# The Radiological Research Accelerator Facility

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**Research Using RARAF**

The "bystander" effect, in which only some cells are irradiated and a response is obtained that is greater than would be expected for the fraction irradiated remains of great interest. Several experiments examining this effect were continued, observing a variety of endpoints to determine the size of the effect and the mechanism by which it is transmitted. There is evidence for both direct cell-cell communication through cell membranes and indirect, longer-range communication through some release by the cells into the cell medium. In some experiments, the unirradiated cells can be identified due to a different staining and scored directly. In other experiments, unirradiated cells are physically separated from the irradiated cells. Both the microbeam and the track segment facilities continue to be utilized in various investigations of this phenomenon. The single-particle microbeam facility provides precise control of the number and location of particles but is somewhat limited in the number of cells that can be irradiated. The track segment facility provides broad beam irradiation that has a random pattern of charged particles but allows large numbers of cells to be irradiated.

In Table I are listed the experiments performed at RARAF from May 1, 2001 through April 30, 2002 and the number of days each was run in this period. Fourteen different experiments were run during this 12-month period, about the same as the average for 1996-2001. Eight experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH) and the Department of Energy (DOE). Six experiments were performed by outside users, supported by grants and awards from the NIH, NASA, the Department of Defense (DoD), the University of Toronto and the Ministry of Education, Science, Sports and Culture of Japan. Brief descriptions of these experiments follow.

Investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells (Exp. 73) were continued by Eric Hall and Stephen Mitchell (who has replaced Satin Sawant) of the CRR. Using the microbeam facility, 10% of the cells were irradiated through the nucleus with 2 to 12 helium ions. Cells were plated at densities of approximately 200 and 2000 per dish to try to observe the relative contribution of cell-cell communication to the bystander

**Table I**

**Experiments Run at RARAF May 1, 2001 - April 30, 2002**

| Exp. No. | Experimenter | Institution | Exp. Type | Title of Experiment | Days Run |
|---|---|---|---|---|---|
| 73 | S. Mitchell, E.J. Hall | CRR | Biology | Neoplastic transformation of C3H10T½ cells by specific numbers of α particles | 1.0 |
| 76 | A. Xu, T.K. Hei | CRR | Biology | Mutation at the S1 locus of human-hamster hybrid ($A_L$) cells by specific numbers of alpha particles | 2.0 |
| 89 | R.H. Mauer, et al. | Johns Hopkins Univ. | Physics | Calibration of a portable real-time neutron spectrometry system | 3.5 |
| 94 | B. Ponnaiya, C.R. Geard | CRR | Biology | Single cell responses in hit and bystander cells: single-cell RT-PCR and protein immunofluorescence | 12.0 |
| 96 | J. Zimbrick, J. Katanic | Purdue Univ. | Chemistry | Response of tooth enamel to neutrons | 2.5 |
| 101 | K. Komatsu (Zhou) | Hiroshima Univ. | Biology | Bystander effect of *Ataxia Telangiectasia* cells | 1.0 |
| 103 | G. Jenkins, C.R. Geard | CRR | Biology | Damage induction and characterization in known hit versus non-hit human cells | 18.0 |
| 106 | B. Ponnaiya, C.R. Geard | CRR | Biology | Track segment alpha particles, cell cocultures and the bystander effect | 8.0 |
| 108 | H. Zhou, T.K. Hei | CRR | Biology | Modulation of adaptive response in alpha-particle-induced bystander effects | 10.5 |
| 109 | A. Balajee, C.R. Geard | CRR | Biology | DNA damage induction in microbeam-irradiated cells assessed by the comet assay | 7.0 |
| 110 | H. Zhou, D. Roy T. K. Hei | CRR | Biology | Identification of molecular signals of alpha particle-induced bystander mutagenesis | 25.0 |
| 111 | R. Bristow, S. Al Rashid | University of Toronto | Biology | Visualization of the localization of repair proteins after microbeam irradiation | 2.0 |
| 112 | Y. Horowitz, M. Zaider | Ben Gurion Univ., Memorial Sloan Kettering | Physics | HCP and neutron irradiation of LiF:Mg, TI TLD chips to determine 5a/5 intensities and characterization of 5a peak as a Q/RBE nanodosimeter | 6.5 |
| 113 | A. Miller | AFRRI | Biology | Role of alpha particle radiation in depleted uranium-induced cellular effects | 0.5 |

*Note: Names in parentheses are CRR members who collaborated with outside experimenters.*

effect. Cell survival was about the same for both cell densities for two particles, but for 12 particles the lower density has a survival of 90% while the higher density has a survival of only 75%. This would imply little or no bystander effect at low density (only the 10% of cells hit die) but a large effect at high density where 2.5 times as many cells as were hit don't survive.

Studies of the mutation of hamster hybrid ($A_L$) cells by specific numbers of helium ions (Exp.76) were extended by An Xu and Tom Hei of the CRR to protons. These irradiations were only preliminary because protons that have a range less than that of the collimator thickness have such a low momentum that they are scattered more easily than the helium ions that are normally used. This increases the effective diameter of the beam at the cell position so that it may be larger than the diameter of the cell nuclei. These experiments will resume as soon as the focused microbeam, which will produce a beam 2.5 μm in diameter for both helium ions and protons, is operational.

Richard Mauer, David Roth and James Kinnison of Johns Hopkins University continued development of a neutron spectrometer for the energy range 0.5 to 100 MeV (Exp. 89). The spectrometer will be used on the International Space Station and the manned mission to Mars. Emphasis is now on determining the efficiencies of the detectors at different neutron energies and investigating the rise times of the pulses generated by gamma rays and neutrons to perform gamma-ray discrimination. In addition, a CCD camera was irradiated with 2.2 MeV neutrons to determine its resolution as a function of neutron fluence. The camera is being considered for a probe that will be sent to Pluto and the asteroid belts beyond. Because solar panels will not be able to produce enough energy that far from the sun, a Radioactive Thermal Generator will be used, producing a spectrum of neutrons with a mean energy of approximately 2.2 MeV. The long-term exposure to neutrons raised questions of the survival and the resolution of the camera.

Brian Ponnaiya and Charles Geard of the CRR continued two studies investigating the bystander effect. In one study (Exp. 94), single cells are observed for gene expression using reverse transcription polymerase chain reaction (RT-PCR) in conjunction with immunofluorescence techniques. This procedure permits observation of individual responses to radiation instead of just the average response of a large number of cells. Irradiated and unirradiated cells can be identified by a differential staining administered prior to irradiation. Individual cells are selected using a micromanipulator on the off-line microscope system of the microbeam facility. Copies of DNA segments are created by reverse transcription (RT) from mRNA produced by each cell. The DNA is then amplified by polymerase chain reaction (PCR) until enough material is available for gel electrophoresis to measure the amount of mRNA. Immunofluorescent staining is used to determine the amount of the protein associated with a particular mRNA for corroboration. Based on previous results indicating the induction of p21/WAF1 in irradiated and bystander normal human fibroblasts, microbeam experiments were conducted to standardize protocols for the analyses of other early response genes. The other investigation involves use of the track segment facility for broad-beam charged particle irradiations of human fibroblasts and epithelial cells immortalized with telomerase (Exp. 106). Special cell dishes are made from stainless steel rings with thin Mylar windows epoxied on both sides. Cells are plated on both inner Mylar surfaces and the dish volume is filled with medium. This eliminates all possibility of cell-cell contact between cells on opposite sides. Cells on one surface are irradiated with $^4$He ions; cells on the opposite surface are unirradiated because the particles are stopped before reaching them. Cells are observed *in situ* after irradiation with doses from 0.1 to 10 Gy of 125-keV/μm $^4$He ions. Plateau phase cells are scored for cell cycle delay and micronucleus production while log phase cells are scored for chromosomal aberrations. It was observed that irradiated fibroblasts can induce micronuclei in bystander fibroblasts, but bystander epithelial cells are refractory to irradiated epithelial cells. Furthermore, epithelial cells are capable of responding to irradiated fibroblasts, which results in the induction of micronuclei in the bystander epithelial cells. Chromosomal analyses of irradiated fibroblast populations and bystander cells at the first cell division post irradiation demonstrated the induction of gross chromosome aberrations in the irradiated population and chromatid aberrations (of the simple type –breaks and gaps) in the bystander population. Elevated yields of similar types of chromatid type aberrations were also observed in both irradiated and bystander fibroblast populations up to 20 population doublings post irradiation.

Studies of the response of tooth enamel to neutrons (Exp. 96) were continued by John Zimbrick and Janine Katanic of Purdue University. Damage to the enamel by X and gamma rays is permanent and can be observed by electron spin measurements, making teeth useful as biological dosimeters. Previous studies by others of the response of tooth enamel to neutrons had large uncertainties. Enamel irradiated at RARAF by 14 MeV neutrons with and without 3 mm of plastic for secondary charged particle equilibrium showed no observable effects. It was hoped that teeth extracted from atomic bomb survivors for medical reasons might be used to help determine the neutron doses received.

Kenshi Komatsu of Hiroshima University in Japan, in collaboration with H. Zhou of the CRR, continued a study of the bystander effect on *Ataxia Telangiectasia* (AT) cells (Exp. 101) using the microbeam facility. There is considerable evidence indicating that p53 may play a crucial role in the bystander effect. Atm , a kinase for the phosphorylation of several proteins including p53, seems to be the sensor of DNA damage or center of signal transduction. AT cells lack Atm and therefore could provide useful information on the role of p53 in the bystander effect. In current experiments, cell survival was measured for radiation sensitive AT cells and normal Hx cells. In addition, the bystander effect for HPRT mutation in Hx cells was determined by irradiating 10% of the cell nuclei with 20 alpha particles.

Hongning Zhou and Tom Hei of the CRR continued to use the single-particle microbeam facility for two experiments investigating the bystander effect. One study is examining adaptive response in bystander effects in human-

hamster hybrid ($A_L$) cells (Exp. 108). After low-dose X-ray irradiation, 10% of the cells are traversed by 1 or 20 helium ions. There is a decrease in the bystander effect for mutation when neighbor cells are traversed by one particle and a somewhat smaller decrease for traversal by 20 particles. . In addition, they found that the bystander cells showed an increase in sensitivity to a subsequent, challenging dose of X-rays. The mutation spectra are being analyzed and should provide some evidence for understanding the mechanism of bystander mutagenesis and adaptive response. With Debasish Roy of the CRR, they are trying to identify the molecular signals of cell-cell communication in bystander mutagenesis (Exp. 110). Hybrid $A_L$ cells, human fibroblasts, and normal human bronchial epithelial cells were irradiated using the microbeam facility. A fraction of the cells was irradiated with a single alpha particle. The irradiated (stained) cells are separated from the unirradiated cells by a cell sorter and accumulated from experiments over four consecutive days. The cells were then analyzed using microarrays. Preliminary data show some gene expression change in the bystander cells.

Investigations of damage induction in human fibroblasts and *Ataxia Telangiectasia* cells (Exp. 109) were extended by Adayabalam Balajee and Charles Geard of the CRR to include a search for foci of damage and repair proteins. Cells irradiated through the nucleus using the microbeam facility are stained and examined to observe these proteins, which should cluster around the helium ion track.

Robert Bristow and Shahnaz Al Rashid of Princess Margaret Hospital, Toronto initiated efforts to visualize the localization of repair proteins around particle tracks (Exp. 111). The microbeam facility was used to irradiate human fibroblast strains with one helium ion per nucleus. At times they observed a discrete sub-nuclear focus with H2AX, but never with rad50, although there was co-localization with one of the rad50 foci and the discrete H2AX. The ser15-p53 foci were diffuse, but observed in both irradiated and unirradiated cells.

Yigal Horowitz of BenGurion University of the Negev in Israel and Marco Zaider of the Memorial Sloan-Kettering Cancer Center in New York are investigating the use of thermoluminescent dosimeter (TLD) chips as nanodosimeters to determine the quality factor of radiations (Exp. 112). Radiation damage to the crystal structure of the LiF:Mg,Ti crystal structure can be removed by heating, with different kinds of damage repaired at different temperatures. As the damage is removed, excess energy is emitted in the form of light (glow peaks). It has been found that the ratio of peak 5a to peak 5 is 7-8% for gamma rays but 25-30% for alpha particle irradiation. The dependence of damage to the crystals on ionization density is similar to that for DNA double strand breaks and the physical structure giving rise to peak 5a is about 2 nm, similar to the diameter of DNA. These features make the TLD system applicable as a nanodosimeter. TLDs were irradiated with 0.22 and 14 MeV neutrons and with charged particles having six different LETs in the range of 10-180 keV/μm to observe the dependence of the ratios of the glow peaks on radiation quality.

The Department of Defense is interested in the biological effects of depleted uranium (DU), especially since its significant use in the Gulf War. The primary focus has been the chemical effects of DU on human cells. Alexandra Miller of the Armed Forces Radiobiological Research Institute has begun a study of neoplastic transformation of immortalized human osteoblast cells by helium ions (Exp. 113). Graded doses of ions were delivered using the track segment facility to try to determine the contribution to cell transformation of the alpha particles emitted by the DU.

**Accelerator Utilization and Operation**

Accelerator usage is summarized in Table II. Use of the accelerator for radiobiology and associated dosimetry decreased by about 10% over last year but was still ~35% higher than the average for 1996-2001. This year less than 80% of the accelerator use for all experiments was for microbeam irradiations because of increased use of the track segment facility and the increase in physics and chemistry usage. Because of the relatively low number of cells that can be irradiated in a day, microbeam experiments often require considerable beam time to obtain sufficient biological material, especially for low probability events such as transformation and mutation.

Utilization of the accelerator by radiological physics and chemistry increased this past year, back to the level in 1999-2000. Two ongoing projects, one for physics and the other for chemistry, resumed this year and two more physics experiments have begun, one very recently.

Use of the accelerator for online development declined somewhat from last year because of the damage to the microbeam lens system. On-line development should increase next year as we finish developing the present lens system and start developing the double lens system to obtain a beam spot 0.5 μm in diameter.

We have continued to minimize the time spent for inspections of the radiation safety system by not inspecting those systems and target stations that are rarely used. Of course, any facility is inspected before it is put back into use, as is the case for the UH area that is presently being used for a physics experiment.

Accelerator reliability declined this year, bringing repair time to a near-record level. Maintenance and repair time more than doubled over last year and was double our long-term average due to recurring problems in the downcharge power supply in the terminal. This supply is used to spray negative charge on the charging belt, sharing the load with the upcharge supply at the ground end of the accelerator. For

**Table II**

**Accelerator Use, May 2001 - April 2002**
**Percent Usage of Available Days**

| | |
|---|---|
| Radiobiology and associated dosimetry | 32.8% |
| Radiological physics and chemistry | 4.9% |
| On-line facility development and testing | 28.8% |
| Off-line facility development | 12.4% |
| Safety system | 2.4% |
| Accelerator-related repairs/maintenance | 18.3% |
| Other repairs and maintenance | 0.4% |

reasons that have not yet been determined, one of two strings of high voltage diodes in the supply short out. No major repairs or modifications to the accelerator were performed. A vacuum leak in one of the sections of the acceleration tube is a problem that has troubled us for several years and will require a permanent repair unless a replacement accelerator is obtained.

**Development of Facilities**

Development of the single-particle microbeam facility is described here briefly:

- Testing of the single electrostatic quadrupole quadruplet continued, using the existing microbeam facility. A beam approximately 2.5 μm in diameter had been obtained in November 2000, just before the rods that make up the lens were severely damaged due to an accidental application of a voltage that caused sparking. New rods have been constructed, the new lens is in place and initial tests are encouraging.
- There has been interest, especially by the DOE, in the effects of radiations with LETs of 10 keV/μm or less. The present gas detector used to count the ions that have passed through the cells has unity gain and the small signal from the protons is lost in the electronic noise. Efforts to reduce the noise level were unsuccessful, so a new detector with gas gain was built. The signal from the protons is now far removed from the noise and proton irradiations with the microbeam facility can commence as soon as the lens system is operational.
- After successful testing of the laser ion source obtained from the University of Arkansas, a system has been designed for use in the Van de Graaff accelerator. This system will use a spherical electrostatic analyzer lens to focus the beam rather than the cylindrical lens used in the Arkansas design. This lens focuses in both the horizontal and vertical directions to better inject the beam into the accelerator. A mock-up of the lens has been machined in plastic to assure that the lens can be constructed in our shop. A new high-power Nd:YAG laser has been purchased to replace the obsolete one obtained from The University of Arkansas. The laser has been installed and tested.
- A feedback system has been developed for the voice coil stage. The position of the stage is monitored using linear variable differential transformers (LVDTs). Circuitry to drive the voice coils based n the error signal from the sensors has been designed and is being tested. This stage should provide both more accurate and faster positioning than the present stage, which is moved by stepping motors.
- Construction of the new microbeam facility on the floor over the exit of the Van de Graaff continued. New on-line and off-line microscopes, a CCD camera with a built-in image intensifier and a bench for the off-line microscope were purchased. The on-line microscope will have to be modified so that it can be moved in and out of place over the irradiation port. A new industrial computer system with two flat panel monitors and an image analysis board have also been purchased. One monitor will display the operating program and the other the image from the image analysis board. Work is being done on integrating the camera, image analysis system and irradiation control program.

**Personnel**

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

Dr. Alan Bigelow, a postdoctoral fellow, is continuing the development of the laser ion source and the voice coil positioning stage for the microbeam facility.

Dr. Alexander Dymnikov, an expert on ion beam transport, left RARAF in June 2002. We will consult with him on the design of the electrostatic lenses for the microbeam facility.

Mr. Mutian Zhang, the accelerator technician, left RARAF in April 2002 for another position at Columbia University.

Dr. Furu Zhan, a postdoctoral fellow from China, arrived in June 2002. He is assisting in performing the microbeam irradiations and developing the facility.

Mr. Kurt Michel, an undergraduate student from Pace University, is a part-time intern assisting with the development of the voice coil positioning stage for the microbeam facility.

Biologists from the Center for Radiological Research not supported by the RARAF grant are stationed at the facility in order to perform experiments:

- Dr. Charles Geard, the Associate Director of the CRR, continues to spend 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. Brian Ponnaiya is an Associate Research Scientist performing experiments using the track segment and microbeam irradiation facilities.
- Ms. Gloria Jenkins, a biology technician, performs experiments on the microbeam facility for Dr. Geard.
- Dr. Steven Mitchell, a postdoctoral fellow who arrived in February 2002 is replacing Dr. Satin Sawant in research involving neoplastic transformation of C3H10T1/2 cells.
- Dr. Oleg Belyakov, another postdoctoral fellow, arrived in April 2002. He is performing experiments on the track segment and microbeam facilities using model tissue culture systems.
- Ms. Allison Groome, an undergraduate student from Pace University, is an intern assisting Drs. Geard and Ponnaiya on a part-time basis.

**Recent Publications of Work Performed at RARAF (2001-2002)**

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2. Brenner DJ and Elliston CD. The potential impact of the bystander effect on radiation risks in a Mars Mission. *Radiat Res*. **156**:1594-7, 2002.
3. Brenner DJ and Hall EJ. Microbeams: A potent mix of physics and biology. *Rad Protec Dosim* **99**:283-6, 2002.
4. Hall EJ. Genomic instability, bystander effect, cytoplasmic irradiation and other phenomena that may achieve fame without fortune. *Physica Medica* **17**(Supp. 1):21-5, 2001.

5. Hei TK, Zhao YL, Roy D, Piao CQ, Calaf G and Hall EJ. Molecular alterations in tumorigenic human bronchial and breast epithelial cells induced by high LET radiation. *Adv Space Res* **27**:411-9, 2001.
6. Piao CQ and Hei TK. Gene amplification and microsatellite instability induced by tumorigenic human bronchial cells by alpha particles and heavy ions. *Radiat Res*. **155**:263-7, 2001.
7. Ponomarev AL, Cucinotta FA, Sachs RK, Brenner DJ and Peterson LE. Extrapolation of the DNA fragment-size distributions in a high-dose PFGE assay to low doses. *Radiat Res* **156**:594-7, 2001.
8. Roy D, Calaf G and Hei TK. Profiling of differentially expressed genes induced by high LET radiation in breast epithelial cells. *Mol Carcin*. **31**:192-203, 2001.
9. Sawant SG, Zheng W, Hopkins KM, Randers-Pehrson G. Lieberman HB and Hall EJ. The radiation-induced bystander effect for clonogenic survival. *Radiat Res* **157**:361-4, 2002.
10. Zhao YL, Piao CQ, Hall EJ and Hei TK. Mechanism of radiation induced transformation of human bronchial epithelial cells. *Radiat Res* **155**:230-4, 2001.
11. Zhou H, Randers-Pehrson G, Suzuki M, Waldren CA and Hei TK. Genotoxic damage in non-irradiated cells: contribution from bystander effect. 13th Symposium on Microdosimetry, Stresa, Italy, May 26-June 1, 2001. *Radiat Prot Dosim* **99**:227-32, 2002.
12. Zhou H, Suzuki M, Geard CR and Hei TK. Effects of irradiated medium with or without cells on bystander cell responses. *Mutat Res* **499**:135-41, 2002.
13. Zhou H, Suzuki M, Randers-Pehrson G, Vannais D, Chen G, Trosko JE, Waldren CA and Hei TK. Radiation risk to low fluences of alpha particles maybe greater than what we thought. *Proc Nat Acad Sci (USA)* **98**:144100-5, 2001.
14. Zhou H, Xu A, Suzuki M, Randers-Pehrson G, Waldren CA and Hei TK. The yin and yan of bystander versus adaptive response: lessons from the microbeam studies. *International Congress Series* **1236**:241-7, 2002. CRR

Gary Johnson, head of the Design and Instrument Shop, working on a CAD-CAM project for RARAF.