THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

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An NIH-Supported Resource Center
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Funding

During this year, we were delighted that NIH funding for continued development of our single-particle microbeam facility was renewed for a further five years.

Research Using RARAF

Table I lists the experiments performed at RARAF during the period May 1, 1998 through April 30, 1999 and the number of days each was run in this period. Twelve different experiments were run during this 12-month period, about the same as the last two years.

Six 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 six by outside users, supported by various grants and awards from NIH, DOE, and NASA. Brief descriptions of these experiments are given here:

Studies of the mutagenesis of human-hamster hybrid (A_L) cells by charged particles (Exp. 43) resumed this year. Tom Hei, Hongning Zhou and Su-Xian Liu of the CRR irradiated cells with ^{4}He particles having an LET of 150 keV/μm using the track segment facility. Cells were treated with graded doses of NNK, a tobacco-specific nitrosamine, or arsenite and given a single particle dose of 0.25 Gy. The mutation rate at the S1 locus of human chromosome 11, a single copy of which is present cells, is then observed.

Charles Geard of the CRR has continued studies using the RARAF single-particle microbeam facility to irradiate cell nuclei with specific numbers of 90 keV/μm ^{4}He ions to observe micronucleus production, cell growth, and progression through the cell cycle in normal human fibroblasts (Exp. 71). The cells are irradiated through the nucleus, through the cytoplasm, or through the surrounding medium. In other experiments, only a fraction of the cell nuclei are irradiated (1 in 2 to 1 in 80) and the unirradiated cells are observed for a "bystander" effect, i.e. a response greater than can be accounted for by only the cells which have been irradiated. Cell densities have been varied from intimate contact between cells to large separations. Brian Ponnaiya is developing a protocol in which small numbers of these cells, ultimately a single cell, can be observed for gene expression.

Investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells (Exp. 73) were continued by Richard Miller and Satin Sawant of the CRR. Cells were irradiated individually through the nucleus or the cytoplasm, or a fraction of the cells were irradiated through the nucleus. In the latter case, when 10% of the cells were irradiated, with 8 helium ions, there was a significant increase in transformation rate compared to what would be predicted if there were no "bystander" effect.

The frequency and types of mutations induced at the S1 locus of human-hamster hybrid (A_L) cells by an exact number of ^{4}He ion traversals (Exp. 76) continue to be investigated by Tom Hei, Hongning Zhou and An Xu of the CRR. Twenty helium ions given to 20% of the cells resulted in a mutation rate 3-fold higher than expected assuming no "bystander" effect. The presence of DMSO had no effect, however lindane, which inhibits cell-to-cell communication, significantly reduced the mutation yield. In other experiments, only the cytoplasm is irradiated and the cells are examined to determine the mechanism by which mutations have been observed even though no particles passed through the cell nuclei. In this case, the addition of DMSO reduced the mutation rate, implying that radicals play a role in the process.

Lucien Wielopolski and colleagues from Brookhaven National Laboratory continued the characterization of an accelerator-based boron neutron capture therapy (BNCT) system (Exp. 81). A moderator/reflector assembly consisting mostly of iron and Teflon is used to moderate neutrons produced by the Li(p,n) reaction. Neutrons with energies from a few eV to 100 keV are captured by the boron, which emits an energetic alpha particle, providing the therapeutic advantage. Ideally the spectrum at the entrance to where the patient's head will be positioned should have few thermal neutrons, which will mostly be absorbed before reaching a tumor, or neutrons with energies above 100 keV, which won't become thermalized and contribute unwanted dose to healthy tissue. The γ -ray dose from the competing Li(p,p`γ) reaction and from neutron capture in hydrogenous material must also be kept at a minimum since this is also unnecessary dose to the healthy tissue. Neutron yields and spectrometry measurements are compared with Monte Carlo calculations used to model the system.

Noelle Metting of Pacific Northwest National Laboratory (PNNL) in Washington State continued to investigate early responses to DNA damage (Exp. 80). HeLa S3 cells were irradiated through the nucleus by ^{4}He ions with an LET of 90 keV/µm using the microbeam facility. The DNA of cells irradiated and then incubated was probed by enzymatic addition of labeled dNTPs to the 3'-OH ends.

William Morgan of the University of California at San Francisco (UCSF) and Frank Petrini, of the University of Wisconsin at Madison, in collaboration with Charles Geard of the CRR, are using the microbeam facility to investigate normal human fibrobalsts derived from people with Nijmejen breakage syndrome (Exp. 84). These cells are deficient in a component of the repair process. The cells are observed for intra-nuclear localization of repair proteins following site-specific irradiation.

Tom Hei and Gloria Calaf of the CRR continued experiments using the track segment facility to develop a model for neoplastic transformation in immortalized human breast epithelial (MCF-10F) cells similar to that used for human bronchial epithelial cells (Exp. 85). Cells from transformed colonies resulting from one or two 0.6 Gy doses of 150 keV/µm ^{4}He ions are observed for altered morphology, increased growth rate, anchorage-independent growth, and invasive capabilities before being implanted into nude mice to assay for tumor formation.

JaeSub Hong and William Craig of the Columbia University Astrophysics Laboratory continued their investigation of materials to shield gamma-ray detectors used in high-altitude balloon flights from neutrons (Exp. 88). Neutron and gamma-ray fluxes and

spectra are being measured for initially monoenergetic neutrons in the energy range from 0.2 to 2 MeV after they have passed through various potential shielding configurations. This research will be the doctoral thesis for Mr. Hong.

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 is being developed by a group at the Applied Physics Laboratory of Johns Hopkins University. Calibration of this system (Exp. 89) is being performed by Richard Maurer, David Roth, Raul Fainchtein and others in their group. The low-energy portion of the neutron spectra are measured using He proportional counters and the higher energy section is measured using a 5-mm thick lithium-drifted silicon detector. Essentially monoenergetic neutrons in the energy range from 0.5 to 18.5 MeV have been provided using the T(p,n), D,d,n) and T(d,n) reactions. The neutron doses delivered to the detectors have been measured with a tissue-equivalent ionization chamber and converted to fluence using standard fluence-to-dose conversion factors so that the efficiency of the detectors as a function of energy can also be determined.

David Boothman of Case Western Reserve University, in collaboration with Charles Geard of the CRR, is examining the expression of radiation-induced proteins associated with apoptosis in human mammary epithelial cells (Exp. 90). Cells with and without a p53 construct are irradiated using the single-particle microbeam. Cells undergoing apoptosis after irradiation are examined to determine protein expression that may be associated with this process.

Transformation of primary human lung epithelial cells by ^{4}He ions (Exp. 91) is being investigated by Tom Hei and Hongning Zhou of the CRR. Explants of cells are grown into cultures and irradiated with 150 keV/μm ^{4}He ions using the track segment facility. Because of the low probability of producing a transformed cell, large numbers of cells must be irradiated for each experiment.

Accelerator Utilization and Operation

Accelerator usage is summarized in Table II. Use of the accelerator for radiobiology and associated dosimetry was very similar to the average for 1992-98. Over 90% of the accelerator use for radiobiology and 75% of the accelerator use for experiments was for microbeam irradiations. These experiments require considerable beam time to obtain sufficient biological data, especially for low probability events such as transformation and mutation.

Utilization of the accelerator by radiological physics and chemistry increased somewhat over last year and was slightly higher than the average for the past 6 years. Two of the projects (Exps 88 and 89) should continue through at least the next year. Long-term physics experiments can require large amounts of beam time and can often be run on relatively short notice if the experimenters do not have a long travel time.

Time spent on radiation safety system inspections was reduced slightly by not inspecting those systems that are rarely, if ever, used, such as the ^{137}Cs source that is used only for chamber calibrations or the 50 kV X-ray source. Any target stations that have not been used for a while are also not inspected. Of course, any facility will be inspected before it is put back into use.

Table I. Experiments Run at RARAF May 1, 1998 - April 30, 1999

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
43	T. K. Hei, H. N. Zhou, S. X. Liu	CRR	Bio	Cellular and Molecular studies on the mutagenesis of charged particles using human-hamster hybrid (A_L) cells	2.5
71	C. R. Geard, B. Ponnaiya	CRR	Bio	Chromosome aberration and micronucleus production in human cells lines by specific numbers of α particles	16.2
73	R. C. Miller, S. Sawant	CRR	Bio	Neoplastic transformation of C3H 10T½ cells by specific numbers of α particles	19.8
76	T. K. Hei, H. N. Zhou, A. Xu	CRR	Bio	Mutation at the S1 locus of human-hamster hybrid (A_L) cells by specific numbers of α particles	21.2
80	N. F. Metting	PNNL	Bio	Early responses to DNA damage	1.0
81	L. Wielopolski, et al.	BNL	Phys	Neutron spectroscopy for moderator assembly for BNCT using Li(p,n) reaction	6.0
84	W. Morgan, J. Petrini	UCSF, Univ. of Wisconsin	Bio	Genomic instability using specific numbers of α particles	2.0
85	T. K. Hei, G. Calaf	CRR	Bio	Neoplastic transformation of human breast epithelial cells by high-LET radiation	2.0
88	W. Craig, J. Hong	Columbia Univ.	Phys	Development of neutron shields for high-altitude gamma-ray detectors	6.2
89	R. H. Mauer, et al.	Johns Hopkins Univ.	Phys	Calibration of a portable real-time neutron spectrometry system	2.5
90	D. Boothman	Case Western Reserve Univ.	Bio	Expression of radiation-induced proteins associated with apoptosis	1.0
91	T. K. Hei, H. N. Zhou	CRR	Bio	Neoplastic transformation of primary human lung epithelial cells by high-LET radiation	0.5

Accelerator reliability was about normal this year. Maintenance and repair time was slightly above the recent average, and less than half that for 1994-95. No major repairs to the accelerator were performed, although there was a modification to the charging control system, which is described in the next section.

Development of Facilities

Development of the microbeam and low-energy neutron facilities are described here briefly. More detailed descriptions of the development of these facilities are given elsewhere in this report.

The single–particle microbeam has a number of developments and modifications that are nearing completion:

Table II.
Accelerator Use, May 1997 - April 1998
Percent Usage of Available Days

Radiobiology and associated dosimetry	27%
Radiological physics and chemistry	6%
On-line facility development and testing	23%
Off-line facility development	33%
Safety system	2%
Accelerator-related repairs / maintenance.	9%

- A quadrupole quadruplet lens to focus the particle beam to ~2μm diameter has been designed, constructed, and successfully tested for high voltage capability
- A high voltage power supply has been purchased for the quadruplet and is being modified to turn off if there is a sudden voltage change (break-down)
- The voice-coil positioner for cell dishes has been refined and a control circuit designed

The low-energy neutron facility produces neutron spectra with dose-mean energies of 86, 56, and 40 keV. It is based on the Li(p,n) reaction and requires a rotating target to avoid melting the lithium at high beam currents. The target system is fully functional:

- The water and vacuum seals do not leak, even at twice the design motor speed
- A beam current of 60 μA for several hours did not reduce the thickness of the lithium
- Neutron spectra show only a moderate amount of scattered higher-energy neutrons
- Neutron dose rates are adequate and the percentage γ-ray dose is acceptable
- Multiple small cell samples can be irradiated simultaneously at the same dose rate

We have partially installed the new voltage control system that was purchased for the Van de Graaff last year. This system is designed to regulate the terminal voltage to ±1 keV whereas the previous system, installed about 1970, can only regulate to ±3-5 keV. The new generating voltmeter (GVM) and corona head have been mounted on the accelerator tank and the signal cables have been run. While the new corona head could be mounted on a spare port, allowing us to keep the old one in place, the new GVM had to replace the old one, so we could not maintain two parallel systems and switch between them. The new GVM has been in use for 2 months but the corona head has not as yet been tried. The control electronics for the new system do not provide some of the features the

old system did, so modifications will be made to the circuitry to obtain a digital readout of the terminal voltage and the position of the corona head.

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, with the assistance of Dr. Haijun Song, a post-doctoral fellow. Staffing at RARAF has increased during the past year to the point that there are no longer offices available and the biology labs have become somewhat crowded.

Dr. Dusan Srdoc, who had been collaborating on measurements of microdosimetric and neutron spectra, left RARAF in March 1999.

Dr. Alexander Dymnikov, an expert on ion beam transport, joined the RARAF staff in February, 1999. He is doing detailed calculations on the design of the electrostatic quadrupole lens systems which are being developed to increase the microbeam resolution initially to 2 μm and eventually to ~0.5 μm.

Mr. Stig Palm from the University of Goteborg, Sweden visited from August through October 1999 to do irradiations with the single-particle microbeam. His experiments were related to the study of radioimmunotherapy cancer treatment using antibodies labeled with ^{211}At, the subject of his recent doctoral thesis.

Mr. Francois Lueg-Althoff, an undergraduate student from the University of Aachen in Jülich, Germany, arrived in October for a nine-month visit to do his Praxissemester and Diplomarbeit (practical semester and undergraduate thesis). He has been assisting the RARAF staff, particularly with microbeam irradiations. As his thesis project, he will irradiate track-etchant plastic using the single-particle microbeam to determine the radial distribution of alpha particles at the location of the cells.

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 a large part of most working days at RARAF. In addition to his own research, he is collaborating with several outside users on experiments using the single-particle microbeam facility.

Dr. Richard Miller worked at RARAF approximately 3-4 days per week until January 1999, when he took a position with the Radiological Society of North America (RSNA). He has returned several times to perform or assist in microbeam experiments.

Dr. Satin Sawant, has taken over Richard Miller's work on transformation using the C3H10T1/2 cell line. He spends essentially all his time at RARAF, primarily doing experiments utilizing the microbeam facility.

Dr. Brian Ponnaiya, a post-doctoral fellow, arrived in April 1999. He works at RARAF full-time, performing microbeam experiments. He has equipped the cell laboratory for molecular characterization of radiation damage.

There is one full-time biology technician, Ms. Gloria Jenkins. Two other technicians, Ms. Mei Wang and Ms. Sonu Dhar, are at RARAF part of the time.

Microbeam Meeting

We organized the *4th International Workshop: Microbeam Probes of Cellular Radiation Response*, held in Killiney Bay, Dublin, July 17-18. Roughly 75 scientists (about

equal numbers of physicists and biologists) attended the workshop, the fourth in a bi-annual series. Extended abstracts from the meeting are in press in the *Radiation Research* journal and are available on the RARAF website (www.raraf.org).

RECENT PUBLICATIONS OF WORK PERFORMED AT RARAF (1998-1999)

1. Calaf, G.M. and Hei, T.K. Establishment of a radiation and estrogen-induced breast cancer model. Carcinogenesis **21** (in press, 2000)
2. Calaf, G.M. and Hei, T.K. Establishment of a radiation and estrogen-induced breast cancer model. Carcinogenesis, in press, 2000
3. Dymnikov, A.D., Brenner, D.J., Johnson, G. and Randers-Pehrson, G. Theoretical study of short electrostatic lens for the Columbia ion microprobe. Rev. Sci. Instr. (In Press, 2000)
4. Dymnikov, A.D., Brenner, D.J., Johnson, G.W. and Randers-Pehrson, G. Electrostatic lens design for the Columbia microbeam. Radiation Research, in press, February 2000.
5. Geard, C.R., Randers-Pehrson, G., Marino, S.A., Jenkins-Baker, G., Hei, T.K., Hall, E.J. and Brenner, D.J. Intra- and inter-cellular responses following cell-site specific microbeam irradiation. Radiation Research, in press, February 2000.
6. Hei, T. K., Roy, D., Piao, C.Q., Calaf, G. and Hall, E. J. Genomic instability in human epithelial cells transformed by high LET radiation. Radiat. Res. **153** (in press, 1999)
7. Mauer, R.H., Roth, D.R., Fainchtein, R., Goldsten, J.O. and Kinnison, J.D. Portable real time neutron spectrometry II, to be published in the proceedings of the International Space Station Conference, Albuquerque, NM, January 30-February 3, 2000.
8. Miller, R.C., Martin, S.G., Geard, C.R., Marino, S.A., Randers-Pehrson, G., Brenner, D.J. and Hall, E.J. High LET-induced Oncogenic Transformation. In *Risk Evaluation of Cosmic-ray Exposure in Long-term Manned Space Mission* (F. Fujitaka, et. al., Eds.) pp. 121-126, Kondasha Scientific Ltd., Tokyo, Japan, 1999.
9. Miller, R.C., Martin, S.G., Hanson, W.R., Marino, S.A. and Hall, E.J. Effect of track structure and radioprotectors on the induction of oncogenic transformation in murine fibroblasts by heavy ions. Adv. Space Res. **22**: 1719-1723 (1998).
10. Miller, R.C., Sawant, S.G., Randers-Pehrson, G.,. Marino, S.A., Geard, C.R., Hall E.J. and Brenner, D.J. Single alpha-particle traversals and tumor promoters. Radiation Research, in press, February 2000.
11. Randers-Pehrson, G., Geard, C.R., Johnson, G.W. and Brenner, D.J. Technical characteristics of the Columbia University single-ion microbeam. Radiation Research, in press, February 2000.
12. Zhou, H.N., Randers-Pehrson, G., Waldren, C., Vannais, D., Hall, E.J. and Hei, T.K. Induction of a bystander mutagenic effect of alpha particles in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **97** (in press, 2000).
13. Zhou, H., Randers-Pehrson, G. and Hei, T.K. Studies of bystander mutagenic response using charged particle microbeam. Radiation Research, in press, February 2000.