THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

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An NIH-Supported Resource Center
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Research Using RARAF

We have reached something of a milestone this year– experiment number 100! The last experiment listed in this report is the 100 th experiment proposal since RARAF moved from Brookhaven National Laboratory to Nevis Laboratories.

There has been considerable interest this year in the "bystander" effect in which only some cells are irradiated and there is a response greater than would be expected for the fraction of cells irradiated. In some experiments, the unirradiated cells can be identified due to a different staining and scored directly. Several experiments with a variety of endpoints have been undertaken to determine the size of the effect and whether the observed effects are due to direct cell-to-cell communication through their membranes or indirect, longer-range communication through some release into the cell medium. Both the microbeam and the track-segment facilities have been utilized in various investigations.

Table 1 lists the experiments performed at RARAF during the period May 1, 1999 through April 30, 2000 and the number of days each was run in this period. Seventeen different experiments were run during this 12-month period, about 20% more than the last two years, but about average for 1992-99. Five experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH) and the Department of Energy (DOE), and twelve by outside users, supported by various grants and awards from NIH and NASA. Brief descriptions of these experiments are given here:

Jamie Milligan of the University of California at San Diego continued his experiment to determine the mean number of damages per cluster in DNA caused by high-LET radiation (Exp.61). Bare SV40 virus DNA is irradiated with ^{4}He ions using the track-segment facility. Samples are treated with graded doses of radical scavengers to observe changes in the cluster sizes of damaged DNA. Large numbers of samples are required because of the number of radiation dose/scavenger concentration combinations and to have macroscopic amounts of single- and double-strand breaks. Doses as high as 1600 Gy were given to individual samples.

Studies using the RARAF single-particle microbeam facility to irradiate cell nuclei with specific numbers of ^{4}He ions to observe micronucleus production, cell growth, and progression through the cell cycle in normal human fibroblasts (Exp. 71) were continued by Charles Geard and Brian Ponnaiya of the CRR. This effort involves the bystander effect. In some experiments only a fraction of the cell nuclei are irradiated (2-20%) and the cells are observed for a result greater than would be expected for the fraction of cells hit. In other experiments, only some of the cells are stained with the dye used for observing nuclei during irradiation; the others are stained with a different vital

dye and are not irradiated because they do not fluoresce with the wavelength of light used for the microbeam and are not visualized. Unirradiated cells can be observed for scoring by using a different excitation wavelength. No difference in result has been observed for the two methods. Cell densities have been varied from intimate contact between cells to large separations. In other experiments, only the cytoplasm of the cells or only the cell medium has been irradiated. While there have been some effects observed for cytoplasmic irradiation, irradiation of only the medium with as many as 100 particles/cell yielded no difference from the controls. Additional studies of the effect of the irradiation of the cell medium are being carried out using the track-segment facility, where larger numbers of cells can be used. Stainless steel rings have Mylar epoxied to both sides, cells are plated on both inner surfaces and the volume is filled with medium. Cells on one surface are irradiated with ^{4}He ions; cells on the opposite surface are unirradiated because the particle range is much too short. This eliminates all possibility of cell-to-cell contact.

Satin Sawant of the CRR continued investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells (Exp. 73). Cells were irradiated individually through the nucleus or the cytoplasm, or a fraction of the cells were irradiated through the nucleus to observe the bystander effect. Because of the low yield of transformation, a considerable number of replicate experiments must be performed to obtain reasonable statistics.

Mutations induced at the S1 locus of human-hamster hybrid (A_L) cells by an exact number of ^{4}He ion traversals using the microbeam facility (Exp. 76) continue to be investigated by Tom Hei, Hongning Zhou, and An Xu of the CRR. The primary focus this year has been on extra-nuclear and extra-cellular targets. To evaluate the role of nitric oxide (NO), an important bioregulatory molecule, in mediating the mutagenicity of cytoplasmic irradiation, cells were irradiated with 8 ^{4}He ions through the cytoplasm in the presence or absence of L-NMMA, which has been shown to competitively inhibit nitric oxide synthases (NOS). Pretreatment with L-NMMA suppressed mutation induction by ~3-fold to near background level. In contrast the treatment had no effect on the mutagenic yield in A_L cells irradiated by 2 alpha particles through the nucleus. In other experiments, irradiation through the nuclei of 5-20% of randomly selected cells with 1-2 alpha particles each results in mutant fractions that are significantly higher than expected assuming no bystander modulation effect. Analysis by multiplex PCR shows that the types of mutants induced are significantly different from those of spontaneous origin. Pre-treatment of cells with the radical scavengers DMSO or NAC only had limited effect on the mutagenic incidence, however, pretreatment with lindane or Octanol, which inhibit gap junction cell-cell communication, significantly decreased the mutant yield.

Development of specialized neutron proportional counters (Exp. 82) was resumed by Gerhard Randers-Pehrson and Haijun Song of the CRR. The first counter being investigated uses a gas mixture consisting primarily of nitrogen. Monoenergetic neutrons produced by the Li(p,n) reaction using a thin target are being used to detect a resonance in the nitrogen at around 430 keV. If this detector is successful, other gas fillings may be tried.

William Morgan of the University of Maryland, in collaboration with Charles Geard of the CRR, continued use of the microbeam facility to investigate normal human fibroblasts derived from people with Nijmejen breakage syndrome (Exp. 84). These cells are deficient in a component of the repair process and are observed for intra-nuclear localization of repair proteins following site-specific irradiation.

Calibration of a portable neutron spectrometry system to cover the energy range from 20 keV to 500 MeV for use on the space shuttle and the manned mission to Mars (Exp. 89) is being performed by Richard Maurer, David Roth, Raul Fainchtein and others at the Applied Physics Laboratory of Johns Hopkins University. The low-energy portion of the neutron spectra is measured using ^{3}He proportional counters and the higher energy section is measured using a 5-mm-thick lithium-drifted silicon detector. This year, measurements were conducted to refine the discrimination in the ^{3}He counters of the pulses produced by neutrons from those produced by gamma rays in order to reduce the low-energy limit of the detector.

David Boothman of the University of Wisconsin at Madison, in collaboration with Charles Geard of the CRR, is examining the expression of radiation-induced proteins associated with apoptosis in human breast cells (Exp. 90). Breast carcinoma MCF-7 cells with and without a p53 construct are irradiated through the nucleus with ^{4}He ions using the single-particle microbeam and assessed for cell cycle progression, the incidence of micronuclei, and apoptosis. Cells undergoing apoptosis are examined to determine protein expression that may be associated with this process.

An experiment employing cDNA microarray technology (Exp. 92) was begun by Sally Amundson of the National Institutes of Health (NIH). ML-1 cells were irradiated with 0.43 MeV neutrons to determine gene induction as a function time after irradiation. RNA was extracted from the cells for use in microarray hybridization. Software is being developed by collaborators in the Human Genome Research Institute to allow cluster analysis of the data to detect genes with coordinate regulation. If this approach is successful, additional experiments will be performed to determine gene induction as a function of neutron energy.

George Sgouros and Ase Ballungrud of the Memorial Sloan-Kettering Cancer Institue along with Edward Lin, a high-school student from New York City, used the track-segment facility to simulate dual-agent radioimmunotherapy treatment of cancer cells (Exp. 93). Alpha-particle emitters such as ^{213}Bi have been used in the treatment of cancer to label antibodies against tumor-cell associated antigens. It has been proposed to follow this treatment with one using antibodies labeled with a short-range (internal conversion) electron emitter, hopefully producing a synergistic effect. Human breast carcinoma MCF-7 cells were irradiated with X rays, 120 keV/ μm ^{4}He ions or alpha particles followed by X rays and observed for survival.

Brian Ponnaiya of the CRR is developing a protocol in which small numbers of cells, as little as a single cell, can be observed for gene expression using reverse transcription polymerase chain reaction (RT-PCR) (Exp. 94). Copies of DNA segments are created by reverse transcription from RNA produced by the cell(s). The DNA is then amplified by PCR until enough material is available for gel electropheresis. This method permits observation of individual responses to radiation instead of just the

average response of a large number of cells. Single cells are obtained using a micromanipulator on the off-line microscope system of the microbeam facility. An efficiency of 80% has been achieved, i.e., 80% of the time a product is obtained when starting with a single cell irradiated using the microbeam facility.

Stig Palm of Göteborg University in Sweden spent two months at RARAF using the microbeam facility to simulate ^{211}At irradiations (Exp. 95). Astatine-211 is an alpha-particle emitter being studied at Göteborg for use in radioimmunotherapy (RIT). The doses delivered to the cells are determined by a complex calculation, and it was desir able to try to obtain some independent data of cell response to small numbers of alpha particles as a test to determine the accuracy of the calculations.

Radiation damage to the enamel of human teeth, observed using electron spin resonance (ESR), does not fade with time, making it useful as a retrospective biological dosimeter. It is of particular interest with respect to exposures of Japanese atomic bomb survivors since there continues to be controversy over the dosimetry calculations. John Zimbrick and Jeanine Katanic of Purdue University are studying the response of tooth enamel to neutrons (Exp. 96), an area in which there has been little investigation and considerable uncertainty. Small samples of powdered enamel irradiated with 10 Gy of 1-MeV neutrons showed no significant differences from the controls. An additional irradiation using 14-MeV neutrons is planned.

John Petrini of the University of Wisconsin and William Morgan of the University of Maryland, in collaboration with Charles Geard and Brian Ponnaiya of the CRR, have begun experiments to investigate repair protein complex localization after irradiation by ^{4}He ions (Exp. 97). Human or mouse fibroblasts are irradiated with a specific number of particles through the cell nucleus using the microbeam facility and examined for localization at DNA damage sites of the Mre11 complex and complex-interacting proteins.

Robert Ullrich of the University of Texas Medical Branch (UTMB) has begun an investigation into chromosomal instability (Exp. 98) in collaboration with Brian Ponnaiya of the CRR. It has been observed that the number of chromosomal aberrations in some irradiated cells decreases to the background level after a few cell passages, but may increase again several passages later. Human breast epithelial cells (MCF-10A) were irradiated using the microbeam facility and observed for chromosomal aberrations during successive passages to determine the amount of instability produced by specific numbers of ^{4}He ions.

Charles Limoli of the University of California at San Francisco and William Morgan of the University of Maryland, in collaboration with Charles Geard of the CRR, initiated another chromosomal instability experiment (Exp. 99). Chinese hamster ovary (CHO) cells were irradiated with ^{4}He ions using the microbeam facility and the number of chromosomal aberrations was assessed at various passages afterward.

A study of the effects of ^{4}He ions on normal and Ataxia telangectasia human fibroblasts using the comet assay (Exp. 100) was begun by T. Kumaravel of the National Institutes of Health in collaboration with Brian Ponnaiya and Adayabalam Balajee of the CRR. This procedure, like the single-cell PCR assay, is a way to observe effects in individual cells. Because the cells are irradiated using the microbeam facility, the

number of ^{4}He ion traversals is known, so variability in response is solely due to individual variability in the cells and the stochastic nature of the radiation.

Table 1. Experiments Run at RARAF May 1, 1999 - April 30, 2000

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
61	S. Milligan	Univ. of California San Diego	Chemistry	Yields of strand breakage produced in DNA by radiation associated with radon decay	4.5
71	C. R. Geard, B. Ponnaiya	CRR	Biology	Chromosome aberration and micronucleus production in human cells lines by specific numbers of α particles	21.5
73	S. Sawant	CRR	Biology	Neoplastic transformation of C3H 10T½ cells by specific numbers of α particles	28.0
76	T. K. Hei, H. Zhou, A. Xu	CRR	Biology	Mutation at the S1 locus of human-hamster hybrid (A_L) cells by specific numbers of α particles	29.5
82	G. Randers-Pehrson, H. Song	CRR	Physics	Neutron detector development	13.0
84	W. Morgan (Geard)	UCSF	Biology	Genomic instability using specific numbers of α particles	2.0
89	R. H. Mauer, et al.	Johns Hopkins Univ.	Physics	Calibration of a portable real-time neutron spectrometry system	4.0
90	D. Boothman (Geard)	Case Western Reserve Univ.	Biology	Expression of radiation-induced proteins associated with apoptosis	2.5
92	S. Amundson	NIH	Biology	Functional genomics of cellular response to high-LET radiation	1.0
93	G. Sgouros	Memorial Sloan-Kettering	Biology	Alpha particle induced radiosensitization: A strategy for targeted therapy of micrometastases.	1.0
94	B. Ponnaiya	CRR	Biology	Development of single cell RT-PCR	5.5
95	S. Palm	Göteborg University	Biology	Simulation of ^{211}At cell irradiation	2.0
96	J. Zimbrick, J. Katanic	Purdue University	Chemistry	Response of tooth enamel to neutrons	1.5
97	J. Petrini, W. Morgan (Geard, Ponnaiya)	Univ. of Wisconsin/ Univ. of Maryland	Biology	Repair protein complex localization after irradiation	0.5
98	R. Ullrich (Ponnaiya)	UTMB	Biology	Chromosomal instability in MCF-10A human mammary epithelial cells	4.5
99	C. Limoli, W. Morgan (Geard)	UCSF/ Univ. of Maryland	Biology	Chromosomal instability in Chinese hamster ovary (CHO) cells	1.0

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
100	T. Kumaravel (Ponnaiya, Balajee)	NIH	Biology	Comet assay of normal and *Ataxia telengectasia* cells irradiated with specific numbers of α particles	1.0

Accelerator Utilization and Operation

Accelerator usage is summarized in Table 2. Use of the accelerator for radiobiology and associated dosimetry increased by about 20% over last year, and is ~30% higher than the average for 1992-98. As was the case last year, almost 90% of the accelerator use for radiobiology and 70% of the accelerator use for all experiments was for microbeam irradiations. These experiments require considerable beam time to obtain sufficient biological material, especially for low-probability events such as trans formation and mutation. In addition, there has been considerable interest in "bystander" experiments that produce low yields even for normally frequent responses.

Utilization of the accelerator by radiological physics and chemistry increased 50% over last year and was considerably higher than the average for the past 7 years. Two of the projects (Exps. 89 and 95) should continue through at least next year.

Time spent on radiation safety system inspections continues to be minimized by not inspecting those systems that are rarely, if ever, used, such as the ^{137}Cs source used only for ionization chamber calibrations or the 50-kV X-ray source. No inspection is performed if the accelerator will be unused during the month due to meetings or installation of modifications. Any target stations that have not been used for a while are also not inspected. Of course, any facility is inspected before it is put back into use.

Accelerator reliability was about normal this year. Maintenance and repair time was about the same as last year. No major repairs or modifications to the accelerator were performed. The Freon 113 that has been used in the past to cool the ion source in the Van de Graaff terminal is no longer manufactured because it is a chlorofluorocarbon and can damage the ozone layer. It has been replaced with DuPont Vertrel XF, a hydrofluorocarbon that is still manufactured because it is much less damaging.

Table 2. Accelerator Use, May 1999 - April 2000. Percent Usage of Available Days.

Radiobiology and associated dosimetry	38%
Radiological physics and chemistry	9%
On-line facility development and testing	17%
Off-line facility development	25%
Safety system	2%
Accelerator-related repairs / maintenance.	11%

Development of Facilities

The considerable development of the single-particle microbeam facility is described here briefly:

- The single electrostatic quadrupole quadruplet constructed last year to focus the particle beam to ~2-μm diameter has been installed in the existing facility. In testing, it reduced the beam size to 20-μm diameter for an object aperture with a diameter of 50 μm, a demagnification factor of 2.5. Further testing will be done to try to obtain the calculated demagnification factor of 4 and the object aperture size will be reduced to reduce the final beam-spot diameter.
- A magnetic quadrupole lens has been installed in the beam line between the last two bending magnets for the microbeam in order to focus the beam on the object aperture of the electrostatic quadrupole system. This should assist in increasing the demagnification factor of the quadruplet lens.
- A fixture has been designed and is being constructed to rotate the ceramic rods used for the quadrupole electrodes so that their surfaces can undergo ion implantation. This will reduce the resistance and hopefully eliminate occasional electrical breakdown in the electrodes.
- The test laser system obtained from the University of Arkansas has been used successfully to oblate aluminum from a solid target. Work will now begin on extracting the ions produced by the laser pulses.
- A specially designed prism has been obtained that will be used along with a light pipe to couple our fast wavelength switcher to the microscope on the microbeam facility so that wavelengths can be changed rapidly under computer control to excite different cell stains.
- Design has been completed of a 90º magnet to be placed between the exit of the Van de Graaff and the switching magnet in order to direct the charged-particle beam to the floor above. It is capable of bending the heavy ion beams that will be eventually produced by the laser ion source under development. The magnet is under construction and should be delivered in December 2000. Because there now will be less room in this region, several new beam-line components have been purchased to replace existing ones in order to minimize the space required. Whereas the older components used o-ring seals, the new components all use metal vacuum seals that should result in better vacuum.
- Construction of a new laboratory on the floor over the exit of the Van de Graaff should begin in early December. This will house the next generation microbeam facility with an ultimate beam diameter of <0.5 μm.
- A new 650-MHz Pentium III computer with a large number of available slots has been purchased to control the microbeam experiment. In addition to being considerably faster than the computer now in use, all the control boards can be mounted in the computer. Presently many of the boards are mounted in an expansion chassis, which slows the system down somewhat.
- A new version of the image-analysis software has been obtained. This should provide "watershed" capability, i.e., the ability to find the boundary between cell

nuclei that are in contact by observing reductions in fluorescence and "pinching" of the outer edge of the image where the nuclei touch.

- Design has begun of an imaging system to observe the size of the focused microbeam using secondary electrons created by the charged particles.

Personnel

The Director of RARAF is Dr. David Brenner. The Van de Graaff accelerator is operated by Mr. Stephen Marino and Dr. Gerhard Randers-Pehrson.

Dr. Haijun Song, a post-doctoral fellow, left in February of this year to pursue a career in medical physics at Thomas Jefferson University Hospital in Philadelphia.

For the first time in six years, RARAF has a physics technician. Mr. Mutian Zhang started as a part-time employee in March of this year and became a full-time employee in July.

Dr. Alexander Dymnikov, an expert on ion beam transport who joined the RARAF staff in February, 1999 as a Visiting Research Scientist to assist in the development of electrostatic lenses for the microbeam, left RARAF in April 2000.

Mr. Francois Lueg-Althoff, an undergraduate student from the University of Aachen in Jülich, Germany, arrived in October 1999 for a nine-month visit to do his Praxissemester (practical semester) and Diplomarbeit (undergraduate thesis). He returned home in July of this year. As his thesis project, he irradiated track- etchant plastic using the single-particle microbeam to determine the radial distribution of alpha particles at the location of the cells.

Dr. Alan Bigelow, a post-doctoral fellow, arrived in August of this year, having recently received his Ph.D. degree from University of North Texas. As part of his duties he is continuing the development of the laser ion source begun by Haijun Song.

Biologists from the Center for Radiological Research not supported by the RARAF grant spend various amounts of time at the facility in order to perform experiments:

- Dr. Charles Geard spends most of each working day at RARAF. In addition to his own research, he is collaborating with several outside users on experiments using the single-particle microbeam facility.
- Dr. Satin Sawant, an Assistant Research Scientist, spends all his time at RARAF, primarily doing experiments utilizing the microbeam facility.
- Dr. Brian Ponnaiya, a post-doctoral fellow, works at RARAF full-time performing microbeam experiments.
- There is one full-time biology technician, Ms. Gloria Jenkins. Another technician, Ms. Mei Wang, spent most of her time at RARAF until she transferred to another department in November 2000. A third technician, Ms. Sonu Dhar, worked at RARAF full time for several months before being reassigned to the CRR.

Microbeam Meeting

The 5th International Workshop on Microbeam Probes of Cellular Radiation Response will be held in Lago Maggiore, Italy May 26-27, 2000 and is being organized in part by RARAF. More than 70 participants from 13 countries attended the previous

meeting, which was jointly sponsored by RARAF and the Massachusetts Institute of Technology. Twenty-four presentations were made, ranging from descriptions of microbeam facilities and biological effects to theoretical predictions of biological results.

RECENT PUBLICATIONS OF WORK PERFORMED AT RARAF (1999-2000)

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14. Zhou, H. N., Zhu, L. X., Li, K. B. and Hei, T. K. Radon, tobacco specific nitrosamine and mutagenicity. Mutation Res. **430**, 145-153 (1999).