

PUERTO RICO NUCLEAR CRUE, 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) ---Page Break--- AEC BIO-MEDICAL PROGRAM DIRECTORS MEETING San Juan, Puerto Rico February 7-8, 1972 ---Page Break--- Puerto Rico Nuclear Center Bio-Medical Building, Río Piedras MONDAY, FEBRUARY 7 9:00 a.m. Bus leaves hotel for meeting Introductory Remarks Jobos Bay Coffee Break Temperature Studies in Tropical Marine Environment International Decade of Ocean Exploration in Marine Biology 2:00 p.m. Terrestrial Radioecology and International Biological Program 3:00 p.m. Visit PRNC research vessel "PALIMBO" TUESDAY, FEBRUARY 8 9:00 a.m. Bus leaves hotel for meeting 9:00 - 9:15 a.m. Introductory Remarks 9:15 - 9:30 a.m. Effect of Gamma Radiation on Interferon Production in Chick Embryos and Mice Effects of Gamma Radiation on Antibodies and on Activation of Coxsackie Virus in Immune Animals Dr. B. Roig Dr. M. Canoy Dr. S. Kolehmainen Dr. W. Forster Dr. P.G. Lowman Dr. R. Clemente Dr. V. Maretat Dr. J.I. Colén Dr. J.T. Golda ---Page Break--- TUESDAY, FEBRUARY 8 (continued) 9:50 - 10:00 a.m. 10:00 - 10:10 a.m. 10:10 - 10:20 a.m. 10:20 - 10:40 a.m. 10:40 - 10:50 a.m. 10:50 - 11:00 a.m. 11:00 - 11:10 a.m. 11:15 a.m. - 12:15 p.m. AFTERNOON Effect of Gamma Radiation on Mice Immunized Against Delayed Effects of Radiation Upon the Schistosoma mansoni or Bacteria in the "Curative" Effect of Henolymph of Biomphalaria glabrata on Schistosoma mansoni-Infected Mice The Use of Slow Release Techniques in a Multile Schistosomiasis Organism Control of Poly I.C. Stabilized with Different Metals Structural and Functional Differences Coffee Break Fasciola hepatica - General Statement An Epidemiologic Observation on Fascioliasis in Puerto Rico On the Immunogenic Effect in Rats of Triple Vaccination with 2,900 R Gamma-Irradiated F. hepatica Metacercariae Sugarcane Borer Heterocyclic Molecules Dr. John R. Totter's meeting Mr. V. De

Re Dr. Re be. J. be. a. Dr. be Drs A. Ay Lopez AL Brown, AL Brown Ritente chiribega Chiribogs Ritchie = de Led - Walker Grimison ---Page Break--- ENVIRONMENTAL STUDIES FOR THE AGUTRRE POWER COMPLEX Michael J. Canoy Tm April 1, 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 proposed study 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 study 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. = ---Page Break--- MATERIAL 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 sustained under controlled laboratory

conditions will be studied. Some preliminary studies have already been made in Guayantla Bay, and these data will be discussed. ---Page Break--- 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. P. Paluabo during the past six months. Samples of water, sediment, plankton, crustacea, and fish will be analyzed for pesticides, heavy metals, nutrients, and hydrocarbons. ---Page Break--- MARINE WARNING 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 organic cobalt and cobalt incorporated into cobalamin have been done with marine sediments, plants, and animals. Investigations on the distribution patterns of ten elements in seagrass and turtle grass ecosystems have been made. The chemical and 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, Naguabo, and Fajardo Bays have been measured and related to seasonal fluctuations, sources and reservoir, 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 Mayagüez to Peñuelas, 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 Tateo Shipbuilding 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 1968 until his untimely death on July 4, 1964.

RAINFALL, INTERCEPTION IN A TROPICAL FOREST Richard C. 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 events showed that 66% 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 determinations for Ca, Mg, Na, and 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 was dependent upon the intensity, duration, and time between storms and wind direction. A shift in wind direction from

casterly €0 northerly caused a tenfold increase in the concentration of the four elements in rainwater, which in turn affected the concentration of those elements in throughfall and streamflow water. ---Page Break--- 'THE CRITICAL IMPORTANCE OF THE FIRST TENTH OF AN INCH OF RAINFALL' *PRELIMINARY REPORT Richard C. Clements * sequential rainfall collector was developed to fractionate incoming rainfall into samples representing 0.01 inch each. 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 $Y = A/N$; where N is the fraction number and Y is the concentration of the element. STUDIES ON THE DIVERSITY AND ECOLOGY OF RAINFOREST PLANTS AND ANIMALS George F. Drewey A brief description is made of studies in four biological aspects of ecology. These include diversity studies of rainforest plants and animals, and the ecology of snails, frogs, and freshwater 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 of radioisotopes in food webs and mineral cycles, etc. ---Page Break--- U.S. INTERNATIONAL BIOLOGICAL PROGRAM TROPICAL FOREST BIOME Richard G. Clements The participation of the Radioecology Division of FNRC in the U.S. INP program is described. The proposed study deals with a complete watershed that encompasses three distinct forest types, cultivated and non-cultivated 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. ---Page Break--- EFFECT OF GAMMA RADIATION ON INTERFERON PRODUCTION IN CHICK EMBRYOS AND MICE J. Ts Colén, 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) were studied. The interferon was assayed by the plaque inhibition test of Sindbis virus in chick embryo fibroblast and in L cells, depending on the route 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 10^0 plaque-forming units (pfu) of NDV, the interferon titer was 160 units per ml. In chick embryos irradiated with 400 rads and inoculated with 10^0 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 aminophylline, 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. ---Page Break--- EFFECTS OF GAMMA RADIATION ON ANTIBODIES AND ON ACTIVATION OF COXSACKIE VIRUS IN IMMUNE ANIMALS 4.1. Colén, M. Toro-Rodríguez, C. Rivera-Campos, and C. 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 A1. 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, Cruze KR, Mareines-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 (Co) and immediately challenged with a virulent strain. An inoculum of 10^0 culture-forms of the avirulent strain was found to protect 50% of the mice (PDsq), but this protection was completely suppressed by the radiation dose. In subsequent experiments, a single protection inoculum of 100 PDsp 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 non-irradiated mice died on the 34th day post-challenge. <a ---Page Break--- 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. <a ---Page Break---

DELAYED EFFECT OF RADIATION UPON THE SCHISTOSOMA

MANSONI WORK R.A, Brom, L, S, Ritthe, 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 fewer worms that 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 is not increased by irradiation of the host. ---Page Break---

BACTERIA IN THE "CURATIVE" EFFECT OF HEMOLYMPH OF BIOMPHALARIA GLABRATA ON SCHISTOSOMA MANSONI-INFECTED MICE
Jorge Chiriboga, Lawrence S. Ritchie, Jost Oltver-consflez, Raymond A. Brown, and Victor A. Sper
The "curative" agent for *Schistosoma mansoni*, occurring in the hemolymph of *Biomphalaria glabrata*, did not pass through either dialysis membrane or millipore filter (0.22). Thoroughly washed anebotd 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 infection. *Klebsiella pneumoniae* cultured from hemolymph did not affect mature worms. *F. mirabilis* injected into normal mice did not produce abscesses. Cultures of *B. mirabilis* derived from worms, taken aseptically from hemolymph...

lymph-treated mice produced liver abscesses within 10 days in other mice infected with *S. mansoni*. Similarly, *K. pneumoniae* cultured from liver abscesses in lymph-treated mice and subsequently injected produced abscesses in one of eight mice, and this mouse was cleared of a bona fide infection of *S. mansoni*. Possible clinical complications for humans when enteric bacterial infections occur simultaneously with schistosomiasis may warrant consideration. 15 ---Page Break---

'HO. USH: OF SLOW RELEASE ORGANOTINS IN A MULTILEVEL CONTROL OF SCHISTOSOMIASIS L. S. Ritchie, V. A. Uspez, 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-namalian stages of the parasite could be continuously exposed. Bis(tri-butyl 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 at levels of 10^{-6} (10 ppb) exerted 100% cercaricidal action as measured by infectivity for mice after a 30-minute treatment with the chemical, while with 10^{-7} and 10^{-8} , the worm recoveries were reduced by 54% and 40% respectively. Niclosamide, CuSO_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 concentrations as low as 10^{-7} ; the development of the embryo was arrested. Molluscicide: Freshly hatched snails were killed after 48 hours exposure to 10^{-6} TBTO. Very low molluscicidal activity was.

obtained with 10^9 after 6 days (172). =. ---Page Break---

POLY Ic STABILIZED MITR DIFFERENT METALS STRUCTURAL AND FUNCTIONAL DIFFERENCES + Chirtbog# 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 µg/ml until no further hypochromicity at 246 nm was observed. A concentration of 50 µM 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 Mn²⁺ ions, atomic absorption values for manganese and a modified Fiske and Subbarow method of phosphorus determination indicated that there was one manganese atom for every two phosphorus atoms. The inhibition of plaque formation by Sindbis virus in 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. ---Page Break---

Two procedures were used to measure the effect of radiation upon the different polynucleotide complexes. In the first method, the hypochromicity observed upon heating the polynucleotide above its melting temperature was analyzed.

melting temperature (T_n) 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 Yn* complex was found to be more radio-resistant than the other complexes. The linear dose response curve obtained for the Yn"* 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 Nn" complex to be the most radioresistant. Surprisingly, the Lithium complex was found to be highly radiosensitive. There is another difference besides radiosensitivity between Min"? and the Poly 1: Poly C complexes. After heating above the T_m, the Ma"* double stranded complex was completely reconstituted upon cooling, and heating for a second time gave data similar to the first heating. The double stranded wn" complex was not reconstituted upon cooling. The hysteresis observed with the Nn" complex merits further study. 18 ---Page Break---

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 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 any conditions for many months. In the laboratory, all stages of the life cycle are maintained. Laboratory experiments suggest 2 possible vaccines may be developed from irradiated metacercariae. ---Page Break---

AN EXTENDED 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 baseline 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 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 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 65%. 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 -20- ---Page Break--- 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.

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"ON THE IMMUNOGENIC EFFECT IN RATS OF TRIPLE VACCINATION WITH 2,500 R GAMMA-IRRADIATED *F. HEPATICA* METACERCARIAE" J. Chirtboga, D. de Loin, F. Lined, A. Velarde, and P. Gómez. Studies on the use of X-irradiated larval vaccines were reviewed by Usquhart 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. of 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 (eo, 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 rad 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.

SECT STERILITY PROGRAM David W. Walker The insect Sterility Program was begun to determine if the sugarcane borer, *Diatraea saccharalis* (F.) (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 kGy 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 kGy. These partially sterile individuals are better mating competitors than normals from the field and they have longer adult life spans 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 {not @ natural gene pool) was explored in considerable detail from the laboratory population ~ mh
---Page Break--- 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 TPS 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 icata* (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, and 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 hemolymph 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. -3-

---Page Break--- **STUDIES OF GAMMA RADIOLYSIS OF HETEROCYCLIC MOLECULES** Alec Grimston 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 stable intermediates formed after the absorption of high energy radiation. This is made possible by utilizing the matrix

{solvation 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 compounds, 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 the 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 photoinization process. Initial attention has been focused on the indole molecule. =e ---Page Break--- ---Page Break--- AEC BIOMEDICAL PROGRAM DIRECTORS MEETING San Juan, Puerto Rico - February 7-8, 1972 ADLER, Toward T, Oak Ridge National Laboratory ALBENESTUS, Edward L, Savannah River Laboratory ALPEN, Edvard Le Battelle Northwest Laboratory BAIR, William J. Battelle Northwest Laboratory BEASLEY, Thomas M. Division of

BMology and Medicine

BORN, Janes L. Lawrence Berkeley Laboratory

BROOKS, Frank T. Division of Biology and Medicine

CARSON, Stanley F. Oak Ridge National Laboratory

COWSER, X. E. Oak Ridge National Laboratory

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JEE, Webster S. University of Utah

OES, Robert K. Lovelace Foundation

KNISLEY, Ralph M. Oak Ridge Associated Universities

LANG, Antong, ABC Plant Research Laboratory Michigan State University

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MCCLELLAN, Roger O.

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WARREN, Shields
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