The Radiological Research Accelerator Facility

THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

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An NIH Supported Resource Center

Research Using RARAF

Table I lists the experiments performed at RARAF during the period May 1, 1997 through April 30, 1998 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. Fourteen different experiments were run during this 12-month period, about the same as the previous year.

Eight experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), and six were by outside users, supported by various grants and awards from NIH and NASA.

Brief descriptions of the experiments that have been run at RARAF between May 1, 1997 and April 30 1998 are given here:

Kirby Johnson and Charles Geard of the CRR continued a series of experiments to measure chromosome aberrations in normal human fibroblasts as a function of charged particle LET (Exp. 7) and neutron energy (Exp. 26). The data will be used to try to determine if there is a difference in the relative rates of production of the various types of chromosome aberrations produced by low and high LET which could be used as a 'fingerprint' of high LET.

A collaboration between James Willey of The Medical College of Ohio at Toledo and Tom Hei and Chang Qing Piao of the CRR continued investigating the oncogenic transformation of an immortalized human bronchial epithelial cell line (BEP2D) using *single doses* of 150 keV/μm ^{4}He ions (Exp. 53). Transformed cells implanted in nude mice have yielded tumors, however repeated irradiations are required to reduce statistical uncertainty.

Several different investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells by Richard Miller of the CRR continued. Additional measurements of the oncogenic transformation of synchronized populations of cells (Exp. 56) were made using single doses (0.6 Gy) of 5.9-MeV neutrons given at various intervals after mitotic shake-off. There is a distinct variation in transformation frequency through the cell cycle, with transformation occurring principally in cells in the G_1 and, to a somewhat lesser extent, G_2 phases. Irradiations of cells with 90 keV/μm ^{4}He ions using the microbeam facility (Exp. 73) also continued. The transformation frequency obtained for asynchronous cells for a single ^{4}He ion traversal through the cell nucleus does not appear to be statistically different from background, while cells with two, four or eight traversals show a definite increase in transformation. This result, if confirmed, has significant implications for radon exposure, in which few cell nuclei in the lung are ever traversed by more than one α particle. Cells treated with the same stain used for the microbeam were irradiated using the track segment facility

and a UV source to confirm that the stain and UV, alone and in combination, were not affecting the response of the cells to the ^{4}He ions.

Charles Geard of the CRR has continued studies using the RARAF microbeam facility to irradiate cell nuclei with specific numbers of 90 keV/µm ^{4}He ions (Exp. 71) to observe chromosome aberration and micronuclei production in normal human fibroblasts.

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 of the CRR with the assistance of Li-Jun Wu, a visitor from the Chinese Academy of Science in Hefei, China. By stretching the cells using cAMP, particles were targeted under computer control on the cytoplasm only. The particle imaging and delivery systems are so designed that the chance of a particle hitting the cell nucleus is less than 0.5%. In comparison to irradiations of cell nuclei, cytoplasmic irradiation induced minimal toxicity. While cytoplasmic traversal with one or two particles induced few mutants, those irradiated with four or more particles had an induced mutant frequency that was more than twice the background incidence, and the majority of induced mutants had deletion patterns similar to the spontaneous ones, consisting mostly of smaller alterations involving only the M1C1 gene. This research is the doctoral thesis for Mr. Wu.

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. Most of the cells incubated for 2 hours labeled in discreet, columnar foci while controls were labeled completely across the nucleus. It is hypothesized that the observed foci are the paths of particles through the cell nucleus and the labeling reflects the movement of cellular endonucleases to sites of highly localized DNA damage.

The development of specialized neutron proportional counters (Exp. 82) was continued by Gerhard Randers-Pehrson of the CRR. The first counter being investigated uses a gas mixture consisting primarily of nitrogen. If this detector is successful, detectors will be developed based on other gas mixtures.

William Morgan of the University of California at San Francisco (UCSF), in collaboration with Charles Geard of the CRR, is using the microbeam facility to investigate genomic instability in a human -hamster hybrid with human chromosome 4 after irradiation by ^{4}He ions (Exp. 84). Genomic instability has been evidenced after particle traversals through the cell nuclei, but not through the cytoplasm.

Tom Hei and Gloria Calaf of the CRR have begun experiments using the track segment facility to develop a model for neoplastic transformation in normal human breast epithelial cells (Exp. 85) similar to that used for human bronchial epithelial cells. Cells from transformed colonies resulting from single doses of 150 keV/µm ^{4}He ions are implanted into nude mice to assay for tumor formation.

Experiments to observe genomic instability of human epithelial cells caused by specific numbers of ^{4}He ions traversing the cell nuclei (Exp. 86) were performed by Joel Bedford of Colorado State University using the microbeam facility.

Eric Shron of the Department of Genetics and Cell Metabolism of Columbia University is studying mitochondrial damage by high-LET radiation (Exp. 87). Initial irradiations have been performed using monoenergetic neutrons.

JaeSub Hong and William Craig of the Columbia University Astrophysics Laboratory are investigating materials to shield gamma-ray detectors used in high-altitude balloon flights from neutrons (Exp. 88). Calibrations of their ^{3}He and proton recoil proportional counters have been performed. Neutron and gamma-ray spectra 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.

Table I. Experiments Run at RARAF May 1, 1997 - April 30, 1998

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	No. Days Run
7	C. R. Geard, K. Johnson	CRR	Bio	Studies of the relationship between chromosomal aberrations and DNA repair: Effects of LET	2
26	C. R. Geard, K. Johnson	CRR	Bio	Monoenergetic neutron induction of chromosome aberrations in human cells of fibroblastic or epithelial origin	2
53	J. C. Willey	Med. College of Ohio at Toledo	Bio	Evaluation of the cytotoxic and oncogenic transforming effects of simulated radon daughter products using human bronchial epithelial cells	2
56	R. C. Miller, E. J. Hall	CRR	Bio	Transformation of synchronized C_3H 10T½ cells by neutrons and charged particles of defined LETs	1
71	C. R. Geard	CRR	Bio	Chromosome aberration and micronucleus production in human cells lines by specific numbers of α particles	24
73	R. C. Miller	CRR	Bio	Neoplastic transformation of C3H 10T½ cells by specific numbers of α particles	14
76	T. K. Hei, L-J. Wu	CRR	Bio	Mutation at the S1 locus of human-hamster hybrid (A_L) cells by specific numbers of α particles	29
80	N. F. Metting	PNNL	Bio	Early responses to DNA damage	3
82	G. Randers-Pehrson	CRR	Phys	Neutron detector development	9
84	W. Morgan	UCSF	Bio	Genomic instability using specific numbers of α particles	11
85	T. Hei, G. Calaf	CRR	Bio	Neoplastic transformation of human breast epithelial cells by high-LET radiation	1.0
86	J. Bedford	Colorado State Univ.	Bio	Genomic instability of human epithelial cells induced by specific numbers of α particles	1
87	E. Shron	Columbia Univ.	Bio	Mitochondrial damage by high-LET radiation	1
88	W. Craig, J. Hong	Columbia Univ.	Phys	Development of neutron shields for high-altitude gamma-ray detectors	1

Accelerator Utilization and Operation

Accelerator usage is summarized in Table II. Use of the accelerator for radiobiology and associated dosimetry was approximately 45% higher than last year, 20% higher than the 1992-96 average, and the same as the average for 1987-96. As was the case last year, most of the accelerator use for radiobiology was for microbeam irradiation experiments.

Utilization of the accelerator by radiological physics and chemistry, which had been increasing slowly over the last few years, reduced somewhat and was only 1/3 of the average use from 1987-96 but about the same as for 1992-96. Long-term physics experiments can require large amounts of beam time as evidenced by the high usage in 1987-88, 1988-89, and 1990-91, mostly due to a single physics experiment.

The accelerator was very reliable again this year. Maintenance and repairs were at a record low, only 40% of the 1987-96 average, and less than half the average if the two report periods which required extraordinarily large amounts of repair (1991-92 and 1994-95) are ignored. All maintenance was routine; no major repairs or modifications to the accelerator were performed.

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

Radiobiology and associated dosimetry	38%
Radiological physics and chemistry	4%
On-line facility development and testing	24%
Off-line facility development	28%
Safety system	3%
Accelerator-related repairs / maintenance.	3%

Development of Facilities

Effort on the development of new capabilities increased significantly this year. Developments on the microbeam and low-energy neutron beam are detailed elsewhere in this report.

A new generating voltmeter and voltage control system has been purchased for the Van de Graaff and will be installed in the coming year. This system is designed to regulate the terminal voltage to ±1 keV whereas the current system, installed about 1970, can only regulate to ±3-5 keV. The lower energy spread of the beam is required for the development

of specialized neutron detectors and will also be beneficial for the low-energy neutron facility.

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. 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 part of most working days at RARAF and is particularly involved with the microbeam facility. Dr. Richard Miller continues to work at RARAF about 3-4 days per week. There is one full-time biology technician, Ms. Gloria Jenkins.

Dr. Andrew Ridsdale, who began a postdoctoral position at RARAF in 1997, has recently left for a permanent position in his native country of Canada. He has been replaced by Dr. Hai-Jun Song, who comes to us from Dr. Jacquelyn Yanch's accelerator lab at MIT.

Dr. Dusan Srdoc, who has been a visiting scientist at the CRR and RARAF several times in the past, has begun to work at RARAF to perform microdosimetry measurements, particularly for the low-energy neutron facility.

Scientific advisory Committee

The second meeting of our scientific advisory committee was held on September 8, 1998. Committee members were briefed on the development of new irradiation facilities, and discussions were held on the role of RARAF as a NIH Supported Resource Center. The committee made a number of useful suggestions with regard to the operation and development of the facilities at RARAF, particularly in regard to the new facilities being planned.

RECENT PUBLICATIONS OF WORK PERFORMED AT RARAF (1997-1998)

1. Weaver DA, Hei TK, Hukku B, McRaven JA, Willey JC. Cytogenetic and molecular genetic analysis of tumorigenic human bronchial epithelial cells induced by radon alpha particles. Carcinogenesis 1997;18(6):1251-7
2. Hei TK, Wu LJ, Liu SX, Vannais D, Waldren CA, Randers-Pehrson G. Mutagenic effects of a single and an exact number of alpha particles in mammalian cells. Proc Natl Acad Sci U S A 1997;94(8):3765-70
3. Ponnaiya B, Cornforth MN, Ullrich RL Induction of chromosomal instability in human mammary cells by neutrons and gamma rays. Radiat Res 1997 147:288-294
4. Azziz, N., Ranogajec-Komor, M., Marino, S. A., Klemic, G. A. and Osvay, M. Fast neutron detection using aluminum oxide TLDs. In *Proc. of the IRPA Regional Symposium on Radiation Protection in Neighboring Countries of Central Europe* (J. Sabol, Ed.) pp. 495-497, Prague, Czech Republic, 1997.

5. Geard, C. R., Randers-Pehrson, G., Hei, T. K., Jenkins, G. J., Miller, R. C., Wu, L. J. Brenner, D. J. and Hall, E. J. Microbeam mediated cellular effects: single α particle induced chromosomal damage, cell cycle delay, mutation and oncogenic transformation. In *Microdosimetry, An Interdisciplinary Approach* (D. T. Goodhead, P. O'Neal and H. G. Menzel, Eds.) pp. 327-330, The Royal Society of Chemistry, Cambridge, UK, 1997.

6. Geard, C.R., and Brenner, D.J. Chromosome aberrations induced by neutrons: Implications for radiation protection. In: Effets biologiques des neutrons: Consequences en radioprotection d'electricite de France No. 10, Paris France, pps. 31-43, 1997.

7. Sachs RK, Brenner DJ, Hahnfeldt PJ, Hlatky LR. A formalism for analysing large-scale clustering of radiation-induced breaks along chromosomes. Int J Radiat Biol 1998;74:185-206

8. Randers-Pehrson, G. and Brenner, D. J. A practical target system for accelerator-based BNCT which may effectively double the dose rate. Med. Phys. **28**, 894-896 (1998).

9. Miller, R. C., Marino, S. A., Napoli, J., Shah, H., Hall, E. J., Geard, C. R. and Brenner, D. J. Oncogenic transformation by low-energy neutrons. Radiation Research, In Press (1999).

10. Miller, R. C., Randers-Pehrson, G., Geard, C. R. Hall, E. J. and Brenner, D. J. The oncogenic transforming potential of the passage of single alpha particles through mammalian cell nuclei. PNAS, 96; 00-00 (1999).

11. Obelic, B., Srdoc, D., Djuric, P. M. and Marino, S. A. The frequency distribution of the number of ion pairs in irradiated tissue. Radiat. Res. **149**, 411-415 (1998).

12. Piao, C. Q., Willey, J. C. and Hei, T. K. Alterations of p53 in tumorigenic human bronchial epithelial cells correlate with metastatic potential. Submitted for publication (1998).

13. Lindgren, A. L., Riley, E. F., Miller, R. C. and Ainsworth, J. Comparison of recovery from G_0/G_1 arrest in lens epithelial cells after X, neutron and ^{56}Fe irradiations. Submitted for publication (1998).

14. Wu, L. J., Randers-Pehrson, G., Waldren, C. A., Geard, C. R., Yu, Y. Z. and Hei, T. K. Biological consequence of cytoplasmic irradiation: role of reactive oxygen species. Submitted for publication (1998).

15. Powell, J., H. Ludewig, M. Todosow and M. Reich, Target/filter concept for accelerator-driven boron neutron capture therapy applications. Nuclear Technology (in press, 1999)

16. Hong, J., Hailey, C. J. and Craig, W. W. Development of neutron .shields in gamma-ray detectors. In *Proceedings of the SPIE*, 3445 (O. Siegmund and M. Gunmin, Eds.) in press, 1999.

17. Hei, T. K., Piao, C. Q., Willey, J. C. and Hall, E. J. Genomic instability and tumorigenic induction in immortalized human bronchial epithelial cells by heavy ions. Adv. Space Research. In press (1998).