto Rico Water Resources Authority and the study which we propose to do in response is outlined. The effects of elevated temperatures will be studied in experimental conditions in the field by exposing different organisms to different temperatures and by studying the species diversity, density and biomass at different temperatures around a fossil-fuel thermoelectric plant that is located in Guayanilla Bay on the south coast of Puerto Rico. In addition, the influence of the elevated temperatures upon the resident ecosystems in the bay and upon plants and animals maintained under controlled laboratory conditions will be studied.

Some preliminary studies have already been made in Guayanilla Bay and these data will be discussed.

INTERNATIONAL DECADE OF OCEANOGRAPHY EXPLORATION

William O. Forster

Two cruises for sampling and measuring baseline-levels of pollutants have been carried out aboard the R. F. Palumbo during the past six months. Samples of water, sediment, plankton, crustacea and fish will be analyzed for pesticides, heavy metals, nutrients and hydrocarbons.

MARINE BIOLOGY

Frank G. Lowman

The prediction of hazard to man and other animals from the introduction of radionuclides into the environment has been based upon two methods using the concept of critical pathway or that of specific activity. The application of the specific activity method depends upon the introduction of radionuclides into the environment in forms which are as equally available to the food webs as the naturally occurring corresponding stable elements. This is not always the case. Many trace elements, for which radionuclides are produced in nuclear technology, occur in the marine environment in more than one form. Cobalt is one of these. Investigations have been carried out during the past year on the forms of cobalt which occur in the estuarine waters of Puerto Rico. In addition, controlled studies of the simultaneous uptake of tagged ionic cobalt and cobalt incorporated into cobalamin have been done with marine sediments, plants and animals.

Investigations on the distribution patterns of ten elements in mangrove and turtle grass ecosystems have been made. The chemical - physical forms of some of the elements in the waters of these systems have been determined.

Seasonal distribution patterns of trace elements in waters from Jobos, Mayaguez and Añasco Bays have been measured and related to seasonal fluctuations, sources and reservoirs and the interactions of river and marine waters in estuarine areas.

During the past year the laboratories and offices of the Marine Biology Program have been moved from the main installation at Mayaguez to Pta. Guanajibo south of town. The laboratory is located in a 20-acre tract

on the point. During the next year a dock will be constructed and the size of the present laboratory building will be doubled.

In April of 1971 the Atomic Energy Commission took delivery of the research vessel "R. F. Palumbo" from the Tatco Ship-building Yard in San Diego, California. Staff members of the Marine Biology Program brought the ship to Puerto Rico through the Panama Canal. Measurements and marine samples were taken throughout the trip. The ship is named after Dr. Ralph F. Palumbo, an algologist, who worked for the Atomic Energy Commission at the Laboratory of Radiation Ecology, University of Washington, from 1948 until his untimely death on July 4, 1964.

RAINFALL INTERCEPTION IN A TROPICAL FOREST

Richard G. Clements

During a one year period, quantitative measurements were made on incoming rain, throughfall and stemflow on a 400 square meter plot in the montane rainforest at El Verde. Predictive equations were developed for the relationship between rainfall-throughfall ($R^2 = 0.97$) and rainfall-stemflow ($R^2 = 0.88$). Original analysis of rainfall-distribution by storm classes showed that 68% of all rain days were of one half inch or less. This distribution suggests that reconsideration must be given to the importance of light rains on the input of isotopes, both radioactive and stable, to natural ecosystems.

THE CHEMISTRY OF RAINFALL, THROUGHFALL AND STEMFLOW IN A TROPICAL FOREST

Richard G. Clements

Chemical determination for Ca, Mg, Na, K were performed on samples of incoming rain, throughfall and stemflow water in conjunction with Rainfall Interception Studies at El Verde. The chemical composition of the samples studied varied considerably and were dependent upon the intensity, duration, and time between storms and wind direction. A shift in wind direction from easterly to northerly caused a tenfold increase in the concentration of the four elements in rain water which in turn affected the concentration of those elements in throughfall and stemflow water.

THE CHEMICAL IMPORTANCE OF THE FIRST TENTH OF
AN INCH OF RAINFALL-PRELIMINARY REPORT

Richard G. Clements

A sequential rainfall collector was developed to fractionate incoming rainfall into samples representing 0.01 inch. These fractions were analyzed by atomic absorption spectrophotometry for Na, K, Ca, and Mg. Results based on the analyses of 35 storm events showed that given the concentration of these elements in a given fraction, the concentration of the other fractions can be determined by the formula $Y = \frac{1}{N}$; where N is the fraction number and Y is the concentration of the element.

STUDIES ON THE DIVERSITY AND ECOLOGY OF RAIN FOREST PLANTS AND ANIMALS

George E. Drewry

A brief description is made of studies in four biological aspects of radioecology. These include diversity studies of rain forest plants and animals, and the ecology of snails, frogs, and fresh water shrimps. Each study is discussed as it relates to the ecosystem as a whole, and to the practical aspects of radioecology involving the gamma irradiation study, or radioisotopes in food webs and mineral cycles.

U. S. INTERNATIONAL BIOLOGICAL PROGRAM TROPICAL FOREST BIOME

Richard G. Clements

The participation of the Radioecology Division of PRNC in the U. S. IBP program is described. The proposed study deals with a complete watershed that encompasses three distinct forest types, cultivated and noncultivated agricultural lands, an estuarine system and a marine system. The overall objective will be to quantify the hydrologic and isotope cycles and determine management alternatives for a tropical watershed.

EFFECT OF GAMMA RADIATION ON INTERFERON PRODUCTION IN CHICK EMBRYOS AND MICE

J. I. Colón, C. Rivera-Campos, and M. Toro Rodríguez

The effect of gamma radiation on the production of interferon by 10 day-old chick embryos and adult mice inoculated with newcastle disease virus (NDV) was studied. The interferon was assayed by the plaque inhibition test of sindbis virus in chick embryo fibroblast and in L cells, depending on the host where it was produced. In experiments using chick embryos, it was found that gamma radiation enhanced the production of interferon in the allantoic fluid and in the embryos. In chick embryos not irradiated and inoculated with 10^6 plaque forming units (pfu) of NDV, the interferon titer was 160 units per ml. In chick embryos irradiated with 400 rads and inoculated with 10^6 pfu of NDV, the interferon titer was 3,850 units per ml. In chick embryos that were irradiated but inoculated with the viral diluent (Saline A), no interferon was produced.

In similar experiments in adult mice, it was found that the amount of interferon produced in the animals that were irradiated (100-600 rads, whole-body) and immediately inoculated intravenously with 10^7 pfu NDV was significantly less than the amount found in the unirradiated controls, while the amount produced in mice that were irradiated with 700, 800, and 900 rads was significantly greater (6-10 times) than the amount produced in the unirradiated controls. With doses of radiation greater than 900 rads, the titer of the interferon produced decreased rapidly. In experiments with aminophyllene, it was found that it has a similar effect on the interferon production as the gamma radiation. This finding could indicate that the radiation is enhancing the interferon production by a mechanism involving cyclic AMP.

EFFECTS OF GAMMA RADIATION ON ANTIBODIES AND ON ACTIVATION
OF COXSACKIE VIRUS IN IMMUNE ANIMALS

J. I. Colón, M. Toro-Rodríguez, C. Rivera-Campos, and G. L. Borrero

Adult white mice were immunized with coxsackie virus type A10 by two intraperitoneal inoculations given 7 days apart. Thirty days after the last inoculation, these animals showed no active virus in the blood nor in any of the organs tested of animals that were sacrificed. All the animals showed a high neutralizing antibody titer. When these animals were irradiated (whole body) with 400 rads, active coxsackie virus was isolated from the blood, spleen, and pancreas. There are two possible explanations for this phenomenon: (1) the virus in the immune animal is in a concentration too low to be detected by our methods, but after radiation the virus freely propagates due to the damage done to the immune mechanism, or (2) the virus in a "latent state" is reactivated by the radiation and again can freely be propagated due to the damage done to the immune mechanism. In another series of experiments, mice were immunized and observed for a period of 6 months after the last viral inoculation. Each one of the animals was bled and the serum titrated for neutralizing antibodies against coxsackie virus A10. Animals with antibodies were divided into two groups. A third group was made with those that did not show neutralizing antibodies. Each group was irradiated with 400 rads (whole body) and the following results were obtained: (1) active virus was isolated from immunized mice that still showed circulating neutralizing antibodies when they were given whole body irradiation; (2) no virus was recovered from the previous group of animals if they were not irradiated; (3) no active virus was recovered from animals that were immunized but at the time the whole body irradiation was given did not have circulating antibodies.

These results showed that radiation reactivates virus only from animals that were immunized and showed circulating antibodies at the time of irradiation. These experiments support the hypothesis that viruses that confer long-lasting immunity remain in a "latent state" in the animal. We can go one step further and postulate that as long as antibodies are present in an animal the virus is also present in some form somewhere in the animal. If an animal is free of antibodies it is also free of virus.

EFFECT OF GAMMA RADIATION ON MICE IMMUNIZED AGAINST T. CRUZI

R. Martínez-Silva, V. A. López, and G. Araujo Ugarte

Preliminary experiments showed that mice inoculated with an avirulent strain of T. cruzi could resist the challenge of a virulent strain, but that the conferred resistance could be suppressed by exposing the animals to sub-lethal doses of gamma radiation. The animals would then become susceptible to the challenging inoculum and die.

The first experimental plan was designed to determine the threshold of reversible protection. Several litters of newborn mice received tenfold increments of an avirulent strain, while a similar group was allowed to grow without the protecting inoculum. Four weeks later one-half of the mice in each of the groups were exposed to 400 rads (^{60}Co) and immediately challenged with a virulent strain. An inoculum of 10^4 culture-forms of the avirulent strain was found to protect 50% of the mice (PD_{50}), but this protection was completely suppressed by the radiation dose.

In subsequent experiments, a single protection inoculum of 100 PD_{50} was used, the same process of radiation and challenging following. The blood parasite levels of the unprotected groups rose to a maximum of 10^7 per ml within 2 weeks after challenge while in the protected groups the irradiated mice rose to 1/4 of this level in 40 days and then decreased to less than the detectable levels. In contrast, the protected mice whose immunity was not reversed by radiation showed only minimal or no parasitemia throughout the experiment. The 100% death rate of unprotected mice, irradiated and not irradiated, was attained within 2 weeks post-challenge while 72% of the protected -irradiated group died within 45 days. Only 3% (1 of 32) of the protected not irradiated mice died on the 34th day post-challenge.

These findings suggest that the humoral and cellular immunity mechanisms triggered by the protection inoculum are suppressed by the sublethal doses of gamma radiation employed.

DELAYED EFFECT OF RADIATION UPON THE SCHISTOSOMA MANSONI WORM

R. A. Brown, L. S. Ritchie, and J. Cora

There have been a number of attempts, not highly successful, to immunize animals to Schistosoma mansoni with irradiated cercariae. These investigators observed that not only were there less worms which survived to adulthood in animals exposed to irradiated cercariae, but that the survivors were substantially smaller than normal. The dose-response curve for survivors is quite different from that observed for reduction in the length of the worm. The survivor curve has a pronounced shoulder and a large extrapolation number suggesting that the worm may have capacity for the repair of radiation damage. The dose response curve for worm-size is a curve which does not appear to become linear at high doses. The stunting of the worm by irradiation can be partially reversed by irradiating the host suggesting that the stunting may be the result of an immune reaction by the host. The number of survivors are not increased by irradiation of the host.

BACTERIA IN THE "CURATIVE" EFFECT OF HEMOLYMPH
OF BIOMPHALARIA GLABRATA ON SCHISTOSOMA MANSONI-INFECTED MICE

Jorge Chiriboga, Lawrence S. Ritchie, José Oliver-González,
Raymond A. Brown, and Víctor A. López

The "curative" agent for Schistosoma mansoni, occurring in the hemolymph of Biomphalaria glabrata, did not pass through either a dialysis membrane or millipore filter (0.22 μ). Thoroughly washed ameboid cells from the hemolymph were inactive. Among bacteria cultured from hemolymph, Proteus mirabilis decimated mature S. mansoni in mice, but it was not effective when injected 1 week after mice were exposed to cercariae. Moreover, the infectivity of cercariae was unaltered by mixing them with P. mirabilis 1 hour before exposure of the mice. Klebsiella pneumoniae cultured from hemolymph did not affect mature S. mansoni, but a laboratory strain of this bacterium killed some worms. P. mirabilis injected into normal mice did not produce abscesses. Cultures of P. mirabilis derived from worms, taken aseptically from hemolymph-treated mice, produced liver abscesses within 10 days in other mice infected with S. mansoni. Similarly, K. pneumoniae cultured from liver abscesses in hemolymph-treated mice and subinjected, produced abscesses in one of eight mice, and this mouse was cleared of a bonafide infection of S. mansoni. Possible clinical complications for man when enteric bacterial infections occur simultaneously with schistosomiasis may warrant consideration.

THE USE OF SLOW RELEASE ORGANOTINS IN A MULTILEVEL CONTROL OF SCHISTOSOMIASIS

L. S. Ritchie, V. A. López, and J. Cora

In recent years organotins have been incorporated in slow-release rubber matrices and have been tested for their molluscicidal potential. The continuous release of low concentrations of these chemicals in snail - infected water bodies is perhaps the ideal tool for the control of schistosomiasis, since all the extra-mammalian stages of the parasite could be continuously exposed. Bis (tributyl tin) oxide (TBTO) was compared in this respect with other commonly used molluscicides against newly hatched snails suppression of egg laying by the snail, suppression of egg development, and its cercaricidal and miracidicidal effects.

Cercaricide: TBTO in levels of 10^{-8} (10 ppeb) exerted 100% cercaricidal action as measured by infectivity for mice after 30-minute treatment with the chemical, while with 10^{-9} and 10^{-10} , the worm recoveries were reduced 54 and 40% respectively. Niclosamide, Cu SO_4 and others showed considerably lower activities in this respect.

Miracidicide: Results pending

Oviposition: A concentration of TBTO of 10^{-8} abruptly stopped egg laying. Some molluscicidal action was noticed after a 24 hr. exposure to this concentration.

Egg hatching: Preliminary experiments show that eggs at different stages of development failed to hatch in concentration as low as 10^{-10} while development of the embryo was arrested.

Molluscicide: Freshly hatched snails were killed after 48 hours exposure to 10^{-8} TBTO. Very low molluscicidal activity was obtained with 10^{-9} after 6 days (17%).

POLY IC STABILIZED WITH DIFFERENT METALS
STRUCTURAL AND FUNCTIONAL DIFFERENCES

J. Chiriboga and R. Ramos-Aliaga

Previous studies of the effect of polyribonucleotides on interferon production, inhibition of parasite growth, and other biological processes have been made using stranded ribonucleic acids with secondary structures stabilized by either sodium or magnesium salts. We are currently interested in the effect of different metallic ions upon the structure and radiosensitivity of Poly I: Poly C complexes and correlating these data with the biological activity of polymers.

Increasing concentrations of different metallic salts were added to solutions containing equimolar quantities of Poly I and Poly C at a total polymer concentration of 20 μ gm/ml until no further hypochromicity at 248 μ was observed. A concentration of 50 mM with the monovalent ions and of 5 mM with the divalent ions was adequate to produce saturation and all the polynucleotides were assumed to be double stranded at these concentrations. To circumvent problems of toxicity in tissue culture studies, divalent ion solutions were precipitated with ethanol and reconstituted in water to the original polynucleotide concentration before incubation with cells. In the case of solutions reconstituted from those with Mn^{++} ions, atomic absorption values for manganese and a modified Fiske and Subarow method of phosphorus determination indicated that there was one manganese atom for every two phosphorous atoms.

The inhibition of plaque formation by Sindbis virus in L cell monolayers pretreated with different Poly I: Poly C metal complexes was tested in three different experiments. The results of these experiments show that the manganese complex is the most efficient inhibitor.

Two procedures were used to measure the effect of radiation upon the different polynucleotide complexes. In the first method, the hyperchromicity observed upon heating the polynucleotide above its melting temperature (T_m) was compared before and after irradiation. The ratio of these two values was used to calculate the amount of double stranded structure remaining after irradiation. The double stranded Mn^{++} complex was found to be more radio-resistant than the other complexes. The linear dose response curve observed for the Mn^{++} complex suggests a single hit process. In the second method, an estimate of chain scission produced by irradiation was made by measuring the amount of ultraviolet absorbing material (probably oligonucleotides containing more than 30 residues) precipitated by 10% trichloroacetic acid (TCA) after irradiation. The amount of soluble oligonucleotides produced by irradiation also showed the Mn^{++} complex to be the most radioresistant. Surprisingly, the lithium complex was found to be highly radiosensitive.

There is another difference besides radiosensitivity between Mn^{++} and Mg^{++} Poly I: Poly C complexes. After heating above the T_m , the Mg^{++} double stranded complex was completely reconstituted upon cooling, and heating for a second time gave data similar to the first heating. The double stranded Mn^{++} complex was not reconstituted upon cooling. The hysteresis observed with the Mn^{++} complex merits further study.

FASCIOLA HEPATICA-GENERAL STATEMENT

Jorge Chiriboga

The liver fluke of cattle, Fasciola hepatica, is a problem to human and animal populations throughout Latin America.

Two cooperative programs involving PRNC, one with the Commonwealth Department of Agriculture and the other with the UPR Agriculture Experiment Station, were begun to study this problem in Puerto Rico. Investigations included both field and laboratory research.

The results of a survey of 19 dairy herds in the Dorado area showed that the percent infection varied between 5 and 90 on 17 of the farms while 2 farms were free of infection. Of the two snail vectors, Lymnaea cubensis and Lymnaea columella, L. cubensis was found to be the dominant vector. The life cycle may be as short as 3 weeks, and population levels are determined by moisture conditions. However, the snails can survive in the soil under dry conditions for many months.

In the laboratory, all stages of the life cycle are maintained. Laboratory experiments suggest a possible vaccine may be developed from irradiated metacercariae.

AN EPIDEMIOLOGIC OBSERVATION ON FASCIOLIASIS IN PUERTO RICO

L. S. Ritchie, P. Gómez, and A. Velarde

A remarkable number of Lymnaea cubensis snails, the intermediate host of Fasciola hepatica, occurred in the Dorado Area of Puerto Rico. This happened in relation to a near-record rainfall that wet the fields for about 6 months. Any low spot became a small swamp, making it suitable for snail propagation. Snail infections were common and it was apparent that fascioliasis might increase greatly. Since we had no previous data on the snail population, collections were started for comparison with subsequent years. On the other hand, we had examined 19 herds of cattle in 1970, which provided a base line for comparison. Six of the 19 herds were chosen to re-examine in 1971.

On five of these six farms the snails had mostly disappeared between March and May (1971), and from August to December the overall population was decreased about 95%. It is already apparent that the snail population of February-March 1971, will not be repeated in the same months of 1972. The 29 colonies observed on 6 farms are probably sufficient to validate the 20-fold range of difference in the population of L. cubensis between March and December 1971.

For the six herds of cattle examined, all showed considerably higher occurrence of F. hepatica in 1971, as compared with 1970. The least increase was from 33 to 57%, and the greatest from 18 to 75%. The overall increase in occurrence for the 6 herds was from 39 to 85%.

It is apparent from these findings that evaluation of the seriousness of fascioliasis in Puerto Rico cannot be based on one or two years of observation. The loss from condemned livers is readily easy to estimate but the

loss in milk production is most difficult to determine, because of secondary, superimposed factors.

If an attempt to control snails had been made in 1970, no more than one potential colony in five would have been detected. Repopulation of treated areas would probably have occurred through flood waters, or by specimens carried on the feet of cattle.

"ON THE IMMUNOGENIC EFFECT IN RATS OF TRIPLE VACCINATION
WITH 2,500 R GAMMA-IRRADIATED F. HEPATICA METACERCARIAE"

J. Chiriboga, D. de León, F. Liard, A. Velarde, and P. Gómez

Studies on the use of X-irradiated larval vaccines were reviewed by Urquhart and co-workers in 1962. They reported that previous inoculation of X-irradiated infective larvae in a number of host-helminth systems will protect the host against a potentially pathogenic challenge of normal infective larvae.

Thorpe and Brown reported some evidence of acquired resistance in rats given a single dose of X-irradiated metacercariae of F. hepatica at 1 kr. or 2.5 kr. However, Hughes reported no evidence of immunity in mice, rabbits, and sheep following single administration of irradiated F. hepatica metacercariae.

Between August 1971, and January 1972, the Medical Sciences and Radiobiology Division undertook an evaluation of vaccination against F. hepatica in rats with three doses of metacercariae that were exposed to gamma radiation (^{60}Co , 2,500 R).

A total of 30 apparently healthy rats were selected and allotted at random in 3 groups of 10. Group I served as control (not given irradiated metacercariae) and was given orally 30 normal metacercariae each 70 days after the start of the experiment. Group II was given 3 oral doses of 30 gamma-irradiated metacercariae at 2,500 R at 2-week intervals but was not challenged. Group III was given 3 oral doses of 30 gamma-irradiated metacercariae at 2,500 R at 2-week intervals and challenged with 30 normal metacercariae each 70 days after the start of the experiment. On the 159th day, all the rats were sacrificed and examined for worm count, pathology, worm morphology and immunity.

Full protection was obtained as indicated by a 100% reduction in worm burden.

Another trial is in progress and histological studies on the effect of 2,500 R gamma-irradiation on the worm and the rat immunological response are being studied.

Our ultimate intention is to determine if cattle can be protected by vaccination with metacercariae attenuated by radiation.

INSECT STERILITY PROGRAM

David W. Walker

The Insect Sterility Program was begun to determine if the sugarcane borer, Diatraea saccharalis (Fab.) (Lepidoptera: Crambidae) could be eradicated by releasing sterile males in an overflooding program. Early work concentrated on determining the sterilizing dose, the killing dose, and behavior changes associated with dose. This species can be sterilized as newly emerged adults with 25 K_{rads} of ⁶⁰Co gamma radiation. Immature stages cannot be effectively sterilized since the killing dose and sterilizing dose are nearly equal. Biological and behavioral aspects of importance to a sterile release program were also investigated: time of mating, adult, life span, developmental time, artificial diets, and rearing methods. The sterilizing dose does not greatly affect mating competitiveness. However, it was found that irradiation at doses below the dosage necessary to produce complete dominant lethality (as scored in embryo development) could be just as effective as releasing fully sterile individuals: i.e. 16 to 20 K_{rads}. These partially sterile individuals are better mating competitors than normals from the field and they have longer adult life-span than field-caught individuals. Nearly one hundred percent mortality of the F₁ generation occurs before these F₁ individuals mature and reproduce. The same effect as the sterile release occurs but over a longer period of time. The main drawback to this approach is that additional crop damage is sustained by the addition of pest individuals to the crop environment.

The concept of overflooding with partially sterile individuals into a natural population (thereby introducing a detrimental gene load into a natural gene pool) was explored in considerable detail from the laboratory population

basis as well as from the theoretical basis. We have called this Inherited Partial Sterility (IPS). We have found that detrimental genetic manifestations (i.e. gene load) can be carried in a laboratory population that is outbred for as many as 7 successive generations. Although the original concept of IPS for population control does not appear to be feasible by itself for eradication, it can be a useful adjunct to integrated control programs.

Current work with additional lepidoptera Lamprosema indicata (bean leaf folder) and Prodenia ornithogalli, (yellow striped armyworm) and with Nezara viridula (Hemiptera: Pentatomidae, southern green stink bug) are confirming most of our observations with the borer. Preliminary tests with fractionated doses are inconclusive. Combinations of juvenile hormone and radiation are under test. Artificial diet and rearing methods are being tested.

Dr. N. Virkki (AEC/UPR) is studying cytological abnormalities, particularly chromosome abnormalities and meiotic disturbances in IPS borer larvae. No translocations have been observed (at meiotic metaphase) even when there is an IPS effect. Differences in haemolymph protein between IPS and normal lines are being studied by disc gel electrophoresis.

Future work will be directed to evaluating Inherited Partial Sterility in pest-crop management systems and to explaining the mechanism of IPS in holokinetic chromosomes.

STUDIES OF GAMMA RADIOLYSIS OF HETEROCYCLIC MOLECULES

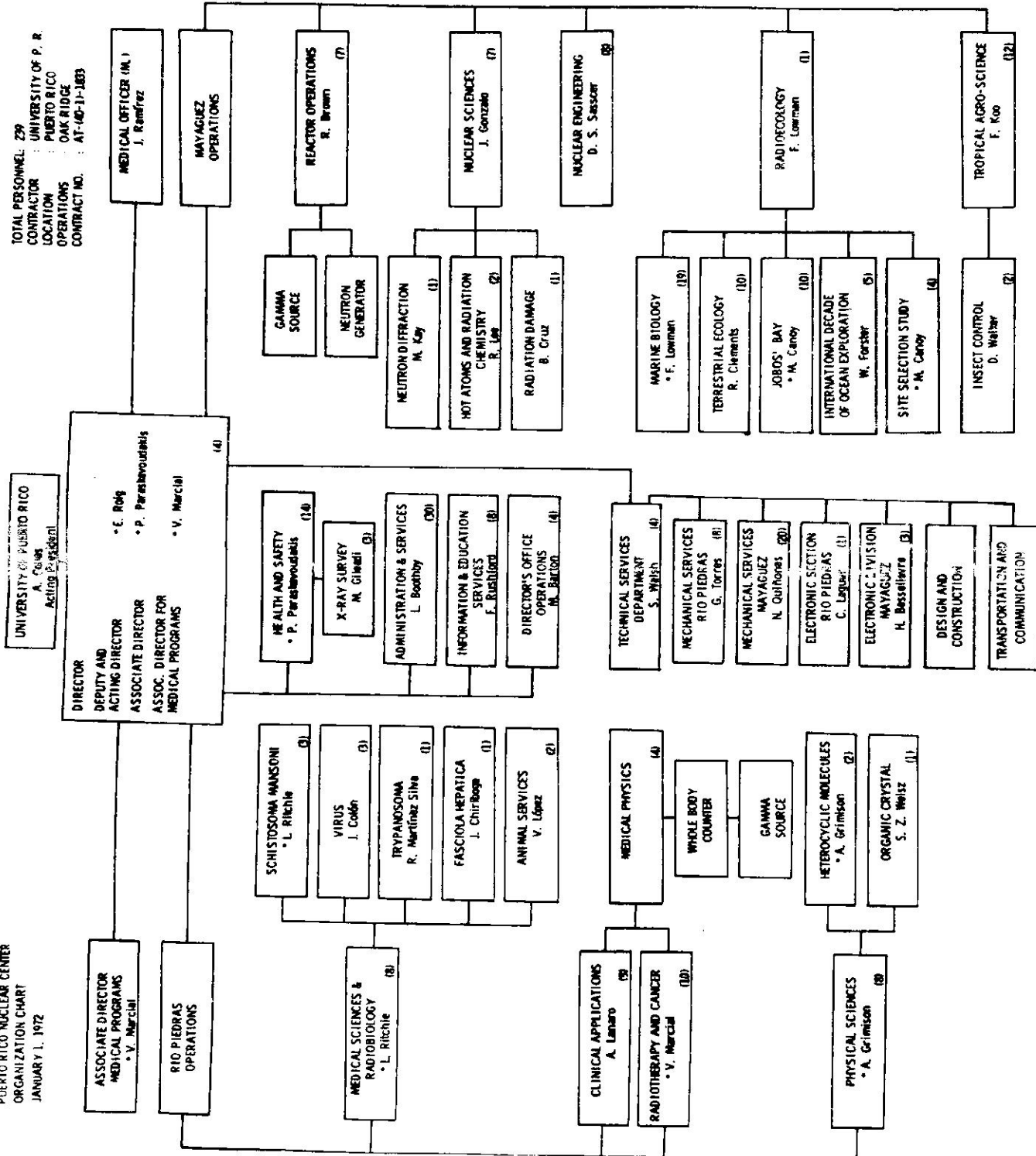
Alec Grimison

The object of these studies is the identification of the species formed by gamma radiolysis of heterocyclic molecules of possible biological importance; therefore, emphasis is on direct observation of the normally labile intermediates formed after the absorption of high energy radiation. This is made possible by utilizing the matrix isolation technique, in which the molecule is irradiated in some form of rigid matrix, normally at low temperature. Under appropriate conditions, radicals and radical ions can thus be stabilized for extended periods and characterized 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 experimentally measured properties to identify unknown intermediates.

Gamma-irradiation of pyridine and the diazines in an organic glass has been shown to produce absorption spectra closely resembling the authentic radical anions of these heterocyclic, prepared by chemical reduction. This, and other supporting evidence, suggests that the attachment of a secondary electron to the heterocyclic substrate can be a significant process. The importance of this data is confirmed by recent studies of pulse-radiolysis of uracil, thymine, and cytosine. These studies yield spectra attributed to the radical anions of the pyrimidines startlingly similar to that attributed here to the radical anion of pyrimidine itself.

Related work concerns the study of thermoluminescence subsequent to gamma-radiolysis or photolysis at 77°K. This luminescence is attributed to

ionization of the heterocyclic molecule to form a trapped radical cation and electron, followed by recombination to give the excited state of the heterocyclic molecule, which emits light. The details of the recombination process are being studied by the methods of flash spectroscopy, as is the photoionization process. Initial attention has been focussed on the indole molecule.



APPROVED FOR AEC
[Signature]
 AREA MANAGER
 PUERTO RICO AREA OFFICE

APPROVED FOR CONTRACTOR
[Signature]
 ACTING DIRECTOR
 PUERTO RICO NUCLEAR CENTER

* Multiple Furlough

AEC BIO-MEDICAL PROGRAM DIRECTORS MEETING
San Juan, Puerto Rico - February 7-8, 1972

ADLER, Howard T.	Oak Ridge National Laboratory
ALBENESIUS, Edward L.	Savannah River Laboratory
ALPEN, Edward L.	Batelle Northwest Laboratory
BAIR, William J.	Batelle Northwest Laboratory
BEASLEY, Thomas M.	Division of Biology and Medicine
BORN, James L.	Lawrence Berkeley Laboratory
BROOKS, Frank T.	Division of Biology and Medicine
CARSON, Stanley F.	Oak Ridge National Laboratory
COWSER, K. E.	Oak Ridge National Laboratory
EDINGTON, Charles W.	Division of Biology and Medicine
EWING, John A.	UT/AEC Agric. Research Laboratory
FRIEDEL, Hymer L.	Case Western Reserve University
GARDINER, Donald M.	Chicago Operations Office
GOLDMAN, Marvin	Los Alamos Scientific Laboratory
GUSTAFSON, Phillip F.	Argonne National Laboratory
HALL, Nathan S.	UT/AEC Agric. Research Laboratory
HARRIS, John W.	University of California
JEE, Webster S.	University of Utah
JONES, Robert K.	Lovelace Foundation
KNISLEY, Ralph M.	Oak Ridge Associated Univer
LANG, Antong	AEC Plant Research Laboratory Michigan State University
LUNT, Owen R.	Nuclear Medicine and Radiation Biology University of California
McCLELLAN, Roger O.	Lovelace Foundation

MILLS, William A.	Environmental Protection Agency
MORGAN, J. P.	AEC/Puerto Rico Area Office
MORGAN, Karl Z.	Union Carbide
NELSON, Daniel J.	Oak Ridge National Laboratory
NEUMANN, William F.	University of Rochester
NIELSEN, Julian M.	Battelle Northwest Laboratory
RICHMOND, Chester R.	Los Alamos Scientific Laboratory
SHORE, Bernard	Lawrence Livermore Laboratory
SHORE, Moris L.	Environmental Protection Agency
SHOUP, Charles	Oak Ridge Operations Office
SINCLAIR, Warren K.	Argonne National Laboratory
SMITHWICK, Grover A.	Savannah River Operations
STEELE, Robert	Brookhaven National Laboratory
TIERNAN, Michael W.	Richland Operations Office
TOTTER, John R.	Division of Biology and Medicine
VAUGHAN, Burton E.	Battelle Pacific Northwest Laboratory
WALKER, Donald	Idaho Operations Office
WARREN, Shields	Cancer Research Institute New England Deaconess Hospital
WEISS, Samuel B.	Argonne Cancer Research Hospital
WEYZEN, Walter W.	Division of Biology and Medicine
WHITNAH, John C.	Division of Biology and Medicine
WILSON, Daniel	Lawrence Livermore Laboratory

N O T E S

