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THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

Progress Report
for the Period December 1, 1993 - November 30, 1994

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Introduction

The Radiological Research Accelerator Facility (RARAF) is based on a 4-MV Van de Graaff accelerator, which is used to generate a variety of well-characterized radiation beams for research in radiobiology, radiological physics, and radiation chemistry. It is part of the Center for Radiological Research (CRR) - formerly the Radiological Research Laboratory (RRL) - of Columbia University, and its operation is supported as a National Facility by the U. S. Department of Energy (DOE). As such, RARAF is available to all potential users on an equal basis and scientists outside the CRR are encouraged to submit proposals for experiments at RARAF. The operation of the Van de Graaff is supported by the DOE, but the research projects themselves must be supported separately.

RARAF was conceived in the mid-1960s by Drs. Victor P. Bond of Brookhaven National Laboratory (BNL) and Harald H. Rossi of Columbia University as a research resource dedicated to radiobiology and radiological physics and was officially established on January 1, 1967. RARAF's Van de Graaff accelerator originally served as the injector for the Cosmotron, a 2-GeV accelerator operated at BNL in the 1950s and early 1960s. The immediate aim was to provide a source of monoenergetic neutrons for studies in radiation biology, dosimetry, and microdosimetry. In other major projects the energetic ions produced were utilized directly. RARAF was located at BNL from 1967 until 1980, when it was dismantled and moved to the Nevis Laboratories of Columbia University, where it was then reassembled and returned to operation.

Data obtained from experiments using RARAF have been of pragmatic value to radiation protection and neutron therapy. At a more fundamental level, the research at RARAF has provided insight into the biological action of radiation and especially its relation to the distribution of energy deposition in the cell. High-LET radiations are an agent of special importance because measurable cellular effects can be caused by single particles, eliminating some of the complexities of multi-event action and more clearly disclosing basic features. This applies particularly to radiation carcinogenesis.

Facilities are available at RARAF providing different radiations having a wide range of linear energy transfers (LETs). In the track-segment facility, monolayer cultures of cells or other thin samples can be exposed to charged-particle tracks with well-defined LETs from 10 to 200 keV/ μm . Cells can be irradiated with spatially correlated pairs or triplets of ions using the molecular-ion facility and cells will be able to be irradiated individually with a precise number of particles using the single-particle facility, nearing completion. There is also a charged-particle beam line for radiological physics or chemistry experiments. The charged-particle beams from the accelerator can be used to produce essentially monoenergetic neutrons in the range 220 keV to 15 MeV and broad-spectrum neutrons with a mean energy as low as 40 keV. Low-LET (photon) radiations are provided by a 250-kVp X-ray machine and a 50-kVp X-ray machine attached to the track-segment fixture. A facility is now available to provide soft X rays (0.3 to 3 keV) using proton-induced X-ray emission (PIXE).

Some of the support facilities available at RARAF include: cell culture laboratories, a minicomputer and a personal computer (PC) for controlling experiments and recording data, a CAMAC data acquisition system, a bio-preparation/animal-holding room, limited electronics for use by experimenters, and office space for users.

Members of the RARAF staff provide a variety of services in addition to operating, maintaining, and developing the accelerator. They perform most irradiations and are responsible for dosimetry and for developing specialized dosimetry devices and methods when needed. They also provide computer support, help design experiments and irradiation fixtures, and do collaborative research with some users. The CRR machine shop is available for the fabrication of necessary dosimetry and irradiation fixtures. Costs for special services and machine shop time are recovered from the user. All principal investigators are encouraged to request funds in their grant applications to cover the cost of accelerator use unless their funding source is the Office of Health and Environmental Research of DOE.

In addition to the RARAF Staff, other CRR scientists are engaged in collaborative research with outside users of RARAF. Some of the most interesting and complex experiments

done by outside users have been made possible only by active and significant in-house collaboration far beyond the operation of the accelerator and provision of dosimetry.

The operation of RARAF through 1982 was described in RRL Annual Progress Reports. From 1983 to 1987 RARAF operated under a separate DOE Contract or Grant (first DE-ACO2-83ER60110, then DE-FG02-86ER60539), and operation of the Facility was described in separate RARAF Progress Reports (1-5). Since 1988 the operation of RARAF has been supported by part of a single grant to the CRR (DE-FG02-88ER60631), but since RARAF is a separate component, the reports are submitted separately (6-11).

RARAF User's Guide

To increase the utilization of RARAF by scientists outside CRR, a RARAF User's Guide was prepared in 1985 for distribution to potential users. The Guide contains detailed information about the Facility's research capabilities and how to plan an experiment at RARAF and includes an Experiment Request Form for submitting proposals for new experiments. The User's Guide was reproduced as an appendix to the RARAF Progress Report for 1985 (3). A copy of the Guide may also be obtained by contacting the Manager of RARAF.

Since the sections of the User's Guide covering the RARAF staff and the Scientific Advisory Committee are currently out of date and new capabilities and facilities have now become or will soon be available, it is expected that a new User's Guide will be prepared after the basic development of these new facilities is completed.

Research Using RARAF

Table 1 lists the experiments performed at RARAF during the period May 1, 1993 through April 30, 1994 and the number of days each was run in this period. The numerical order of experiments is based on the date of submission of the Experiment Request Forms. Fractional days were assigned to experiments when two or more were run on the same day or

Table 1. Experiments Run at RARAF, May 1, 1993 - April 30, 1994

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
37	D. J. Brenner	CRR	Phys/Bio	Towards the new Q	3.0
41	R. C. Miller	CRR	Bio	Oncogenic transformation induction by high-LET radiation	2.0
43	T. K. Hei	CRR	Bio	Cellular and molecular studies on the mutagenesis of charged particles using human-hamster hybrid [A _L] cells	4.8
52	R. C. Miller	CRR	Bio	Short-range radiation induced oncogenic transformation	2.0
53	J. C. Willey	University of Rochester	Bio	Evaluation of the cytotoxic and oncogenic transforming effects of simulated radon daughter products using human bronchial epithelial cells	0.5
54	L.G. Littlefield	ORISE (Oak Ridge)	Bio	Cytogenic indices - direct vs. indirect action	2.0
56	R. C. Miller/ J. Cao	CRR	Bio	Transformation of synchronized C ₃ H 10T½ cells by neutrons and charged particles of defined LETs	6.0
58	B. Loucas	CRR	Bio	Breakage and rejoining of interphase chromatin in non-cycling human fibroblasts as a function of LET	3.5

Table 1. (Continued)

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
59	D. Grunberger	Columbia University	Bio	Induction by radon daughter alpha particles of mutations in the dihydrofolate reductase gene of Chinese hamster ovary cells in culture	4.0
64	S.G. Martin	CRR	Bio	Human epithelial cell transformation	6.3
66	G.B. Spector	FIBERTEK, Inc.	Phys	Scintillator fiber optic long counter response to ~14-MeV neutrons	1.0
67	M. Salasky	Landauer, Inc.	Phys	Characterization of MR3 plastic and polycarbonate response to neutrons	7.0
68	C-Z. Chen	CRR	Bio	Measurement by comet assay of DNA strand breaks in human cells induced by high-LET alpha particles	7.8
---	Y. Zhao	CRR	Bio	Shuttle vector system for identification of alpha-particle-induced mutations in fission yeast	1.0
---	B. Loucas	CRR	Bio	Single-particle facility irradiations	8.8

TOTAL 59.7

when an experiment ran more than 10 hours on a single day. Fifteen different experiments were run during this 12-month period. This is about the same as the average from 1986-93.

Ten experiments were proposed by members of the CRR, supported by grants from NIH and DOE DE-FG02-88ER60631, and five were proposed by outside users, supported by private industry and various grants and awards from DOE and the National Institutes of Health (NIH). In addition to research conducted by another group from Columbia University, experiments were performed by researchers from one other university, a national laboratory, and two private corporations. Approximately one quarter of the experimental time was used by outside researchers, a 50% increase over last year but still below the average from 1986-93 of 40% of the experimental time.

The problem of radon exposure has continued to be a topic of considerable interest. More than one third (6) of the experiments run at RARAF this year used ^3He and ^4He ions to investigate the effects of radon-progeny alpha particles (Exp. 41, 53, 59, 68, shuttle vector, single-particle facility). Another four experiments (Exp. 37, 43, 58, 64) were not specifically looking at radon effects but included a range of He-ion LETs which would provide sufficient data for that purpose. Among these were measurements of both microdosimetric and nanodosimetric spectra for He ions with various LETs. Thus two-thirds of the experiments and the experimental use this past year were at least partially concerned with the effects of radon progeny.

A list of recent publications at least partially resulting from experiments performed at RARAF is given near the end of this report. Of the eleven papers listed, four were published by outside users of RARAF and two involve experiments performed before RARAF moved from BNL!

A brief description of the experiments that have been run at RARAF since May 1, 1993 is given below.

The final microdosimetry measurements were completed this year for a project proposed by David Brenner in collaboration with other CRR scientists to obtain some of the basic

data necessary for the estimation of the quality factor, Q , as a function of the microdosimetric quantity lineal energy, y (Exp. 37). Measurements of the yield of chromosome aberrations, mutations, and neoplastic transformation in mammalian cells exposed to a series of radiations (mostly charged-particle track segments) have been made over the past 5 years spanning the entire range of lineal energies of interest in radiation protection. About half of the radiations were obtained at RARAF (10 to 200 keV/ μ m) and those lineal energies not available at RARAF were obtained at the Tandem Van de Graaff Accelerator at BNL. Paul Kliauga has measured the microdosimetric spectra for each radiation using a cylindrical proportional counter consisting only of a center wire and a 1/8-inch diameter helix in a 9-inch diameter brass and aluminum housing. Spectra for 0.5- and 1- μ m diameter simulated site sizes were measured at RARAF this year for several ^4He -ion LETs. Biological results are being unfolded using the measured microdosimetric spectra to give a response function, $q(y)$, which can be used to predict the effect of any radiation whose microdosimetric spectrum is known. The quality of the predictions will be tested using the results of irradiations with 14-MeV neutrons which produce secondary particles having a wide range of LETs.

Richard Miller of the CRR performed three different investigations this year, all involving oncogenic neoplastic transformation of C3H 10T $\frac{1}{2}$ cells. A study using ^4He ions to simulate radon progeny (Exp. 41) was completed this year. Irradiations with particles having LETs of 90 and 150 keV/ μ m were made to confirm earlier results. ^4He ions were more carcinogenic at 90 keV/ μ m than at other LETs even though the RBEs for cell inactivation were similar. An experiment investigating the effects of short-range radiations (Exp. 52) using low-energy neutrons continued. Cells were irradiated with 0.35-MeV neutrons either attached to the surface of a standard T-25 cell flask or as a centrifuged pellet at the bottom of a thin plastic tube in order to determine if there is a difference in cell survival or mutation between the two methods. No significant differences were observed. In the future, cells will be irradiated in the plastic tubes with neutron spectra having maximum energies of 100 keV or less. An experiment to determine cell transformation as a function of cell cycle (Exp. 56) was also continued. Cells synchronized by

mitotic shake-off were irradiated with 0.6 Gy of 6-MeV neutrons at various times after synchronization. Little variation in cell survival was observed with position in the cell cycle. Results indicate that the radiosensitive period for transformation of cells by 6-MeV neutrons is primarily the G_1 phase with a secondary peak in G_2 as opposed to x rays, for which transformation peaks only in G_2 . Jianmin Cao of the CRR is continuing this investigation using charged particles. Initially, 40-keV/ μm deuterons are being used because the y -value is the same as the mean y for 6-MeV neutrons. Eventually the experiment will be repeated with alpha-particles at one or more LETs.

Li Zhu and Tom Hei of the CRR, in collaboration with Charles Waldren of Colorado State University, are examining the response of the human-hamster hybrid (A_L) cells containing a single copy of human chromosome 11 to a combination of cigarette smoke condensate (CSC) and irradiation with alpha particles (Exp. 43). Cells are treated with CSC before irradiation and then scored for mutation at both the HGPRT and S1 loci. Southern blot analysis of both types of mutants is being performed to determine if there is a difference in the type of mutations caused by alpha particles and those caused by CSC. The cytotoxic and mutagenic effects of the combined treatment seem to be simply additive of the two agents given separately.

A collaboration between James Willey of the University of Rochester and Chang Qing Piao and Tom Hei of the CRR is investigating the oncogenic transformation of an immortalized human bronchial epithelial cell line using single doses of 150-keV/ μm ^4He ions (Exp. 53). Cells cloned from tumors resulting from implantation of cells from earlier experiments are being examined for overexpression of p53 and the cyclin D1 gene.

The examination of chromosome aberrations in human lymphocytes irradiated with monoenergetic neutrons (Exp. 54) by Gayle Littlefield of Oak Ridge Institute for Science and Education (ORISE, formerly part of Oak Ridge Associated Universities) continued this year. Cells were irradiated using 0.34-MeV monoenergetic neutrons with and without the addition of the OH radical scavengers DMSO and WR-1065. Changes in cell cycle delay with neutron energy are being observed as well as how cell cycle kinetics are ameliorated under conditions of

maximum OH-radical scavenging. Micronuclei production as a function of dose is also being determined. Preliminary results for lymphocytes irradiated without scavengers indicate an RBE of about 2.5 for chromosome aberrations and 1.4 for dicentrics, essentially the same as were obtained previously for 0.44-MeV neutrons. Protection by the radical scavengers was approximately 15% for DMSO and 25% for WR-1065, also nearly identical to the results for 0.44-MeV neutrons.

Bradford Loucas of the CRR continued a study of chromosome breakage and rejoining kinetics as a function of alpha-particle LET (Exp. 58). Using the technique of premature chromosome condensation (PCC), chromosome damage can be observed without requiring cells to be in mitosis and avoiding a long time delay between the radiation damage and the observation, during which repair may occur. Confluent cultures of AG1522 human fibroblasts in G_0 phase are placed in ice within 5 minutes of irradiation to observe "initial" damage or incubated for various times after irradiation to observe repair. Initial breaks as a function of dose increase with LET, peaking at about 90-120 keV/ μ m. As a function of fluence, initial breaks increase with increasing LET, possibly peaking around 180 to 200 keV/ μ m. Studies of chromosome rejoining kinetics and initial breaks have been completed and PCCs and chromosome aberrations 24 hours after irradiation will be investigated this year.

An experiment to examine the mutational effects of radon progeny alpha particles (Exp. 59), proposed by Dezider Grunberger of the Institute of Cancer Research of Columbia University, continued. Adelaide Carothers of the Institute has irradiated Chinese hamster ovary (CHO) cells hemizygous for the dihydrofolate reductase (*DHFR*) gene with 150-keV/ μ m ^4He ions using the track-segment facility. DNA is isolated and hydrolysed by enzyme digestion and damaged nucleosides are being separated by Christina Frankel at NYU using HPLC. They have observed what appears to be a novel damage product not seen with chemicals and as yet unidentified. This compound is detectable by both ultra-violet and electro-chemical means, the quantity is proportional to dose, and it is detectable at 10 mGy. The product is also observed after x-irradiation but at twice the dose only half the yield is produced as for ^4He ions. Experiments this

past year have been aimed at obtaining a sufficient amount of DNA to permit identification of the damage product after separation by HPLC.

Stewart Martin of the CRR continued studies of the human epidermal keratinocyte (RHEK-1) cell system developed by J. Rhim (Exp. 64) in which irradiated cells are injected into nude mice as the assay for tumorigenicity. Irradiations were performed with 0.4-MeV neutrons and alpha particles with several different LETs. RHEK-1 cells formed tumors at the injection sites in nude mice with a latency period of about 4 weeks compared to the 6 months required for human uroepithelial (HUC) cells (described last year), making this system considerably more efficient both in time and expense due to the significant cost of maintaining nude mice during the latency period. The rate of tumor formation was considerably higher for the RHEK cells than for the HUC cells, however there were also spontaneous tumors which had a longer latency time and were less aggressive than those induced by radiation. Explanted cell lines from tumors obtained as a result of single-dose irradiation are being examined for radiation sensitivity, chromosomal alterations, and oncogene activation/suppressor gene loss.

Gary Spector of FIBERTEK, Inc. investigated the response of a fiber-optic neutron long counter to ~14-MeV neutrons (Exp. 66). Unlike a standard long counter which uses a BF_3 tube to absorb and count neutrons thermalized by the surrounding moderator material, this design uses ^6Li -loaded, Tb^{3+} -doped glass fibers. Photomultiplier tubes at each end of the fibers measure the amplitude of the light emitted in the fibers and pulse height rejection is used to discriminate against gamma rays. The light is attenuated as it travels through the fibers so that the two photomultiplier tubes produce pulses of different heights. The difference, $A-B$, of the pulse heights divided by the sum, $A+B$, is inversely proportional to the distance from the end of the counter near the source at which the neutrons thermalized. This allows a low-resolution energy spectrum of the neutrons to be measured since the depth in the counter that a neutron thermalizes is related to its energy.

A characterization of track-etchant plastics to monoenergetic neutrons (Exp. 67) was performed by Mark Salasky of Landauer, Inc. Samples of a new formulation (MR3) of allyl

diglycol carbonate polymer as well as samples of polycarbonate were irradiated with neutrons at six different energies from 0.2 to 14 MeV. Doses delivered in all cases were only 50 mrad to provide approximately 500 mrem. The MR3 plastic will be used in Landauer's personnel dosimeters for neutron monitoring, including those used here at RARAF.

Chung-Zhang Chen of the CRR has begun a study of DNA strand breaks caused by alpha particles in AG1522 human fibroblasts using a relatively new technique called "comet assay" (Exp. 68). Cells in plateau phase were irradiated on Mylar dishes, transferred to slides, immersed in an alkaline buffer (pH 12), then subjected to electrophoresis and stained with a fluorescent dye. A fluorescence microscope was used to examine the nuclear DNA of individual cells visualized as a "comet". Using the ratio of width to length of the comet as the end point, linear dose-response curves were obtained. The slopes of the curves decrease with increasing LET of the alpha particles. When compared with X rays, RBE values are less than 1 for all three alpha-particle LETs used. This study will continue with the determination of DNA double-strand breaks using neutral buffer and may also include irradiations with particles having lower LETs.

A pilot experiment was performed by Yuqi Zhao of CRR to investigate mutagenesis and the repair of DNA damage in fission yeast. A shuttle vector (pCRR1) was irradiated by alpha particles in a 10- μ m thick layer of solution using the track-segment facility. The mutation target on the vector is an Escherichia coli supF suppressor tRNA gene. After irradiation, the vector is inducted into the yeast Schizosaccharomyces pombe for DNA damage processing and mutation induction. The repaired DNA is then recovered and transformed into an E. coli KS40/pKY241 indicator system. This permits the selection of plasmids containing an altered supF by conferring nalidixic acid resistance on the bacteria.

Brad Loucas of the CRR has begun the initial studies using the RARAF single-particle irradiation facility which is still under development (see Development of Facilities). AG1522 human fibroblasts and C3H 10T $\frac{1}{2}$ and HeLa cells are being irradiated only through the cell nucleus by a predetermined number of alpha particles and will be scored for survival and

micronucleus formation. After the system is further refined, chromosome aberrations will be examined.

Accelerator Utilization and Operation

Accelerator usage is summarized in Table 2 and Figure 1 for the current report period as well as the seven most recent periods. The accelerator was used 39% of the normal schedule time (8 hours per day, 5 days per week, excluding holidays) this year, slightly more than last year and almost 60% of the average use from 1986 to 1993.

Maintenance and repairs were kept just below the 1986-93 average, in part due to the reduced accelerator use. Sixty percent of the repair time occurred in March, 1994 due to the vacuum failure of an acceleration tube section at the terminal end of the Van de Graaff. The acceleration tube consists of specially formed stainless electrodes alternating with ceramic insulators. These components held together in sections only using glue; there are no fasteners. It was the glue joint closest to the terminal that developed the vacuum leak which we were unable to seal in place and necessitated replacement of the section. Unfortunately, this is a major operation since the entire acceleration tube must be removed very carefully since it is extremely fragile. This type of failure is a rare event and is believed to have been caused in part by the radiation generated in that area. This is the first tube section we have had to replace since the accelerator began operation at Nevis Laboratories.

Accelerator use for radiobiology and associated dosimetry was essentially the same as last year and about 60% of the average since 1986. No new experiments requiring large amounts of beam time have been proposed. Typically the large experiments have involved neutron irradiations which require considerable time due to the relatively low dose rates and the difficulties in performing dosimetry. The amount of radiobiology should increase

Table 2. Accelerator Use (percent of normal schedule time¹)

	July 1986- April 1987	May 1987- April 1988	May 1988- April 1989	May 1989- April 1990	May 1990- April 1991	May 1991- April 1992	May 1992- April 1993	May 1993- April 1994
Radiobiology and associated dosimetry	34.4	40.8	34.3	31.5	43.8	36.2	20.2	19.6
Radiological physics and chemistry	26.7	27.0	35.1	2.4	29.9	12.5	2.8	4.4
Facility development and testing	1.4	0	0.4	3.2	0	0.8	3.2	4.4
Safety system tests	2.4	2.4	2.4	2.4	2.4	2.8	2.4	2.4
Accelerator-related repairs and maintenance	4.8	10.7	7.9	8.0	7.3	18.2	7.2	8.1
Total used²	69.7	80.9	80.1	47.5	83.4	70.5	35.8	38.9
Unused³	31.5	9.7	27.1	54.0	22.6	36.8	64.7	62.3
Unavailable due to 1/18/88 fire	---	12.3	2.2	---	---	---	---	---
Total days	208	248	247	250	248	253	249	248

¹ 8 hours per day, 5 days per week, excluding holidays.

² The sum of the used and unused time may add to more than 100 percent due to experiments which run significantly longer than the normal eight-hour scheduled time per day or are run on weekends.

³ Includes user cancellations, meetings, and building repairs.

Fig. 1 RARAF Accelerator Use

markedly this coming year as the single-particle facility becomes operational and new neutron targets become available.

Utilization of the accelerator by radiological physics and chemistry was 50% higher this year than last but only 20% of the average use from 1986-93. Use for radiological physics should increase somewhat this year since construction of a new ultra-miniature nanodosimetry counter for Paul Kliauga should be completed soon. Experiments will then begin to check the functionality of the counter and examine its gain characteristics.

Development of Facilities

As in the previous several years, there has been considerable effort spent on the development of new capabilities. This level of effort should gradually decrease this year as the single-particle facility nears completion.

The attempt to produce a usable beam of boron x rays (183 eV) using protons incident on a solid target has been temporarily abandoned. Since boron is not available except as powder or granules, a target was constructed consisting of an aluminum disc with a circular recess into which boron powder was compressed. Even though high pressure was used, the boron did not fuse into a single piece of material nor did it make very good contact with the aluminum. Boron is also a very poor conductor of heat. Consequently when a beam of protons was incident on the boron, it was heated as individual grains which quickly reached very high temperatures. The boron began to fuse together, however significant amounts of it were evaporated from the target. As the beam current was increased, the boron was eventually removed completely. A thin layer of boron plated onto an aluminum disc might withstand the high beam power, however it was determined that this would be both difficult and quite expensive to obtain. Development was postponed in order to concentrate on the single-particle facility.

Development of a facility to irradiate cells with low energy neutrons, described in last year's Annual Report, has also been postponed. The effort projected to bring the design to completion would have greatly interfered with the development and construction of the single-

particle facility (see below) for which a greater priority was given. Instead of attempting to get a sufficient dose rate of low-energy "monoenergetic" neutrons with a large spread in energies, low-energy spectra will be obtained in the same manner as has been done at RARAF in the past. A tritium target will be bombarded with protons having an energy just above the back threshold of the $T(p,n)^3\text{He}$ reaction (1.150 MeV). By using a triatomic proton beam, an effective beam current of 360-450 μA can be obtained. At reaction angles greater than 90° , neutrons are emitted with energies from 0 keV to a maximum which is dependent on the angle and the incident proton energy. In the past, spectra with maximum energies of 60 and 110 keV have been produced having dose-weighted mean energies of about 40 and 75 keV respectively. The dose rates are quite low (1-2 rad/hr at 10 cm) so irradiations are performed using cells spun down in small tubes 3 cm or less from the target. Spectra with lower maximum neutron energies are possible, but dose rates would decline below even minimally acceptable levels. This procedure has become feasible now that a source of tritium targets is available.

There has been considerable interest in recent years in radon exposure and the radiobiology and molecular biology of carcinogenesis and mutations caused by radon-progeny alpha particles. The CRR received a grant from NIH in 1990 to improve the radon-simulation facilities at RARAF. Construction of a single-particle facility to irradiate the nuclei of individual cells with a precise number of charged particles, a part of this project, is nearing completion.

All the major components of the single-particle facility are now operational. The final collimator consists of two .0005" thick stainless steel apertures, 6 μm and 5 μm in diameter, separated by a 300- μm thick spacer. The holes are drilled by a laser after the parts have been assembled. The final (6- μm) hole in the collimator is covered with 4- μm thick polystyrene as a vacuum seal.

Cells dishes have been constructed by gluing 2.5- μm thick pieces of mica over holes made in the bottoms of standard 60-mm diameter Petri dishes because Mylar, which is used for track-segment dishes, has too much fluorescent background. The holes are only 0.1" x 0.1", the view size on a 14" television monitor when using a 4x microscope objective. Cells are plated in

this area and the nuclei are stained with Hoescht's 33342 fluorescent dye which emits visible light when excited using ultraviolet light. The positions of the all cell nuclei are determined in a single microscope field using optical recognition software and the cell medium is then removed.

Irradiations are performed using a 40x power microscope objective, which allows accurate positioning of the nuclei over the exit aperture. A gas proportional counter with unity gain attached to the front of the high-power objective is used to count the particles passing through the cells. There is no requirement to change the position of the microscope turret or to darken the viewing field during irradiation. The computer performs the initial positioning of the cell nucleus and the final positioning is done manually using a joystick. Currently a mechanical beam stop requiring about 40 msec for insertion is used to control the particle beam and therefore the beam is limited to about 1 particle per second to reduce the probability of a particle during the closing of the shutter. Eventually this will be replaced by an electrostatic system requiring only about 10 μ s to deflect the beam thereby permitting higher fluence rates and shorter irradiation times. When the button on the joystick is depressed, the beam stop is removed, reinserted when the correct number of particles have been detected, and the microscope stage is positioned for the next cell nucleus. This process now requires about 10 seconds per cell but the time will be reduced as the system becomes better automated and the particle rate is increased.

Tritium targets have finally been ordered from the DOE facility in Pinellas, Florida. Unfortunately the paperwork required to get a cost estimate took 6 months. New targets should be received before the end of June.

Personnel

The Principal Investigator of the RARAF Grant is Eric J. Hall. The Van de Graaf accelerator is operated by Mr. Stephen Marino and Dr. Gerhard Randers-Pehrson, assisted by an accelerator technician, Mr. Jesus Perez. Physicists and biologists from the Center for Radiological Research spend various amounts of time at RARAF in order to perform experiments. Dr. Charles Geard spends part of every working day at RARAF.

Several people not supported by the RARAF Grant also work at the Facility. Dr. Jianmin Cao, a new arrival, works here full-time doing some of the experiments originated by Dr. Richard Miller, who continues at RARAF about three days per week. In addition there is a full-time biology technician, Ms. Marcia Richards, and two part-time biology technicians, Mr. Marcus Geard and Ms. Noreen Duddy. Dr. Bradford Loucas is spending considerably more time at RARAF since the single-particle facility is nearing completion. Dr. Stewart Martin is now spending most of his time at the CRR, but spends one day here each week.

RECENT PUBLICATIONS OF WORK PERFORMED AT RARAF

- Geard, C.R., Miller, R.C., Brenner, D.J. and Hall, E.J. Oncogenic transformation through the cell cycle and the LET dependent inverse dose rate effect. *Radiat. Protect. and Dosim.* [in press].
- Hei, T.K., Krauss, R., Hall, E.J., and Weinstein, I.B. Protein kinase C level and oncogenic transforming potential. *Carcinogenesis* 15(2): 369-374, 1994.
- Jones, G.D.D., Milligan, J.R., Ward, J.F., Calabro-Jones, P.M. and Aguilera, J.A. Yield of strand breaks as a function of scavenger concentration and LET for SV40 irradiated with ^4He ions. *Radiat. Res.* 136: 190-196, 1993.
- Loucas, B.D. and Geard, C.R. Initial damage in human interphase chromosomes from α -particles with LETs relevant to radon exposure. *Radiat. Res.* [in press].
- Loucas, B.D. and Geard, C.R. Kinetics of chromosome rejoining in normal human fibroblasts after exposure to low- and high-LET radiations. *Radiat. Res.* [in press].
- Miller, R.C., Geard, C.R., Geard, M.J., and Hall, E.J. Cell-cycle-dependent radiation-induced oncogenic transformation in C3H 10T $\frac{1}{2}$ cells. *Radiat. Res.* 130: 129-133, 1992.
- Miller, R.C., Geard, C.R., Martin, S.G., Marino, S.A. and Hall, E.J. Neutron-induced cell cycle dependent oncogenic transformation of C3H 10T $\frac{1}{2}$ cells. *Radiat. Res.* [submitted].
- Miller, R.C. and Hall, E.J. Cell-cycle dependent radiation-induced transformation of C3H 10T $\frac{1}{2}$ cells In: Low Dose Irradiation and Biological Defense Mechanisms, T. Sugahara, L.A. Sagan and T. Aoyama, eds., Elseviers Science Pubs. pp. 347-350, 1992.
- Solomon, H.M., Beckman, D.A., Buck, S.J., Gorson, R.O., Mills, R.E., and Brent, R.L. Comparative effects of neutron irradiation and X irradiation on the embryonic development of the rat. *Radiat. Res.* 137: 226-230, 1994.
- Spector, G.B., McCollum, T., and Spowart, A.R. Scintillator fiber optic long counter response to neutrons from 0.5 - 15.1 MeV. *Nucl. Inst. Meth. in Phys. Res. A* [in press].
- Srdoc, D. and Marino, S.A. Microdosimetry of monoenergetic neutrons. *Radiat. Res.* [submitted].

LITERATURE CITED

1. Rossi, H.H. and Hall, E.J. Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60110-1 (1984).
2. Rossi, H.H. Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60110-2 (1985).
3. Rossi, H.H., Goldhagen, P., and Hall, E.J. Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60110-3 (1986).
4. Hall, E. J. and Goldhagen, P. Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60439-1 (1986).
5. Hall, E.J. and Goldhagen, P. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60439-2 (1987).
6. Hall, E.J. and Goldhagen, P. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-T1 (1988).
7. Hall, E.J., Goldhagen, P., and Marino, S.A. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-T4 (1989).
8. Hall, E.J., and Marino, S.A. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-6 (1990).
9. Hall, E.J., and Marino, S.A. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-8 (1991).
10. Hall, E.J., and Marino, S.A. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-9 (1992).
11. Hall, E.J., and Marino, S.A. The Radiological Research Accelerator Facility. USDOE Progress Report DOE/ER/60631-12 (1993).

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