EXHIBITS ANNEXED TO WRITTEN DISCLOSURE

Exhibit 1. Dr. Hill Curriculum Vitae

Exhibit 2. Dr. Howell Curriculum Vitae

Exhibit 3. Dr. Bishayee Curriculum Vitae

Exhibit 4. Grant Application

Exhibit 5. The September 20, 1999 Experiment

Exhibit 6. Dr.Hill's Observations for the Period of October 11-23, 1999

Exhibit 7. September 6, 1999 experiment

Exhibit 8. Memo to Dr. Raveché from Dr. Hill, dated May 22, 2001

Exhibit 9. Graph Entitled "Cell Count as a Function of Dose on Day 3

Exhibit 10."Terminal Digits and the Examination of Questioned Data" by James E. Mosimann et.al.

Exhibit 11."Data Fabrication: Can People Generate Random Digits?" by James E. Mosimann et.al.

Exhibit 12. Analysis of Coulter Counter Counts by Dr.Bishayee, Dr. Hill and Dr. Lenarczyk

Exhibit 13. Analysis of Scintillation Counts

Exhibit 14. Comparisons of Means and Standard Deviations - Data of Dr. Lenarczyk and Dr. Bishayee

Exhibit 15. Graph Entitled "Is There a Bystander Effect for ^{3}H?"

Exhibit 16. "Evidence for Pronounced Bystander Effects Caused by Nonuniform Distributions of Radioactivity using a Novel Three-Dimensional Tissue Culture Model", by Bishayee et.al.

Exhibit 17. "Free Radical-Initiated and Gap Junction-Mediated Bystander Effect due to Nonuniform Distribution of Incorporated Radioactivity in a Three-Dimensional Tissue Culture Model", by Bishayee et.al.

Exhibit 18. Pub Med Search

Exhibit 19. Log of Observations for Period March 23, 2001 - April 7, 2001.

Exhibit 20. List of Photographs

Exhibit 21. Seventeen (17) photographs identified in Exhibit 20

Exhibit 22. Radioactive Materials Inventory and Disposition Record

Exhibit 23. Pages from Dr. Howell's Notebook

Exhibit 24. List of Radioactivity Counts of the Aliquots taken from the Helena Tubes in the 10.5° incubator on Saturday, March 31,2001

Exhibit 25. Schema for Dr. Howell's protocal

Exhibit 26. Map

Exhibit 27. Letter from Dr. Raveche to Dr. Hill dated April 12, 2001

Exhibit 28. Letter from Dr. Raveche to Dr. Hill dated April 16, 2001

Exhibit 29. UMDNJ's Policy on Misconduct in Science

Exhibit 30. Letter from Dr. Raveche to Dr. Hill dated June 22, 2001

Exhibit 31. Letter from Dr. Saporito, Senior Vice-President for Academic Affairs to Dr. Hill dated July 2, 2001

Exhibit 32. Copy of Dr.Baker's Email sent to Radiology Department and the Attachment to Email dated July 11, 2001 and July 2, 2001, respectively

Exhibit 33. Memorandum from Dr. Howell to Dr. Hill dated July 30, 2001, "Assignment of Laboratory Space".

Exhibit 34. Memorandum from Dr. Howell to Dr. Hill dated July 31, 2001, "Access to Laboratory Space"

Exhibit 35. August 13, 2001 Fax and memo to Dr. Putterman from Dr. Hill

Exhibit 36. Letter from Dr. Hill to Dr. Fields dated August 23, 2001

Exhibit 37. Letter from Dr. Alan Price to Dr. Hill dated August 27, 2001

Exhibit 38. Letter from Chris B. Pascal, JD to Dr. Hill dated September 5, 2002

Exhibit 39. Letter from Dr. Hill to Dr.Fields dated November 3, 2001

Exhibit 40. Email from Dr.Hill to Dr. Field dated December 12, 2001.

Exhibit 41. Letter from Dr. Hill to Dr.Price dated August 22, 2002

Exhibit 42. Letter from Dr. Price to Hill dated September 5, 2002

Exhibit 43. Letter to Dr. Hill from Dr. Forrester dated November 25, 2002

Exhibit 44. UMDNJ Policy on Misconduct in Science

Exhibit 45. Letter from Dr. Saporito to Dr. Hill dated March 21, 2003

Hill, Helene

October 2003

CURRICULUM VITAE

NAME: Helene Z. Hill, Ph.D.
SSN: 011-28-8632

HOME ADDRESS: 3 Silver Spring Road
West Orange, NJ 07052

Tel: 973-736-0738

OFFICE ADDRESS: Section of Cancer Biology, MSB-F452

Department of Radiology
New Jersey Medical School
185 South Orange Avenue
Newark, NJ 07103-2714

Tel: 973-972-3421
Fax: 973-972-5592
E-mail: hill @umdnj.edu

EDUCATION:

Undergraduate: Smith College, Northampton, MA **A.B.** 1950
Graduate: Brandeis University, Waltham, MA **Ph.D.** 1964

POSTDOCTORAL TRAINING:

Harvard Medical School, Boston, MA 1964-66, USPHS Fellow, M.B. Hoagland, M.D., Supervisor: Protein synthesis
University of Colorado Medical Center, Denver, CO 1966-67, USPHS Fellow, T.T. Puck, Ph.D., Supervisor: Mammalian cell genetics

UNIVERSITY APPOINTMENTS:

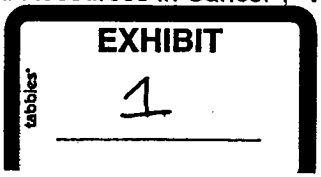
1967-72	Assistant Professor of Biophysics and Genetics University of Colorado School of Medicine, Denver, CO
1973-76	Associate Professor of Radiology, Section of Cancer Biology Washington University School of Medicine, St. Louis, MO
1976-81	Associate Professor of Biochemistry, Marshall University School of Medicine, Huntington, WV
1981	Professor of Biochemistry, Marshall University School of Medicine, Huntington, WV
1981-	Professor of Radiology, UMDNJ-New Jersey Medical School, Newark, NJ
1981-	Professor of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School
1991-	Professor of Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School

OTHER PROFESSIONAL POSITIONS AND MAJOR VISITING APPOINTMENTS:

1963-64	Instructor of Biology, Brandeis University, Waltham, MA
1964-66	Fellow, Department of Microbiology and Immunology, Harvard Medical School, Boston, MA
1966-67	Instructor of Biophysics, University of Colorado School of Medicine
1972	Visiting Professor, Saigon University School of Medicine, Saigon, South Vietnam
1974-76	Adjunct Associate Professor of Biology, Washington University
1976-81	Biochemist, Huntington Veterans' Administration Medical Center, Huntington, WV
1977-81	Member of the Graduate Faculty, West Virginia University, Morgantown, WV
1981-2001	Head, Section of Cancer Biology, Department of Radiology, New Jersey Medical School
1984-	Member of the faculty of the Graduate School of Biomedical Sciences, UMDNJ
1984-	Research Scientist, East Orange Veterans' Administration Medical Center, East Orange, NJ
1985	Visiting Scientist, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland,
1987	Visiting Scientist, Medical Research Division, Argonne National Laboratory, Argonne, IL
1988-	Guest Scientist, Biology Department, Brookhaven National Laboratory, Upton, NY
1996-1997	Visiting Faculty, Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA

AWARDS AND HONORS:

NSF Honor Roll, 1958
President, Univ. of Colorado Medical Center Women's Association, 1971-72
Chapter President, Society of the Sigma Xi, Washington Univ., 1975-76
Baccalaureate Speaker, Chatham Hall, Chatham, VA, May 1980
First recipient, Life Achievement Award, The Baldwin School, Bryn Mawr, PA, 1991
Listed in "American Men and Women in Science," "Who's Who in the East", "Who's Who of American women", "Who's Who Directory of Professionals and Resources in Cancer", "Who's Who in Science and Engineering"
Smith College Medal, February, 1997



Gallo Award for Outstanding Research, presented at the 1998 Annual Retreat on Cancer Research in New Jersey, Princeton, NJ May, 1998

BOARDS OF DIRECTORS/TRUSTEES: Secretary, Faculty Council, Washington Univ. School of Medicine, 1974-76; Board of Directors, South Mountain YMCA, South Orange, NJ (1992-1996); Executive Committee, NJ Academy of Science, 1995-1996; National Board of Advisors, The Baldwin School, Bryn Mawr, PA (1993-99; 2001- ; Executive Committee Member, 1997-99)

MAJOR COMMITTEE ASSIGNMENTS:

a. National and Regional:

New Jersey Institute of Technology: Thesis research supervisor for Grace Coffey, MS, 1985
Rutgers University and UMDNJ: Department of Biochemistry: Thesis research supervisor for Christine Huselton,Ph.D. candidate, 1985-88
East Orange Veterans' Administration Medical Center: Institutional Review Board (1982-83); Research and Development Committee (1983-87)
American Cancer Society - New Jersey Division: Public Education Committee (1987-93); Melanoma and Skin Cancer Detection Awareness Subcommittee (1985-93); Professional Education Committee (1993-94)
American Board of Radiology: Radiobiology Item Writing Task Force 1993-1997
American Society for Photobiology: Council Member (1994-1997; Executive Committee: Secretary 1999-)
NJ Academy of Science: President-elect (1995-1996); President (1996)
PanAmerican Society for Pigment Cell Research: Nominating Committee 1997-1998; 1998-1999; Council, 1999-2002
Ruth Estrin Goldberg Memorial for Cancer Research: Board Member, 1998-

b. Medical School:

UMDNJ-New Jersey Medical School: Biohazards Committee (1981-present, Chairman, 1984-86); Faculty By-Laws Committee (1982-83, 1984-88; 1990-92); Radiation Safety Committee (1981-86); Academic Programs and Policies Committee (1984-86); Cancer Education Committee (1981-88); Faculty Committee on Appointments and Promotions (1984-87); Summer Research Program Review Committee (1983); Institutional Research Review Committee (1986); Cancer Prevention and Control Subcommittee (1988-89); Focus Group on Community-based and International Health Education (1989-90); Institutional Planning and Development Committee (1990-); Institutional Review Board (1991-2); Biomedical Research Support Committee (1994-1997); Cancer Education Program Committee (1992-1998); Campus Security Committee (1993-pres); Dean's Committee on Termination for Cause (1995-pres); Student Affairs Committee (2000-2001); Medical Student interviewer 2001-

UMDNJ-Graduate School of Biomedical Sciences

Ph.D. Thesis Defense Committee for: Wasil Sydor, Biochemistry Department, 1982: Dwight Lee, Pathology Department, 1984; Preliminary Examination Committee and Thesis Advisory Committee for Dorothy McCabe, Ph.D. candidate, Pharmacology Department, 1982-86; Preliminary Examination Committee for Nayantara Kothari, Microbiology and Molecular Genetics Department, 1991

c. Hospital: University Hospital: Oncology Committee (1982-86)

d. Department: Departmental Tenured Faculty Committee on Appointments and Promotions

e. Editorial Boards: Manuscripts reviewed for Plant Physiology, Journal of Cellular Physiology, Science, Journal of the National Cancer Institute, Cancer Letters, Cancer Research, Journal of Investigative Dermatology, Radiation Research, Photochemistry and Photobiology, Pigment Cell Research, Scanning Microscopy International, British Journal of Cancer, Nature, Journal of Photochemistry and Photobiology B. Biology, Journal of Theoretical Biology, Mutation Research, Journal of Inorganic Biochemistry

MEMBERSHIPS, OFFICES AND COMMITTEE ASSIGNMENTS IN PROFESSIONAL SOCIETIES:

American Association for the Advancement of Science
American Association for Cancer Research
American Association of University Professors
American Institute of Biological Sciences
American Society for Photobiology (Council member, 1994-1997; Secretary, 1999-)

Publications Committee (1994-1997)
Education Committee (1994-1997)
Mentoring Committee (1997-)
Executive Committee (1999-)
Secretariat Review Committee (1999-)
Association of Women in Science
Environmental Mutagen Society
European Society for Pigment Cell Research
International Society for Pigment Cell Research
International Association for Women Bioscientists
NJ Academy of Science
Pan-American Society for Pigment Cell Research
Awards Committee (1996)
Nominating Committee (1997, 1998)
Council Member (1999-2002)
Photobiology Foundation (Founding Board member, Secretary-Treasurer, 1999-2001)
Radiation Research Society
Society of the Sigma Xi (President, Washington University Chapter, 1975-1976)
Tissue Culture Association
American Society of Therapeutic Radiologists and Oncologists (Associate Member)

MAJOR RESEARCH INTERESTS:

Cancer Biology, Radiation Biology, Photobiology: Role of melanin in prevention of solar carcinogenesis of skin, Role of autocrine factors in the therapeutic responses of melanomas; Induction of mutations in mitochondria by radiation

GRANT HISTORY:

a. **Principal Investigator:**

USPHS-NIGMS: Gene Expression in Mammalian Cells, 1971-74
American Cancer Society:
Institutional Award, 1974
The Role of DNA Repair in the Therapeutic Responses of Melanoma, 1981-83
Ruth Estrin Goldberg Memorial for Cancer Research:
Scheduling of Cancer Chemotherapeutic Agents Using an Intra-operative Radiation Therapy Mouse Melanoma Model, 1983-84
New Jersey Cancer Research Commission:
Role of Oxygen Radical Damage to DNA in Carcinogenesis, 1984-89
Novel Approaches to Melanoma Therapy, 1988-91
Biomedical Research Support Grant: 1992
USPHS-NCI: Photoprotection and Photosensitization of DNA by Melanin, 1992-1996
Foundation of the UMDNJ:
Preliminary characterization of a radiation resistance factor (RF) from melanoma, 1992-1993
Autocrine rescue of tumors from death by radiation, 1996-1998
Dean's Bridging Support: 1999-2001
Foundation of the UMDNJ: Research Support Grant, 2002-

b. **Co-Principal Investigator:**

VA Research Advisory Group Award and **Merit Review Awards:**
Radiation and Chemotherapy Studies in Melanomas, 1976-88, with George J. Hill, M.D.
Elsa U. Pardee Foundation: Studies of a new multi-therapy resistance factor, 1993- with George J. Hill, M.D.

c. Co-Investigator:
UHPHS: Effects of non-uniform distributions of radioactivity, Roger W. Howell, Principal Investigator 2000-2005
NJ Cancer Research Commission: Effects of low-LET radiation in normal human cells and their progeny, Edouard I. Azzam, Principal Investigator, 2001-2003
USPHS: Damage signaling from irradiated to non-irradiated cells, Edouard I. Azzam, Ph.D., Principal

Investigator 2002-2005

d. Fellowships Sponsored:

NJ Commission for Cancer Research: Fellow: H. Colleen Silva, M.D., Chief of Surgical Oncology, Jersey City Medical Center, Jersey City, NJ Clinical Relevance of a multi-therapy resistance factor 1994-1996

Society for Surgical Oncology: Summer Fellowship for Medical Students awarded to Mauricio Zapiach, NJMS '97 Role of a melanoma multi-therapy resistance factor in apoptosis inhibition

NJ Commission for Cancer Research: Summer Fellowship for Midical Sutdents awarded to Joseph Grossman NJMS '98. Role of a melanoma multi-therapy resistance factor in apoptosis inhibition

Cancer Education Institutional Grant: Summer Fellowship for Undergraduate Students awarded to Cynthia Quainoo, Smith College '99; Summer, 1996, 1997; Fellowships for incoming Medical Students, awarded to Gayatri Rao NJMS '02 and David Muccino NJMS '02, 1998

MAJOR TEACHING EXPERIENCE:

UMDNJ-GSBMS: Molecular Pathobiology of Cancer (J. Cholon, Department of Pathology, Coursemaster); Biochemistry of Cancer (M.A. Lea, Dept. of Biochemistry, Coursemaster); DNA Repair (M. Lambert, Department of Pathology, Coursemaster); Radio-isotopes and Radiation Biology (Co-coursemaster with S. Gertner); Analytical Methods in Biochemistry (B.J. Wagner, Coursemaster); Current Topics in Biochemistry (MA Lea, Coursemaster)
UMDNJ-NJMS: Radiation Biology and Physics (R. Howell, Coursemaster)
Tutorial in Cancer Biology for surgical and medical oncology residents (H.Z.H., Coursemaster); Erythrocyte Biochemistry, Radiation Biology (lectures in Medical Biochemistry); Facilitator in Problem Based Learning in Medical Biochemistry; Biological Effects of Radiation (lecture in Introduction to Clinical Sciences)
UMDNJ-NJDS: Erythrocyte Biochemistry (lecture in Dental Biochemistry)
St. Barnabas Medical Center, Livingston, NJ: Radiation Biology for Radiation Oncology Residents
Rutgers University: Photobiology (W. Ward and B. Zilinskas, Departments of Biochemistry and Microbiology, Coursemasters)
Marshall University: Undergraduate Courses: Biochemistry, Advanced Biochemistry, Human Physiology. Graduate Courses: Cellular and Molecular Biology (Coursemaster for 2 years), Human Biochemistry, Graduate Seminar, Nucleic Acids and Protein Synthesis (Coursemaster for 1 year), Introduction to Research. Medical School Courses: Medical Biochemistry, Medical Genetics (Coursemaster for 4 years)
Washington University: Undergraduate Courses: Introductory Biology, Fundamentals of Cellular and Molecular Biology, Genetics Graduate Courses: Cell Biology, Radiation Biology, Laboratory in Cancer Biology, Mechanisms of Disease - Cancer Medical School Courses: Pathophysiology of Cancer, Genetics and Cancer
University of Colorado: Graduate Courses: Developmental Biology, Human Biochemical Genetics, Human Genetics, Medical School Courses: Medical Biophysics, Biophysics and Genetics
Brandeis University: Undergraduate Courses: Microbiology, General Biology, Introductory Biology

Research Supervision:

Research Associates:

1993-94 Indu Chowdhary (Ph.D. Delhi University, Delhi, INDIA)
1993-95 Shuangwen Zhou (M.S., M.D. Harbin Medical University, Heilongjiang; Ph.D. Tongji Medical University, Wuhan, CHINA)

Post-doctoral Fellows:

1973-84 Anthony T. Morrissey (Ph.D., Harvard Medical School)
1981-82 Nancy Nolan (Ph.D., Catholic Medical School)
1982-83 Hyranne Grimmond (Ph.D., SUNY-Albany)
1981-83 Martha Andersen (Ph.D., SUNY-Buffalo)
1983 Elizabeth Gerges (M.D., Cairo University School of Medicine)
1985 Santosh Raina (M.B.B.S., Topiwala National Medical College, Bombay)
1988 Karen Hubbard-Smith (Ph.D., Illinois Institute of Technology, Chicago, IL)
1989-90 Denise Mammolito (M.D., NJ Medical School, Newark, NJ), Surgical Oncology Fellow; Zoltan Trizna M.D., Semmelweis Medical University, Budapest, HUNGARY); Lincoln Pranikoff (M.D., Tufts University Medical School, Boston, MA), Surgical Oncology Fellow; Uma T. Shankavraman (Ph.D., Osmania University, Hyderabad, INDIA)
1991-92 Uwe Schlehaider (M.D., Ludwig Maximillian Universitaet, Munich, GERMANY)

1992-93 Helina Orgacka (Ph.D., Silesia Academy of Medicine, Katowice, POLAND)
1992-93 Indu Chowdhary (Ph.D., Delhi University, Delhi, INDIA)
1992-96 Colleen Silva, M.D., Fellow in Surgical Oncology, Department of Surgery, NJMS 1992-1994
1993-94 Krystyna Cieszka (Ph.D., Jagiellonian University, Krakow, POLAND)
1993-94 Xiang Ao (M.D., Zunyi Medical College, Zunyi, CHINA)
1993-95 Robert Goodman (Ph.D., New York Medical College, Valhalla, NY)
1994-96 Weixiong Li (M.D. Hunan Medical University; Ph.D. Medical Science and Beijing Union MedicalUniversity, Beijing, CHINA)
1994 Yanhui Liu (M.D. Hunan Medical University, Beijing, CHINA)
1996-97 Hongbing Tang (M.D. Nanjing Medical University, Nanjing, CHINA; M.S., Molecular Biology, Institute of Virology, Chinese Acadmy of Preventive Medicine, Nanjing, CHINA)

Graduate Students:

1980 Shariar Arasteh (M.S. candidate, Marshall University School of Medicine)
1980-81 Rodney Hagley (Ph.D. candidate, Marshall University School of Medicine)
1983-85 Grace Coffey (M.S., New Jersey Institute of Technology, 1985)
1984(summer)Barbara Pilas (Ph.D. candidate, Jagiellonian University, Krakow, POLAND)
1985-88 Research supervisor for Christine Huselton (Ph.D. candidate, Rutgers University/UMDNJ-GSBS; degree conferred 1988)
1993- Research supervisor for Jaskiran Kaur (PH.D. candidate, Punjab University, Punjab, INDIA; degree conferred, Mar, 1999)
1998 Eric Kinnaert (PhD candidate, LOCE, Institut Jules Bordet, Universite Libre, Brussels, BELGIUM)

Medical Students:

Washington University:
1973 Carl Hsieh
1975 Frank Kwong
1976 Michael Pfaller

Marshall University:
1978 David Otto
1980 James Wolf
1979-80 James Banks

New Jersey Medical School:
1983-85 Sharon Kurowski
1983-84 Richard Winne
1984 John Anton
Philip Scarpa
Christine Huselton
1985 Anne Wittenberg
1986 Michael Biunno
Harish Patel
1987 Michael Biunno
1989 Kathleen Nell Cathcart
1990 Joseph Bargellini
1991 Patrice Lahiti
1993 John Cosmi-2nd place – Student Cancer Research Presentations
Diane Carlson
1994-96 Mauricio Zapiach
1995 Joseph Grossman
1996 Valerie Gafori
1997 David Muccino
Gayatri Rao-2nd place – Student Cancer Research Presentations
1998 Stephen Cohen

Undergraduate Students:

1976 Muge Galin
1975 Stuart Brown (Summer)
Helen Kong
Sarah Anscheutz (Smith College January Intern)
1973-74 Roy Philips (Honors Project)
1974 Mark Irwin
1974-76 Matthew Bodner (Honors Project)
1975-76 Kevin Weiss (Honors Project)
1976 Rosaria Salazar
1979 Sonya Vasiri (Summer)
1980 Laura Hawes (Summer)
1981 Timothy Weible
1982 Susan Hyatt (Summer)
1983 Susan Hyatt, Elias Najem
(Summer) 1984 Jeanne Gapac, Anne Felston
(Summer) 1989 David Levi (Summer)
1990-91 Anne Wojtowicz
Bohdan Olesnicky
1992 Tracy Barberi
1993 Ali Pashapour
1994 Neeta Ginde
1995 Michelle Kim (Smith College)
1996,97 Cynthia Quainoo (Smith College)
1998 Glinys Caceres (Wellesley College)
James Netterwald, SHRP
George Ng, Rutgers Univ

High School Students:

1984-85 Andy Chen, Frank Chen, Saroja Rao
1990-92 Samina Ali
1992 Elizabeth Hawkins
1993 Priya Ramashawar
1994 Monifa English
Katharine Hawkins

1995 Shakira Adams

BIBLIOGRAPHY

ARTICLES:

Doctoral Dissertation:

HZ HILL. UV inactivation, photoreactivation and the decay of photoreactivability of the green-colony forming system of Euglena gracilis, Brandeis University, 1964.

Articles:

1. **HZ HILL, JA Schiff and HT Epstein. Studies of chloroplast development in Euglena XII. Variation of** ultraviolet sensitivity with extent of chloroplast development. Biophys J 6:125-34, 1966.
2. **HZ HILL**, JA Schiff and HT Epstein. Studies of chloroplast development in Euglena green colony formation. Biophys J 6:135-44, 1966.
3. **HZ HILL**, HT Epstein and JA Schiff. Studies of chloroplast development in Euglena XV. Factors influencing the decay of photoreactivability of green colony formation. Biophys J 6:373-83, 1966.
4. SH Wilson, **HZ HILL** and MB Hoagland. Physiology of rat liver polysomes III. Protein synthesis by stable polysomes. Biochem J 103:567-72, 1967.
5. TT Puck and **HZ HILL**. Enzyme kinetics in mammalian cells I. Rate constants for galactose metabolism in erythrocytes of normal, galactosemic and heterozygous subjects. Proc Natl Acad Sci USA 57:1676-83, 1967.
6. **HZ HILL** and DW Alling. A model for ultraviolet and photoreactivating light effect in Euglena. Biophys J 9:347-69, 1969.
7. **HZ HILL** and TT Puck. Enzyme kinetics in mammalian cells I. Simultaneous determination of rate constants for the first three steps of galactose metabolism in red cells. J Cell Physiol 75:49-56, 1970.
8. **HZ HILL**. Enzyme kinetics in mammalian cells III. Regulation of activities of galactokinase, galactose-1-phosphate uridyl transferase and uridine diphosphogalactose-4-epimerase in human erythrocytes. J Cell Physiol 78:419-30, 1971.
9. **HZ HILL**, SH Wilson and MB Hoagland. Patterns of albumin and general protein synthesis in rat liver as revealed by gel electro- phoresis. Biochim Biophys Acta 269:477-84, 1972.
10. **HZ HILL** and MB Halcrow. Expression of galactose genes in mammalian cells I. Galactose enzymes in Chinese hamster ovary cell hybrids. Biochem Genet 7:117-26, 1972.
11. **HZ HILL** and TT Puck. Detection of inborn errors of metabolism: Galactosemia. Science 179:1136-39, 1973.
12. TB Friedman, RJ Yarkin, CR Merril, **HZ HILL** and C-Y Young. Galactosemia and galactonolactone: Further biochemical observations. Science 183:764-66, 1974.
13. **HZ HILL** and SI Goodman. Detection of inborn errors of metabolism II. Defects in propionic acid metabolism. Clinical Genetics 6:73-78, 1974. Front article.
14. **HZ HILL** and SI Goodman. Detection of inborn errors of metabolism III. Defects in urea cycle metabolism. Clinical Genetics 6:79-81, 1974.
15. **HZ HILL**. Detection of inborn errors of metabolism IV. Galactokinase deficiency. Int J Clinical Genetics 8:179-82, 1975.
16. TF Smith, M Grunwald, R Artwich and **HZ HILL**. A simple statistical analysis of Indian muntjac giemsa band patterns. Cytogenetics and Cell Genetics 15:153-65, 1975.
17. **HZ HILL**. The effects of pH on incorporation of galactose by a normal human cell line and cell lines from patients with defective galactose metabolism. J Cell Physiol 87:313-20, 1976.
18. GJ Hill, T Shine, **HZ HILL** and CF Miller. Failure of amygdalin to arrest B16 melanoma and BW5147 leukemia. Cancer Research 36:2102-7, 1976.
19. **HZ HILL** and CF Miller. Plating efficiency of mouse embryo cells as a function of gestational age. Experientia 32:1054-55, 1976.
20. M. Grunwald and **HZ HILL**. Characterization of the glucose-6-phosphate dehydrogenase activity in rat liver mitochondria. Biochem J 159:683-87, 1976.
21. **HZ HILL** and R Phillips. A survey of commercially available tissue culture media for the propagation of Indian muntjac cells. Tissue Culture Manual 4:831-32, 1978.
22. **HZ HILL**, GJ Hill and J Szramowski. Dacarbazine and melphalan: Enhancement by dosage scheduling of the effect of combination treatment on the Harding-Passey melanoma in C3D2F1 mice. Arch Surg 114:135-38, 1979.
23. **HZ HILL**, GJ Hill, CF Miller, M Pfaller, K Weiss and M Galin. Effects of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide, 1-(2-chloroethyl)-(4-methylcyclohexyl)-1-nitrosourea and L-phenyl- alanine

mustard on B16, Cloudman S91 and Harding-Passey mouse melanomas. Cancer Research 39:934-39, 1979.

24. **HZ HILL**, GJ Hill, CF Miller, F Kwong and J Purdy. Radiation and melanoma. Response of B16 mouse tumor cells and clonal lines to x-radiation. Radiation Research 80:259-76, 1979.
25. **HZ HILL**, R Backer and GJ Hill. Blood cyanide levels in mice after administration of amygdalin. Biopharmaceutics and Drug Disposition 1:211-20, 1980.
26. **HZ HILL** and RB Setlow. Post-replication repair in 3 murine melanomas, EMT6 and a normal mouse lung fibroblast line. Cancer Research 40:1867-72, 1980
27. **HZ HILL** and RB Setlow. Comparative action spectra for pyrimidine dimer formation in Cloudman S91 mouse melanoma and EMT6 mouse mammary carcinoma cells. Photochemistry and Photobiology 35:681-84, 1982.
28. **HZ HILL** and GJ Hill. Effect of scheduling of combinations of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide and 1-(2-chloro- ethyl)-3-(4-methylcyclohexyl)-1-nitrosourea on the Harding-Passey and the Cloudman S91 mouse melanomas. Cancer Research 42:838-42, 1982.
29. GJ Hill and **HZ HILL**. Cyclophosphamide activity against B16 melanoma in a rapid in vitro test system. J Surg Oncol 24:73-78, 1983.
30. GJ Hill, ET Krementz and **HZ HILL**. DTIC and combination therapy for melanoma: IV. Late results after complete response to chemotherapy (COG protocols 7130, 7131 and 7131A). Cancer 53:1299-1305, 1084.
31. S. Raina, **HZ HILL**, GJ Hill and BF Rush Jr. Scheduling of combination chemotherapy for a murine melanoma with the sub-renal capsular assay. J Surg Oncol 26:51-52, 1984.
32. **HZ HILL** and GJ Hill. In vitro activation of cyclophosphamide for an in vitro assay system. J Surg Oncol 26:225-29, 1984.
33. BH Fadem and **HZ HILL**. The gray opossum (Monodelphis domestica): a marsupial model for xenogeneic neoplasms. Cancer Letters 27:233-38, 1985.
34. DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and **HZ HILL**. In vivo effects of iron-55 and iron-59 on mouse testes: Biophysical dosimetry of Auger-electrons. J Nuclear Medicine 26:1456-65, 1985.
35. DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and **HZ HILL**. Radiobiological effects of Auger electron emitters in vivo: Spermatogenesis in mice as an experimental model. Radiat Prot Dosimetry 13:245-49, 1985.
36. KA Zirvi, GJ Hill and **HZ HILL**. Comparative studies of chemotherapy of human tumor cells in vitro by 3HThd uptake inhibition and soft agar clonogenic assay. J Surg Oncol 31:123-26, 1986.
37. MA Lea, A Luke, O Velezquez, L Carpenter, CF Martinson, **HZ HILL** and GJ Hill. Effects of sodium cyanate in mice bearing B16 melanoma. Cancer Chemother and Pharmacol 17:231-35, 1986.
38. KS Dasmahapatra, **HZ HILL**, A Dasmahapatra and S Suarez. Evaluation of adenosine deaminase activity in patients with head and neck cancer. J Surg Research 40:368-73, 1986.
39. DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and **HZ HILL**. In vivo effects of iron-55 and iron-59 on mouse testes: Efficacy of Auger electrons vs beta rays. Radiat Prot Dosimetry, 1986.
40. GJ Hill, KA Zirvi, **HZ HILL** and S Raina. Chemosensitivity testing with antineoplastic agents. J Med Soc NJ 84:437-41, 1987.
41. **HZ HILL**, M Ohanian, GJ Hill and R Winne. The use of the 90Sr applicator for intraoperative radiation therapy in a mouse tumor model. J Surg Oncol 34:264-67, 1987.
42. **HZ HILL**, CA Huselton, B Pilas and GJ Hill. The ability of melanins to protect against the radiolysis of thymine and thymidine. Pigment Cell Research 1:81-86, 1987.
43. **HZ HILL** and GJ Hill. Eumelanin causes strand breaks and kills cells. Pigment Cell Research 1:163-70, 1987.
44. BH Fadem, **HZ HILL**, CA Huselton and GJ Hill. Transplantation, growth and regression of mouse melanoma xenografts in neonatal marsupials. Cancer Investigation 6:403-8-1988.
45. **HZ HILL**, JG Peak and MJ Peak. Induction of DNA-protein crosslinks in melanotic Cloudman S91 mouse melanoma cells and EMT6 mouse mammary carcinoma cells by monochromatic 254 and 405 nm light. Pigment Cell Research 2:427-30, 1989.
46. CA Huselton and **HZ HILL**. Melanin photosensitizes UVC DNA damage in pigmented cells. Environmental and Molecular Mutagenesis 16:37-43, 1990.
47. **HZ HILL** and GJ Hill. Irradiation of cells attached or suspended by rubber-policeman or by trypsin influences the extent of ionizing radiation-induced DNA strand breaks. Radiation Research 125:343-345, 1991.
48. GP Studzinski, KB Reddy, **HZ HILL** and AK Bhandal. Potentiation of ara-C cytotoxicity to HL 60 cells by $1,25(OH)_2$ vitamin D_3 correlates with reduced rate of maturation of DNA relication intermediates. Cancer Research 51: 3451-3455, 1991.

49. **HZ HILL**, KN Cathcart, J Bargellini, Z Trizna, GJ Hill, KU Schallreuter and JM Wood. Does melanin affect the low LET radiation response of Cloudman S91 mouse melanoma cell lines? Pigment Cell Research 4: 80-86, 1991.
50. **HZ HILL**, Z Trizna, M Ali and GJ Hill. A radiation resistance factor in cultured Cloudman mouse melanoma cells. Radiation Research 129: 43-47, 1992.
51. K Hubbard-Smith, **HZ HILL** and GJ Hill. Melanin both causes and prevents oxidative base damage in DNA: Quantitation by anti-thymine glycol antibody. Radiation Research 130: 160-165, 1992.
52. UK Schlehaider, GJ Hill and **HZ HILL**. Influence of an autocrine diffusible resistance factor on cell survival after exposure to therapeutic agents. Melanoma Research 3: 357-362, 1993.
53. U Schlehaider, **HZ HILL**, A Pashapour and GJ Hill. Influence of an autocrine multi-therapy resistance factor on radiation responses of melanoma cells. Melanoma Research 4:21-27, 1994
54. KU Schallreuter, KR Lemke, **HZ HILL** and JM Wood. Thioredoxin reductase induction coincides with melanin biosynthesis in brown and black guinea pigs and in murine melanoma cells J Invest Dermatol 103: 820-824, 1994.
55. KA Cieszka, **HZ HILL**, GJ Hill and PM Plonka. Growth and pigmentation in genetically related Cloudman S91 melanoma cell lines treated with 3-isobutyl-1-methyl-xanthine and β-melanocyte stimulating hormone. Experimental Dermatology, 4: 192-198,1995
56. **HZ HILL**, GJ Hill, K Cieszka, M Azure, I Chowdhary and RM Sayre. A multi-therapy resistance factor from melanoma reveals that killing by near UV is different from genotoxic agents. Photochemistry and Photobiology 61: 479-483, 1995.
57. RM Goodman and **HZ HILL**. Interference by cellular melanin with assay of DNA-protein crosslinks by the potassium dodecyl sulfate precipitation method. Pigment Cell Research 9:68-71, 1996.
58. GJ Hill, BF Rush, Jr, **HZ HILL**, LF Pliner, S Raina, S Cook, K Klein, C Gerne and R Slimowitz. Breast cancer in the inner city: Intensive efforts may be succeeding. J. Medicine 27: 135-151, 1996.
59. **HZ HILL**. The photobiology of melanin. Introduction. Photochemistry and Photobiology 65: 471, 1997.
60. W. Li and **HZ HILL**. Induced melanin reduces mutations and cell killing in mouse melanoma. Photochemistry and Photobiology 65:480-485, 1997. *Symposium-in-Print*, **HZHILL**, editor.
61. **HZ HILL**, GJ Hill, K Cieszka, PM Plonka, DL Mitchell, MF Meyenhofer, P. Xin and RE Boissy. Comparative action spectrum for ultraviolet light killing of mouse melanocytes from different genetic coat color backgrounds. Photochemistry and Photobiology 65: 983-989, 1997.
62. K. Cieszka, **HZ HILL**, P. Xin, M. Azure, GJ Hill, MF Meyenhofer, RE Boissy and DL Mitchell. Survival of Cloudman mouse melanoma cells after irradiation by solar wavelengths of light. Pigment Cell Research 10: 193-200, 1997.
63. **HZ HILL**, W. Li, P Xin and DL Mitchell. Melanin: A two edged sword? Pigment Cell Research 10: 158-161, 1997.
64. S Zhou, W Li, **HZ HILL**, GJ Hill, F Kueppers, LJ Wolansky and HC Silva. Therapeutic resistance: Characterization and inactivation by specific antiserum of a putative protein family produced by tumor cells. Melanoma Research 9: 1-11, 1999.
65. **HZ HILL** and GJ Hill. UVA, pheomelanin, and the carcinogenesis of melanoma. Pigment Cell Research 13: 140-144, 2000.
66. E. Kinnaert, R. Morandini, S. Simon, **HZ HILL**, G. Ghanem, P Van Houtte. The degree of pigmentation modulates the radiosensitivity of human melanoma cells. Radiation Research 154: 497-502, 2000.
67. GJ Hill and **HZ HILL**. A four-way test of mortality: A useful tool for cancer education. J Cancer Education 16: 185-18, 2001.
68. J. Kaur and **HZ Hill**. Transfection of non-melanocytic cells with tyrosinase gene constructs for survival studies. Environmental and Molecular Mutatgenesis 38:216-222, 2001
69. HZ HILL, GJ Hill, C Cathcart. Photo-recall of sunburn induced by radiation therapy 50 years later. J. Medicine 33:115-118, 2002.
70. SR Baker, S Mann, CM Mele and H Hill. The distribution of emigrant radiologists, revisited: Characteristics of the cohort arriving after 1980. Asian Ocieanian J Radiology 7: 104-110, 2002.

BOOKS, MONOGRAPHS AND CHAPTERS:

1. **HZ HILL** and H-S Lin. "Carcinogenesis and tumor growth" in Clinical Oncology, J Horton and GJ Hill, eds., Saunders, Phila., 1977, pp 1-33.
2. GJ Hill and **HZ HILL**. "Persisting cancer: indications for chemotherapy" in Surgical Care: A Physiological Approach to Clinical Management. R Condon and JJ DeCosse, Eds., Lea and Febiger, 1980.
3. GJ Hill, **HZ HILL** and M Blumenreich. "Treatment of melanoma" in Skin Cancer, Recognition and Management, RA Schwartz, ed., Springer Verlag, 1988, pp 390-417.

4. **HZ HILL.** The relationship of the photobiology of skin cancer and melanins to the radiation biology of melanomas. Comments on Molecular and Cellular Biophysics 6:141-74, 1989.
5. **HZ HILL.** Melanins in the photobiology of skin cancer and the radiobiology of melanomas. In **Cancer Biology and Biosynthesis**, SH Wilson, ed., Telford Press, Caldwell, NJ, 1991, pp 31-53.
6. **HZ HILL.** The function of melanin or six blind people examine an elephant. BioEssays 14: 49-56, 1992.
7. **HZ HILL.** Is melanin photoprotective or is it photosensitizing? in *Melanin, its role in human photoprotection* (L Zeise, M Chedekel and T.B. Fitzpatrick, eds.) Valdenmar Publishing Co, Overland Park, KS. 1995 pp 81-89
8. K Cieszka, **HZ HILL** and GJ Hill. Melanin content in genetically related Cloudman melanoma cells stimulated to differentiate, in *Melanin, its role in human photoprotection* (L Zeise and M Chedekel, eds.) Valdenmar Publishing Co, Overland Park, KS. 1995 pp 151-154

ABSTRACTS:

1. **HZ HILL**, HT Epstein and JA Schiff. Studies of the photoreactivation of ultra-violet inhibition of chloroplast formation in Euglena gracilis. Biophys J 2:1962.
2. **HZ HILL**, HT Epstein and JA Schiff. Studies of the photo- reactivation of ultra-violet inhibition of chloroplast formation in Euglena gracilis. Northwestern Plant Physiology meeting, May 1962.
3. **HZ HILL**, DW Alling, HT Epstein and JA Schiff. The kinetics of photoreactivation in the UV-damaged chloroplast system of Euglena. Biophys J 3:1963
4. **HZ HILL**, DW Alling, HT Epstein and JA Schiff. The loss of photoreactivability in the UV-damaged chloroplast system of Euglena with time. Biophys J 3:1963.
5. TT Puck and **HZ HILL**. Kinetics of galactose metabolism in mammalian cells. Biophys J 7:102a, 1968.
6. **HZ HILL** and TT Puck. Enzyme chain analysis in mammalian cells. Biophys J 8:163a, 1968.
7. TT Puck and **HZ HILL**. Galactose enzyme kinetics in normal, galacto- semic and heterozygous mammalian cells. Genetics Society of America, 1968.
8. **HZ HILL** and TT Puck. Kinetics of galactose metabolism in mammalian cells III. Biophys J 9:257a, 1970.
9. **HZ HILL** and TT Puck. Kinetics of galactose metabolism in mammalian cells IV. Biophys J 10:163a, 1970.
10. **HZ HILL**. Galactokinase and galactose-1-phosphate uridyl transferase in diploid and tetraploid Chinese hamster ovary cells. Biophys J 11:161a, 1971.
11. **HZ HILL**. Gene expression in mammalian cells and its relation to human molecular disease. IVth Int Congr on Human Genetics, Paris, September 1971.
12. **HZ HILL** and TT Puck. Incorporation of 14C-galactose into tissue culture cells of various origins. 10th Annual Somatic Cell Genetics Conf, Aspen, CO, January 1972.
13. **HZ HILL**. Detection of inborn errors of metabolism: Galactosemia. Biophys J 13:331a, 1973.
14. **HZ HILL** and SI Goodman. Detection of inborn errors of metabolism: Methylmalonicacidemia. Am J Human Genetics 25:32a, 1973.
15. **HZ HILL**. Plating efficiency of primary mouse embryo cells as a function of gestational age. J Cell Biology 63:138a, 1974.
16. **HZ HILL**. Studies of B16 melanoma grown in vivo and plated in vitro: feeder-layer effects. Proc Amer Assoc Cancer Research 16:198, 1975.
17. **HZ HILL**, F Kwong and CF Miller. Radiation response of B16 mouse melanoma cells. Biophys J 16:52a, 1976.
18. **HZ HILL**, GJ Hill, F Kwong and CF Miller. Radiation response of B16 mouse melanoma cells in vitro. Proc Amer Assoc Cancer Research 17:22, 1976.
19. GJ Hill, **HZ HILL** and TM Vietti. Response of B16 melanoma to DTIC, MeCCNU and LPAM. Proc Amer Assoc Cancer Research 17:22, 1976.
20. **HZ HILL**, GJ Hill, M Pfaller, K Weiss and M Galin. Comparative effects of DTIC, MeCCNU and LPAM on 3 transplantable mouse melanomas. Proc Amer Assoc Cancer Research 18:9, 1977.
21. **HZ HILL** and GJ Hill. Effects of DTIC and LPAM alone and in combination on Harding-Passey melanoma in CDF1 mice. Proc Amer Assoc Cancer Research 19:46, 1978.
22. **HZ HILL**, GJ Hill and J Szramowski. Synergistic effect of DTIC and LPAM combination treatment on the Harding-Passey melanoma in CD2F1 mice: importance of dosage scheduling. Assoc of VA Surgeons, May 1978.
23. **HZ HILL** and RB Setlow. Post-replication repair in B16, Cloudman S91 and Harding-Passey mouse melanomas compared to EMT6 and a normal mouse lung fibroblast line. Proc Amer Assoc Cancer Research 20:221, 1979.

24. GJ Hill and **HZ HILL**. Synergism between MECCNU and DTIC in treatment of B16, Cloudman S91 and Harding-Passey mouse melanomas. Proc Amer Assoc Cancer Research 20:271, 1980.
25. **HZ HILL** and GJ Hill. Comparison of in vitro drug response of B16 mouse cells and tumors as a predictor of therapeutic effectiveness. Proc Amer Assoc Cancer Research 21:189, 1980.
26. **HZ HILL** and GJ Hill. Differential sensitivity of post-replication repair and replicative fork migration to inhibition by hydroxyurea in mouse melanoma cells. J Cell Biol 87:103a, 1980.
27. **HZ HILL**. Differential effect of hydroxyurea on replicon joining and post-replication repair in mouse melanoma cells. J Supramolecular Structure Suppl 5:189, 1981.
28. **HZ HILL**. Hydroxyurea affects post-replication repair less than replicon-joining in mouse melanoma cells. Symposium on Molecular and Cellular Mechanism of Mutagenesis. Gatlinberg, TN, April 1981.
29. **HZ HILL** and GJ Hill. Hydroxyurea inhibits replicon-joining more than post-replication repair. Proc Amer Assoc Cancer Research 22:32, 1981.
30. **HZ HILL**. Differential inhibition of two steps in replicon-joining and daughter strand gap repair by aphidicolin, cytosine arabinoside and hydroxyurea. J Cellular Biochem Suppl 6:371, 1982.
31. **HZ HILL** and GJ Hill. Aphidicolin and ara-C inhibit DNA chain elongation more than replicon joining and daughter strand gap repair after UV in B16CL4 mouse melanoma cells. Proc Amer Assoc Cancer Research 23:12, 1982.
32. K. Zirvi, **HZ HILL** and GJ Hill. Comparative studies of chemotherapy of human tumor cells in vitro by tritiated thymidine uptake inhibition and clonogenic assay. Proc Amer Assoc Cancer Research 24:302, 1983.
33. HE Grimmond and **HZ HILL**. Accessibility to micrococcal and S1 nucleases of regions in chromatin of MLF mouse lung fibroblast and B16CL4 melanoma cells treated with BCNU. Proc Amer Assoc Cancer Research 24:152, 1983.
34. K. Zirvi, GJ Hill, **HZ HILL** and NK Cheung. A rapid short term assay for in vitro drug sensitivity of human tumor cells. Assoc for VA Surgeons, June 1983.
35. GJ Hill, ET Krementz and **HZ HILL**. Long term control of metastatic melanoma with chemotherapy, implications for surgical adjuvant therapy. Soc Surg Oncol Annual Meeting, May 1983.
36. **HZ HILL**. Interactive effects of dideoxythymidine and DNA polymerase alpha inhibitors on DNA synthesis in untreated and UV-treated B16CL4 mouse melanoma cells. Radiation Research Society Abstracts, p 64, 1984.
37. **HZ HILL**, GJ Hill and SM Kurowski. Post-replication repair after UV in human amelanotic and melanotic melanomas. Proc Amer Assoc Cancer Research 25:108, 1984.
38. **HZ HILL**. The use of the Sr-90 applicator for intraoperative radiation therapy in a mouse model. Radiology 153(P):323-24, 1984.
39. **HZ HILL**, M Ohanian, GJ Hill and R Winne. Development of an intra-operative radiation therapy model in mice using the Sr-90 applicator. Presented to Oncol Soc of NJ, West Orange, NJ, 1984.
40. GJ Hill, **HZ HILL**, KA Zirvi and S Raina. Chemosensitivity testing with antineoplastic agents. Present to Oncol Soc of NJ, West Orange, NJ, 1984.
41. GJ Hill, KA Zirvi and **HZ HILL**. Continuing education for Cancer Research Scientists. Presented to Amer Assoc Cancer Education, Tarrytown, NY, 1984.
42. DV Rao, DSR Sastry, GF Govelitz, HE Grimmond and **HZ HILL**. Radiobiological effects of auger electron emitters in vivo: Spermatogenesis in mice as an experimental model. Presented to the Ninth Symposium on Microdosimetry, Brussels, 1985.
43. GJ Hill, **HZ HILL**, K Zirvi and S Raina. Chemosensitivity testing with antineoplastic agents. Presented to Soc of Surg Oncol, 1985.
44. MA Mohit-Tabatabai, BH Natelson, JM Burke, **HZ HILL**, WN Tapp, JE Ottenweller and GJ Hill. Effect of stress, surgery and age on survival in B16 melanoma. Proc Amer Assoc Cancer Research 26:69, 1985.
45. **HZ HILL**, GJ Hill, M Ohanian and R Winne. Intraoperative radiation therapy (IORT) of renal implants of B16 melanoma in C57BL/6 mice. proc Amer Assoc Cancer Research 26:67, 1985.
46. **HZ HILL**, M Ohanian, GJ Hill and R Winne. Use of the 90Sr applicator (90SA) in a mouse model for intraoperative radiation therapy (IORT). Radiation Research Soc Abstr:112, 1985.
47. MA Lea, A Luke, O Velazquez, L Carpenter, CF Martinson, **HZ HILL** and GJ Hill. Action of cyanate on B16 melanoma. Proc Amer Assoc Cancer Research 26:321, 1985.
48. MA Mohit-Tabatabai, BH Natelson, MJ Burke, **HZ HILL**, WN Tapp, JE Ottenweller and GJ Hill. The effect of cold-restraint stress on the outcome of cancer after surgery. Fed Proc 44:910, 1985.
49. CA Huselton and **HZ HILL**. Effect of gamma irradiation in the presence of melanin upon solutions of thymine and thymidine. Presented to NJ Academy of Science, March 1985.
50. CA Huselton and **HZ HILL**. Effect of gamma irradiation in the presence of melanin upon solutions of thymine and thymidine. Photochem Photobiol 41:14s, 1985.

51. **HZ HILL**, HS Newmark, CA Huselton, K Mercer and B Pilas. Melanins as free-radical scavengers. Presented at the VIth European Workshop on Melanin Pigmentation, Murcia, SPAIN, September 1985.
52. KS Dasmahapatra, **HZ HILL**, A Dasmahapatra and S Suarez. Evaluation of adenosine deaminase activity in patients with head and neck cancer. Presented to Assoc for Acad Surg, Cincinnati, November 1985.
53. RW Howell, KSR Sastry, **HZ HILL** and DV Rao. Cis-Platinum-193m: Its microdosimetry and potential for chemo-Auger combination therapy of cancer. 4th Int Pharmaceutical Dosimetry Symp, Oak Ridge, TN, p 495, 1985.
54. **HZ HILL** and CA Huselton. The effectiveness of melanin at protecting DNA components from ionizing radiation damage. Radiation Research Soc Abstr:40, 1986.
55. **HZ HILL** and CA Huselton. Eumelanin is a free radical scavenger in irradiated solutions of thymidine. Photochem and Photobiol 43:114s, 1986.
56. **HZ HILL**, CA Huselton and SS Raina. The role of melanin in the radiation resistance of melanoma and the prevention of skin cancer. Abstr of the 14th Int Cancer Congr, Budapest, HUNGARY, p 614, 1986.
57. **HZ HILL**, B Pilas and GJ Hill. Eumelanin enhances DNA strand breaks caused by ionizing radiation in B16CL4 melanoma cells. Abstr of XIIIth International Pigment Cell Conference, Tucson, AZ, p 70, 1986.
58. **HZ HILL**. Exogenous melanin causes DNA strand breaks and is synergistic with ionizing radiation damage. Workshop on Cancer Research in New Jersey, November 1986.
59. **HZ HILL**, B Pilas and GJ Hill. Exogenous melanin and ionizing radiation are synergistic in producing strand breaks in DNA. Radiation Research Soc Abstr:101, 1987.
60. CA Huselton and **HZ HILL**. Preliminary studies on the efficiency of UVC radiation in producing thymine base damage in a melanotic and an amelanotic S91 cell line. Photochem Photobiol 45:23S, 1987.
61. CA Huselton and **HZ HILL**. UVC produced thymine base damage in a melanotic and an amelanotic S91 cell line - a preliminary report. Bull NJ Acad Sci 32:37-38, 1987.
62. **HZ HILL**, CA Huselton and GJ Hill. DNA damage prevented and caused by melanin. Pigment Cell Research 1:263-264, 1987.
63. CA Huselton, **HZ HILL** and GJ Hill. Enhancement of UVC-induced indirect and protection against direct effect DNA damage in melanotic Cloudman S91 mouse melanoma cells. Pigment Cell Research 1:279, 1987.
64. **HZ HILL**, CA Huselton and GJ Hill. Eumelanin from various sources causes strand break damage in DNA of B16CL4 mouse melanoma cells. Pigment Cell Research 1:299, 1987.
65. **HZ HILL**. A possible ambiguous role for melanin in solar carcinogenesis. Workshop on Cancer Research in New Jersey, November 1987.
66. CA Huselton and **HZ HILL**. UVC-produced thymine dimer in amelanotic, melanotic and non-melanotic cells. Workshop on Cancer Research in New Jersey, November 1987.
67. **HZ HILL**, JG Peak and MJ Peak. Differential responses of a mouse melanoma cell line and a mammary carcinoma line to DNA-protein crosslinking by 254 and 405 nm radiation. J Cellular Biochem Suppl 12A:288, 1988.
68. CA Huselton and **HZ HILL**. UVC-induced thymine damage present in melanotic, amelanotic and non-melanotic cells. Photochem Photobiol 47 Suppl:47S, 1988.
69. **HZ HILL**, JG Peak and MJ Peak. Intracellular melanin in Cloudman S91 melanoma cells protects against 405 nm but not 254 nm induction of DNA-protein crosslinks. Photochem Photobiol 47 Suppl:60S, 1988.
70. **HZ HILL**, MJ Peak and JG Peak. DNA-protein crosslinks induced by UVA but not by UVC are reduced in melanotic melanoma cells. Radiation Research Soc Abstr 102:64, 1988.
71. CA Huselton and **HZ HILL**. UVC-induced thymine damage present in melanotic, amelanotic and non-melanotic cells. Radiation Research Soc Abstr 102:65, 1988.
72. RB Setlow and **HZ HILL**. Melanotic Cloudman S91 mouse melanoma cells are more sensitive to killing by a solar lamp than isogenic hypomelanotic cells. PanAmerican Pigment Cell Biology Meeting Abstracts:IV-6, 1988.
73. **HZ HILL**, MJ Peak and JG Peak. Fewer DNA-protein crosslinks (DPC) are induced in melanotic mouse melanoma cells than in mouse mammary carcinoma cells by 405 nm radiation but not by 254 nm radiation. PanAmerican Pigment Cell Biology Meeting Abstracts:IV-7, 1988.
74. GJ Hill and **HZ HILL**. Clinical implications of DNA damage induced in melanotic melanoma cells by various radiations. PanAmerican Pigment Cell Biology Meeting Abstracts:IV-8, 1988.
75. CA Huselton and **HZ HILL**. Thymine adducts produced when melanotic and non-melanotic cells are exposed to UVC radiation. PanAmerican Pigment Cell Biology Meeting Abstracts:VI-8, 1988.
76. K Hubbard-Smith and **HZ HILL**. Oxidative damage in DNA induced by melanin. Radiation Research Society Abstracts:120, 1989.

77. **HZ HILL**, K. Hubbard-Smith, CA Huselton and GJ Hill. Melanin is a photo- and radiosensitizer for oxidative damage in DNA of Cloudman S91 melanotic melanoma cells and in DNA in vitro. J Cellular Biochemistry Suppl 13B:17, 1989.
78. K Hubbard-Smith and **HZ HILL**. Immunological quantification of oxidative base damage in DNA induced by melanin in vitro with and without ionizing radiation. PanAmerican Society for Pigment Cell Research Abstracts:69, 1989.
79. **HZ HILL**. Melanotic Cloudman S91 mouse melanoma cells are more sensitive to UV and less sensitive to ionizing radiation killing than isogenic hypomelanotic cells. European Society for Pigment Cell Research Abstracts:29, 1989.
80. **HZ HILL**. Identification of thymine glycol damage in melanoma cell DNA after UVC and its dependence on intracellular melanin concentration. European Society for Pigment Cell Research Abstracts:30, 1989.
81. K Hubbard-Smith and **HZ HILL**. The two edged sword: Induction of thymine glycol (TG) base damage in DNA by melanin and protection by melanin of ionizing radiation induced by TG base damage: An immunological study. European Society for Pigment Cell Research Abstracts:48, 1989.
82. **HZ HILL**. Melanotic Cloudman S91 mouse melanoma cells are more sensitive to UV and less sensitive to ionizing radiation killing than isogenic hypomelanotic cells. Photochemistry and Photobiology 49:73s, 1989.
83. **HZ HILL**. Gamma-ray survival of Cloudman S91 melanoma cells varying in intracellular melanin content. J Cellular Biochem 14A(suppl):66, 1990.
84. K. Hubbard-Smith and **HZ HILL**. Quantitation of oxidative base damage in DNA induced by melanin in vitro. J Cellular Biochem 14A(suppl):74, 1990.
85. **HZ HILL**, KNS Cathcart, Z Trizna and DM Mammolito. Gamma radiation survival of Cloudman S91 mouse melanoma subclones that differ in melanin content. Radiation Research Society Abstracts:190, 1990.
86. **HZ HILL**, GJ Hill and K. Hubbard-Smith. Relationship of intracellular melanin concentration to DNA protection or DNA damage from ultraviolet and ionizing radiation. Amer Assoc Cancer Research Proc 31:74, 1990.
87. GJ Hill, **HZ HILL**, C Huselton and K Hubbard-Smith. DNA damage, gamma radiation effects and UVC photosensitization caused by melanin. Proc ASCO 9:279, 1990.
88. **HZ HILL**, Z Trizna and DM Mammolito. Low LET ionizing radiation response of mouse melanoma cells is influenced by induction of melanin synthesis. J Cancer Research & Clin Oncol 116(suppl):392, 1990.
89. **HZ HILL**, J Bargellini, Z Trizna and GJ Hill. Induction of double strand breaks by low LET ionizing radiation in DNA of mouse melanoma cells varying in intracellular melanin. NJ Commission on Cancer Research 4th Ann Workshop on Cancer Research in NJ Abstracts: 17, 1990.
90. **HZ HILL**, GJ Hill and Z Trizna. Radioresistant melanotic Cloudman S91 melanoma cells elaborate a factor that makes clonally related amelanotic radiosensitive cells become radioresistant. J Cellular Biochem Suppl 15D:102, 1991.
91. **HZ HILL**, M Ali and GJ Hill. Transfer of radioresistance to radiosensitive mouse melanoma cells. Photochem Photobiol 53 (Suppl):27S, 1991.
92. **HZ HILL**, M Ali, Z Trizna and GJ Hill. Transfer of radioresistance to radiosensitive melanoma cells by a diffusible facttor from radioresistant cells. Radiation Research: A Twentieth-Century Perspective, Vol 1: 449, 1991.
93. **HZ HILL**, M Ali and GJ Hill. The resurrection factor: Transfer of radioresistance in melanoma cell lines. IIIrd Meeting PanAmerican Soc for Pigment Cell Research, Edmonton, Alberta, CANADA, July 11-13, 1991.
94. **HZ HILL** and GJ Hill. The resurrection factor: characterization of a diffusible factor that decreases the radiation sensitivity of melanoma cells. Radioresistance of Human Tumors: Mechanisms and Prediction, Montreal, CANADA, July 13-14, 1991.
95. **HZ HILL**, M Ali and GJ Hill. The resurrection factor: Transfer of radioresistance in melanoma cell lines. 3rd Annual Meeting of the European Society for Pigment Cell Research, Amsterdam, NETHERLANDS, Sept 8-11, 1991.
96. **HZ HILL**, GJ Hill, M Ali and Uwe Schlehaider. Characteriztion of cellular responses to a radiation resistance factor elaborated by melanoma cells. J Cellular Biochem Suppl 168: 309, 1992.
97. **HZ HILL**, GJ Hill, M Ali and U Schlehaider. Target cell responses to a radiation resistance factor from Cloudman S91 mouse melanoma cells. Abstracts Radiation Research Society, Salt Lake City, UT: 37, 1992.
98. Uwe Schlehaider, GJ Hill and **HZ HILL**. Cellular responses to a radiation resistance factor produced by melanoma cells. Annual Meeting of the Pan American Society for Pigment Cell Research, Cincinnati, OH, June 7-10, 1992.
99. **HZ HILL**, GJ Hill, M Ali and S Ali. Mutation rates are enhanced in Cloudman S91 mouse melanoma cell treated for 5 days with an inducer of melanin synthesis. 4th Annual Meeting of the European Society for Pigment Cell Research, Berlin, GERMANY, Sept 17-20, 1992

100. UK Schlehaider, GJ Hill and **HZ HILL**. Characteristics of an autocrine factor enhancing resistance of melanoma cells to different therapeutics. 4th Annual Meeting of the European Society for Pigment Cell Research, Berlin, GERMANY, Sept 17-20, 1992
101. **HZ HILL**, UK Schlehaider, I Chowdhary and GJ Hill. An autocrine resistance factor from melanoma that rescues cells from toxic agents. J Cellular Biochem Suppl 17D:154, 1993.
102. I Chowdhary, U Schlehaider, GJ Hill and **HZ HILL**. A therapy resistance factor produced by mouse melanoma cells in vitro. Amer Assoc Cancer Research Proc 34:23, 1993.
103. I Chowdhary, KA Zirvi, U Schlehaider, GJ Hill and **HZ HILL**. A therapy resistance factor produced by mousemelanoma cells in vitro. The Cancer Institute of NJ 1st Annual Scientific Retreat, Princeton, NJ June 9, 1993
104. **HZ HILL**, GJ Hill, U Schlehaider and K Cieszka. Survival of Cloudman S91 mouse melanoma cells of varying melanin content after exposure to UVA, UVB, UVC and solar simulated (FS20) light. Photochem and Photobiol 57 Suppl: 21S-22S, 1993.
105. **HZ HILL**, GJ Hill and K Cieszka. Survival of Cloudman mouse melanoma cell lines of differing melanin content after exposure to various UV wavelengths. XVth International Pigment Cell Conference, London, ENGLAND, Sep 93
106. **HZ HILL**, GJ Hill, I Chowdhary, U Schlehaider. Split dose recovery (SDR) and double strand break (DSB) repair of radiation sensitive and resistant melanoma cells after gamma ray exposure. XVth International Pigment Cell Conference, London, ENGLAND, Sep 93.
107. GJ Hill, **HZ HILL**, RM Beazley and V Krawiek. The American Cancer Society Latin America Exhange Program -- For Example: Bolivia. The Oncology Society of NJ 1994 Scientific Symposium, Mar 94
108. GJ Hill, I Chowdhary, U Schlehaider, **HZ HILL**. A multi-therapy resistance factor produced by mouse melanoma cells. Society of Surgical Oncology Annual Meeting, Houston, TX, Mar 94
109. **HZ HILL**, GJ Hill, U Schlehaider and I Chowdhary. Further studies of a multi-therapy resistance factor (MTRF) produced by mouse melanoma cells in vitro. Proc AACR 35:1, 1994
110. **HZ HILL**, GJ Hill, I Chowdhary and U Schlehaider. Influence of a multi-therapy resistance factor (MTRF) on PLDR and SDR in melanoma cells. Abstr Radiation Research Society, Apr, 1994
111. **HZ HILL**, GJ Hill, K Cieszka and M Azure. Effect of UVC, UVB, UVA and a solar simulator on survival of mouse melanoma cell lines differing in melanin content. Environmental and Molecular Mutageneis 23, Suppl 23: 26, 1994
112. RM Goodman, **HZ HILL**, K Cieszka and GJ Hill. Photoprotection of UVC induced DNA-protein cross-links (DPC) in Cloudman S91 melanoma cells treated with isobutyl methyl samthine (IBMX) and melanocyte stimulating hormone. Photochem Photobiol 59: 60s, 1994.
113. S Zhou, **HZ HILL** and GJ Hill. Further studies of a multi-therapy resistance factor produced by Cloudman S91 mouse melanoma cells. Pigment Cell Research Suppl. 3:19, 1994.
114. **HZ HILL**, GJ Hill, K Cieszka, U Schlehaider and I Chowdhary. XVI International Cancer Congress 1994, Abstract Book-I: 325, Nov 1994.
115. GJ Hill and **HZ HILL**. The American Cancer Society Latin America exchange program -- for example: Bolivia. J Cancer Education 9, suppl to #3: 15, Nov 1994.
116. **HZ HILL**, S Zhou and GJ Hill. The multi-therapy resistance factor (MTRF) of melanoma cells: Similar activity in other cell lines and in substance P, a neuropeptide. Melanoma Research 4:27, 1994.
117. **HZ HILL**, K Cieszka, P Xin and GJ Hill. Differential sensitivities of Cloudman S91 mouse melanoma cell lines to different wave lengths of ultraviolet light. Melanoma Research 4: 35, 1994.
118. W Li and **HZ HILL**. Characterization of ethylmethane sulphonate (EMS) induced ouabain-resistant mutation in Cloudman S91 melanoma cell lines. Abstrs 1995 Annual Workshop on Cancer Research in New Jersey, Mar 1995.
119. S. Zhou, **HZ HILL**, GJ Hill, N Ginde, HC Silva, R Sotomayor, M. Zapicach and P Rameshar. Recent studies of a multi-therapy resistance factor (MTRF). Abstr 1995 Annual Workshop on Cancer Research in New Jersey, Mar 1995.
120. S Zhou, **HZ HILL** and GJ Hill. Further characteriation and purification of a multi-therapy resistance factor (MTRF). Abstr Radiation Research Society, Apr, 1995.
121. S Zhou, **HZ HILL** and GJ Hill. A multi-therapy resistance factor produced by some tumor cells. J Invest Medicine 43, suppl. 2: 371A, 1995.
122. **HZ HILL**, P Xin and GJ Hill. Survival of mouse coat color mutant melanocytes after UV irradiation at various wavelengths. Photochem. Photobiol. 67 Suppl. 15S, 1995.
123. W Li, P Xin, GJ Hill and **HZ HILL**. Effect of induced pigmentation in Cloudman S91 mouse melanoma cells on mutation to ouabain resistance after UVC. Pigment Cell Research Suppl. 4: 27, 1995.

124. S Zhou, F Kueppers, HC Silva, GJ Hill and **HZ HILL**. The multi-therapy resistance factor (MTRF) porduced by Cloudman S91 melanoma cells is inhibited by rabbit antiserum. Pigment Cell Research Suppl. 4: 30, 1995.
125 GJ Hill and **HZ HILL**. Physical and demographic and behavioral observations related to the "ozone hole" in the antactic summer of 1994. J. Cancer Education 10 (Suppl.): 16, 1995.
126. S Zhou, HC Silva, W Li, F Kueppers, GJ Hill and **HZ HILL**. A novel autocrine factor from melanoma rescues cells from radiation and chemotherapy death. Abstracts of the Radiation Research Society, April, 1996, p. 147.
127. J Kaur (with **HZ HILL**). Survival of Chinese hamster ovary (CHOK1-AL) cells transfected with tyrosinase after near and far uv and γ-rays. Photochem. Photobiol 63, 110s, 1996.
128. P Xin, GJ Hill and **HZ HILL**. Induced pigment in Cloudman S91 melanoma cells is photoprotective with respect to the lethal effects of sloar UV wavelengths. The Bulletin NJ Academy of Science 41: 50, 1996.
129. J Kaur and **HZ HILL** Survival of Chinese hamster ovary (CHOK1-AL) cells transfected with tyrosinase after near and far uv and g-rays. The Bulletin NJ Academy of Science 41: 48, 1996.
130. HC Silva, **HZ HILL**, GJ Hill, W Li, S Zhou and F Kueppers. A multi-therapy resistance factor from melanoma-purification and immuno-inactivation. The Bulletin NJ Academy of Science 41: 50, 1996.
131. J Kaur and **HZ HILL**. Transfection of Chinese hamster ovary (CHOK1-AL) cells with tyrosinase gene to study survival after far and near UV rays and γ-rays. The Annual Retreat on Cancer Research in New Jersey, May, 1996, p.38.
132. P Xin, GJ Hill and **HZ HILL**. Melanin induced in Cloudman S91 melanoma cells by IBMX and melanotan-1® is photoprotective with respect to irradiation by UVC, UVB, UVA and FS20 lamps. . The Annual Retreat on Cancer Research in New Jersey, May, 1996, p.39.
133. HC Silva, **HZ HILL**, GJ Hill, W Li, S Zhou and F Kueppers Purification and immunological inactivation of a multi-therapy resistance factor from melanoma. The Annual Retreat on Cancer Research in New Jersey, May, 1996, p.54.
134. H CSilva, **HZ HILL**, GJ Hill, W Li, S Zhou and F Kueppers. Purification and immunological neutralization of a multi-therapy resistance factor from melanoma. Oncology Society of New Jersey, Mar, 1996.
135. P Xin, GJ Hill and **HZ HILL**. Melanin induced in cloudman s91 melanoma cells by ibmx and melanotan-1® is photoprotective with respect to irradiation by UVC, UVB, UVA and FS20 lamps. Photochem. Photobiol. 63: 41s, 1996.
136. **HZ HILL** Melanin --the two-edged sword? Photochem. Photobiol. 63: 41s, 1996.
137. S Zhou, F Kueppers, **HZ HILL** and G Hill. Studies of a multi-therapy resistance factor (MTRF). Presented by HC Silva, Soc. Surg. Oncol. 49th Cancer Symp. Abstract Book, Mar, 1996.
138. **HZ HILL** Melanin - the Two-Edged Sword? Pigment Cell Research Suppl. 5: 19, 1996.
139. HC Silva, **HZ HILL**, GJ Hill, W Li, S Zhou and F Kueppers Purification and immunological neutralization of a multitherapy resistance factor family from melanoma. Pigment Cell Research Suppl. 5: 43, 1996.
140. **HZ HILL**, GJ Hill and P Xin Role of melanin pigments in the response of melanocytes to solar irradiation. Pigment Cell Research Suppl. 5: 62, 1996.
141. **HZ HILL**, GJ Hill, P Xin and W Li Induced eumelanin protects Cloudman S91 mouse melanoma cells from solar radiation killing and mutations. Pigment Cell Research Suppl. 5: 63, 1996.
142. **HZ HILL**, W Li, S Zhou, H Tang, P Xin, HC Silva and F Kueppers. A multitherapy resistance factor from melanoma. Keystone Symposium on Discovery and Development of Novel Therapeutic Agents for the 21st Century, Tamarron, CO, March, 1997.
143. **HZ HILL**, S Zhou, F Kueppers and H Tang. A multi-therapy resistance factor from melanoma. Abstracts of an International Workshop on the Tumor Microenvironment: An important paradigm in cancer etiology and treatment, Edgartown, MA, April, 1997.
144. HZ HILL, H Tang, S Zhou, HC Silva, W Li and F Kueppers. Immunological inactivation of an autocrine multitherapy resistance factor (MTRF) from melanoma. Abstracts of the 45th Annual Meeting of the Radiation Research Society, p 204, Providence, RI, May, 1997.
145. **HZ HILL**, W. Li and J. Kaur. Role of intracellular pigment in responses to solar radiation: Are we barking up the wrong tree? Pigment Cell Research 10: 116, 1997
146. J. Kaur and **HZ HILL**. Photobiology of tyrosinase-transfected Chinese hamster ovary cells. Photochemistry and Photobiology 65: 104s, 1997.
147. **HZ HILL** and H Tang. Western blot analysis of serum-free conditioned medium (SFCM) that rescues cultured melanoma cells from radiation and chemotherapy. 46th Ann Meeting Radiation Research Soc., p 170, Louisville, KY, April, 1998
148. **HZ HILL**. Purification of a multi-therapy resistance factor from melanoma. Abstracts of the 1998 Annual Retreat on Cancer Research in New Jersey, Princeton, NJ. May, 1998

149. **HZ HILL**, H. Tang, H.C. Silva and L.J. Wolansky. Purification of a complex protein that rescues melanoma cells from radiation and chemotherapy death. Photochemistry and Photobiology 67: 44S (1998)
150. G. Ghanem, E. Kinnaert and **HZ HILL**. Induction of both phaeomelanin and eumelanin decreases killing of melanoma cells by reactive oxygen species. Photochemistry and Photobiology 67: 3S-4S (1998)
151. **HZ HILL**, WG McKenna and RJ Muschel. Western blot analysis of serum-free conditioned medium (SFCM) that rescues cultured melanoma cells from radiation and chemotherapy. Pigment Cell Research 11: 238 (1998)
152. GJ Hill and **HZ HILL**. Edison and Cancer: Relationships between the great inventor and his companies with X-rays, radium, and other carcinogens. In *Clinical Cancer Research in New Jersey*, West Orange, NJ, March, 1999.
153. **HZ HILL**, GJ Hill, WG McKenna and RJ Muschel. The 100 kD multi-therapy resistance proteins (MTRPs) appear to be composed of previously undescribed 40 kD glycoprotein subunits. . In *Clinical Cancer Research in New Jersey*, West Orange, NJ, March, 1999.
154. **HZ HILL**, WG McKenna and RJ Muschel. Analysis of a protein from serum-free conditioned medium (SFCM) that rescues cultured melanoma cells from radiation and chemotherapy. Keystone Symposium on the Molecular Basis of Cancer, Taos, NM, March, 1999.
155. GJ Hill and **HZ HILL**. Illuminating cancer education with history and literature: for example, *Edison and Cancer*. Abstracts of the American Association for Cancer Education, Cleveland, OH, October, 1999.
156. **HZ HILL** and GJ Hill. Defining the role of melanins in photoprotection. Pigment Cell Research Suppl 7: 37, 1999.
157. **HZ HILL** and GJ Hill. Increased eumelanin is photoprotective for survival of Cloudman S91 mouse melanoma cells in UVB and photosensitizing in UVA. Pigment Cell Research 13: 206, 2000.
158. **HZ HILL**, GJ Hill and D.L. Mitchell. Increased eumelanin is photoprotective for survival of Cloudman S91 mouse melanoma cells in UVB and photosensitizing in UVA. Abstracts of the 13th International Congress on Photobiology, San Francisco, CA: 92, 2000.
159. **HZ HILL**, C. Cathcart and GJ Hill. Intermittent sunburn photo-recall evoked by radiation therapy 50 years later. Abstracts of the 13th International Congress on Photobiology, San Francisco, CA: 205, 2000.
160. **HZ HILL**, GJ Hill and C Cathcart. Photorecall of sunburn 50 years later by radiation therapy. Clinical Research Symposium 'Clinical Cancer Research in NJ', West Orange, NJ: p33, March, 2001.
161. **HZ HILL**, M Lenarczyk, CA Waldren and RW Howell. Preliminary radiobiological characterization of connexin mutants of the human-hamster hybrid cell line A_L. 48th Annual Meeting of the Radiation Research Society, San Juan, PR: 176, 2001.
162. **HZ HILL**, RW Howell, El Azzam, SM de Toledo and M Lenarczyk. The bystander effect: ionizing radiation and UVA. 29th Annual Meeting of the American Society for Photobiology, Chicago, IL: p58, 2001.
163. M Lenarczyk, **HZ HILL** and RW Howell. Can low-LET radiation from incorporated radionuclides induce mutagenic effects in unirradiated bystander cells? 49th Annual Meeting of the Radiation Research Society, Reno, NV: 173, 2002.
164. HZ HILL, K Hubbard, M Steinber, J Locitzer, A Ogbonnaya and N Eslamdoust. Mitochondrial DNA damage induced by solar simulated light. Annual Meeting of the American Society for Photobiology, Baltimore, MD; 53, 2003.
165. ML Steinberg, J Locitzer, M Berwick and H HILL. Patterns of gene expression related to solar exposure in keratinocytes from melanoma patients: cDNA microarray analyses. Annual Meeting of the American Society for Photobiology, Baltimore, MD; 99, 2003.
166. HZ HILL, J Locitzer, N Eslamdoust, A Ogbonnaya, K Hubbard. Solar Radiation and mitochondrial DNA damage. 12th International Congress Of Radiation Research, Brisbane, Queensland, AUSTRALIA: 191, 2003.
167. HZHILL, K Hubbard, M Steinberg and D Oulianov. The common deletion in human mitochondria is induced by low doses of γ-rays, 12th International Congress Of Radiation Research, Brisbane, Queensland, AUSTRALIA: P08/1702, 2003.

REVIEWS

1. **HZ HILL**. Book review of Short-term interactions between cell surfaces by L Weiss and JP Jarlos in Progress in Surface Science 1:355-405, 1972, J Colloid and Interface Science 41:394, 1972.
2. **HZ HILL**. "Peer review: preventing 'nepotism'". Letter: Science 194:894 1976.
3. **HZ HILL**. Book review of Cell Cycle Regulation (Cell Biology Monograph Series), edited by JR Jeter, IL Cameron, GM Padilla and AM Zimmerman, BioScience 29:46, 1979.
4. **HZHILL**, Editorial Comments re: Detection of X-ray damage repair by the immediate versus delayed plating technique is dependent on cell shape and cell concentration by NMS Reddy, M Kapiszewska and CS Lange. Scanning Microscopy 6: 543-559, 1992.

INVITED TALKS:

NIH, NIAID, Bethesda, MD: "A model for UV and photoreactivation effects in Euglena", February 1963
Colorado Medical Society Annual Meeting, Colorado Springs: "Porphyria", September 1968
National Jewish Hospital, Denver, CO: "Galactose metabolism and its relation to human genetic disease", April 1971
University of Colorado Medical Center, Denver, CO: "Galactose gene action in mammalian cells", May 1971, Faculty Seminar
Saigon University School of Medicine, Saigon, South Vietnam: "Galactosemia - Antenatal diagnosis", April 1972
Mallinkrodt Institute of Radiology, St. Louis, MO: "A model system for the pre-natal diagnosis of inherited biochemical diseases:galactosemia, September 1974
Washington University, Department of Biology, St. Louis, MO: "Ante-natal diagnosis and human molecular disease", September 1973
Washington University School of Medicine, Department of Obstetrics and Gynecology: "Detection of inborn errors of metabolism, March 1975
Washington University, Department of Biology, Plant Seminar, St. Louis, MO: "UV and photoreactivation effects in Euglena", January 1976
Southern Illinois University, Chemistry Department, Edwardsville, IL: "Radiation response in melanoma", April 1976
Society of the Sigma Xi, Marshall University Club, Huntington, WV: "Amygdalin and cancer", March 1978
Association of VA Surgeons, St. Louis, MO: "Synergistic effect of DTIC and L-PAM combination treatment on the Harding-Passey melanoma in C3D2F1 mice", May 1978
Tumor Registrars' Workshop, Huntington, WV: "Update on research in malignant melanoma", September 1979
Marshall University School of Medicine, Biochemistry Department, Huntington, WV: "Radiation and melanoma", October 1979
Society of the Sigma Xi, Marshall University Club, Huntington, WV: "Melanoma: A biologist looks at the black cancer", February 1981
New Jersey Society of Radiation Technologists, Radiation Therapy District, Fall Seminar, Clark, NJ: "Radiation research and the technologist", September 1981
New Jersey Medical School, Biochemistry Department: "Does replicon joining use the same enzymatic machinery as post-replication repair? December, 1981
New Jersey Medical School, Anatomy Department: "Combination chemotherapy studies using a mouse melanoma model", February 1982
American Cancer Society, New Jersey Division, 1983 Volunteer Conference, New Brunswick, NJ: "What can professional education do for you?" February 1983
American Cancer Society, New Jersey Division, 1983 Staff Conference, Spring Lake, NJ: "Of mice and men", June 1983
American Cancer Society, Mercer County Annual Meeting, Trenton, NJ: "Of mice and men", October 1983
Ruth Estrin Goldberg Memorial for Cancer Research Annual Meeting: "An intra-operative radiation therapy mouse model", April 1984
Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland: "Radiation and chemotherapy effects in melanoma", January 1985
VIth European Workshop on Melanin Pigmentation, Murcia, Spain: "Melanins as free-radical scavengers", September 1985
Continuing Seminars in Education, sponsored by Tumor Registrars Association of NJ: "Cancer in the Family? Preventive Measures", American Cancer Society, North Brunswick, NJ, October 1985
Gelb Foundation Symposium on Melanin: "Exogenous Melanin Causes DNA Strand Breaks and is Synergistic with Ionizing Radiation", Stamford, CT, September 1986
Brookhaven National Laboratories, Biology Department: "Melanin, Solar Carcinogenesis and Radiation Therapy", Upton, NY, March 1987
Dermatology Grand Rounds, NJ Medical School, "Radiobiology of Skin", Newark, NJ, March 1987
Argonne National Laboratories, Division of Biological and Medical Research: "Photo- and Radiobiology of Melanin", Argonne, IL, September 1987
European Society for Pigment Cell Research, participant in Symposium on "Chemistry and Photobiology of Melanin", Sorrento, Italy, October 1987
American Cancer Society, NJ Division, Conference on Major Currents in Breast and Gynecological Cancers: "Research - What the future holds", with GJ Hill, West Orange, NJ, November 1987
Marshall University School of Medicine, Biomedical Sciences Seminar: "Melanin, Photoprotector or Photosensitizer?" Huntington, WV, October 1988

NJ Medical School, Cancer Research Colloquium: "Melanin, Photoprotector or Photosensitizer?" Newark, NJ, October 1988

UMDNJ-University Hospital Blood Bank lecture series: "Application of Radiation Biology to Blood Banking", Newark, NJ, February 1989

National Institutes of Health, National Cancer Institute: "Melanin: Photoprotector or Photosensitizer?" Bethesda, MD, February 1989

American Cancer Society, NJ Division, Cancer Survivors Day speaker: "Cancer research", North Brunswick, NJ, April 1989

Cambridge University: "Photobiology of melanin and the radiation biology of melanoma", Cambridge, UK, June 1989

Eleanor Roosevelt Institute for Cancer Research: "Melanin in the photobiology of skin cancer and the radiobiology of melanoma", Denver, CO, January 1990

Participant, Mini-symposium: "DNA Damage Repair, Mutation and Disease: Molecular and Cellular Aspects", Colorado State University, January 1990, Fort Collins, CO

Rutgers University College of Pharmacy, Department of Chemical Biology and Pharmaconosy: "Melanin in the photobiology of skin cancer and the radiobiology of melanoma", Piscataway, NJ, June 1990

Rutgers University, Department of Biological Sciences: "Melanin, skin cancer and melanoma", Newark, NJ, September 1990

Participant, 2nd annual Mini-symposium: "DNA Damage Repair, Mutation and Disease: Molecular and Cellular Aspects", Colorado State University, February, 1991, Fort Collins, CO

Temple University School of Medicine, "The Resurrection Factor: Transfer of Radioresistance in melanoma", Philadelphia, PA, June, 1991

Plenary session speaker, IIIrd Meeting PanAmer Soc Pigment Cell Research, July 11, 1991, Edmonton, Alberta, CANADA

Plenary session speaker, 3rd annual meeting, European Society for Pigment Cell Research, Amsterdam, NETHERLANDS, Sept, 1991

Participant, 4nd annual Mini-symposium: "DNA Damage Repair, Mutation and Disease: Molecular and Cellular Aspects", Colorado State University, February, 1993, Fort Collins, CO

State University of New York Health Science Center at Brooklyn, "Interesting findings in melanoma" Nov 18, 1991

Plenary session speaker, AACR Special Conference on Cellular Responses to Environmental DNA damage, Banff, Alberta, CANADA, Dec, 1991: "The role of melanin in the photo- and radiobiology of malignant melanoma"

Univ Massachusetts Dept of Biochemistry and Molecular Biology "Photo- and Radiobiology of melanin and melanoma" Feb 18, 1992

Brookhaven National Laboratory Dept of Biology "Is tanning carcinogenic?" Aug 3, 1992

National Cancer Institute, NIH, Laboratory of Cellular Biology "Is tanning carcinogenic?" Sep 11, 1992

Participant, 4nd annual Mini-symposium: "DNA Damage Repair, Mutation and Disease: Molecular and Cellular Aspects", Colorado State University, February, 1993, Fort Collins, CO

Lecture series sponsored by the American Cancer Society, the NCI, Bolivian Cancer Society and the Cancer Institute of Eastern Bolivia on "Current Aspects of Surgical Oncology and Cancer Biology" presented at the Departments of Medicine and Surgery, Faculty of Medicine, La Paz and the Cancer Institute of Eastern Bolivia, Santa Cruz, BOLIVIA. Lectures on 8/12, 8/13, 8/14 (La Paz) and 8/16, 8/17 (Santa Cruz) 1993

Plenary presentation: **HZ HILL**, GJ Hill, I Chowdhary, U Schlehaider. Split dose recovery (SDR) and double strand break (DSB) repair of radiation sensitive and resistant melanoma cells after gamma ray exposure. XVth International Pigment Cell Conference, London, ENGLAND, 29 Sep 93

'Role of Melanin in the Photobiology of Melanoma', Rutgers University School of Pharmacy, Mar 1994

'Is melanin photoprotective or is it photosensitizing? Lunch time debate, Melanin Symposium, Washington, DC, Mar 1994

'A multi-therapy resistance factor from melanoma' Biochemistry Department Faculty Seminar, NJ Medical School, Jan 1996

'A multi-therapy resistance factor from melanoma'. Department of Surgery, NJ Medical School, Mar, 1996.

'A multi-therapy resistance factor from melanoma'. Centers for Laboratory Investigation and Continuing Education, NJ Medical School, Mar, 1996.

'A multi-therapy resistance factor from melanoma'. Program of Molecular and Cellular Biology, Wistar Institute, Phila., PA May, 1996.

'Melanins and photoprotection'. Fondation Rene Touraine, Scientific Meeting, Paris, France, Oct, 1996.

'Melanin -- the two-edged sword?' Workshop on Extracutaneous Melanin, Melanocytes and Melanogenesis, XVIth International Pigment Cell Conference, Anaheim, CA, Oct, 1996

'A novel mechanism for radiation and chemotherapy resistance in melanoma' Department of Radiation Oncology, University of Pennsylvania Medical Center, Philadelphia, PA Jan, 1997

'Radiation resistance in melanoma' Colorado State University, Cell and Molecular Biology Graduate Program, Ft. Collins, CO, Mar, 1997.

'A factor that rescues tumor cells from radiation death' Seminar Series: Current Topics in Cancer Research – Student Summer Research Program, July,1997

'A Radiation and Chemotherapy Resistance Factor from Melanoma' Department of Medicine, UMDNJ-NJ Medical School, March, 1998

'Purification of a multi-therapy resistance factor from melanoma'. Focus Session of the Annual Retreat on Cancer Research in New Jersey, Princeton, NJ. May, 1998

'Purification of a complex protein that rescues melanoma cells from radiation and chemotherapy death. Symposium on the Mechanism f Radiation Resistance. Annual Meeting of the American Society for Photocbiology, Snowbird, UT, July, 1998

'Induction of both phaeomelanin and eumelanin decreases killing of melanoma cells by reactive oxygen species. ' Workshop on UV, Accessory to Melanoma – If So, How? Annual Meeting of the American Society for Photocbiology, Snowbird, UT, July, 1998

'A factor that rescues tumor cells from radiation death' Seminar Series: Current Topics in Cancer Research – Student Summer Research Program, 1998

'A radiation and chemotherapy rescue factor from melanoma' Coriell Institute for Medical Research, Camden, NJ, Dec 8, 1998

'Radiation resistance factor from melanoma' Thomas Jefferson University, Philadelphia, PA, Feb 1, 1999.

'Defining the role of melanins in photoprotection.' XVIIth International Pigment Cell Conference, Nagoya, JAPAN, Nov 2, 1999

'Review of the radiobiology of pigment cells.' 9th Eur Soc Pigment Cell Research Meeting, Ulm, GERMANY, Sept 29, 2000

OTHER ACTIVITIES:

Discussion of genetic engineering and superbabies on Station KSD, Huntington, WV, March 1980

Discussion of Interferon for the American Cancer Society, West Virginia Division, WSAZ-TV, April 1980

Discussion leader at Smith College Alumnae College, Northampton, MA, May 1980

Poster presentation: "Is post-replication repair a unique process in mammalian cells?" Gordon Conference on Mutagenesis, Biological and Chemical Mechanisms, Andover, NH, July 1980

Organizer and panel discussion Chairman, Continuing Medical Education Symposium on "Genetic Programs in West Virginia Today", December 1980

Television interview to discuss the basic biology of cancer therapy, WOWK-TV, Huntington, WV, January 1981

Guest expert for the Essex County Medical Society on the Weekly Health Hour, Station WNJR, May 1982

Poster presentation: "Effects of caffeine on DNA replication in mouse melanoma cells", Gordon Conference on Chemotherapy of Experimental and Clinical Cancer, New London, NH, July 1982

Interviewed for radio station WRVM, Haslett, NJ, to discuss skin cancer, November 1984

Consultant to the American Cancer Society, New Jersey Division, on Cancer of the Skin, 1984-present

Television discussion on "Skin Cancer", Health Information Network, sponsored by the American Cancer Society, July 31, 1985

Chairman, poster session on Radiosensitizers and Radioprotectors, 14th International Cancer Congress, Budapest, HUNGARY, August 25, 1986

Chairman, platform session, New Jersey Commission on Cancer Research Workshop on Cancer Research in New Jersey, 1986

Poster presentation, Johns Hopkins University School of Hygiene and Public Health: **HZ HILL**, CA Huselton and GJ Hill, "Melanin protects against direct effect and enhances indirect effect photodamage in DNA of Cloudman S91 mouse melanoma cells{", Laurel, MD, June 1987

Member, American Cancer Society, New Jersey Division, Public Education Committee, 1987- ; Subcommittee on Melanoma and Skin Cancer Detection, 1986-

Co-chairman, mini-symposium, **Ultraviolet Effects**, Radiation Research Society, Annual Meeting, April 1988, Philadelphia, PA

Co-chairman, mini-symposium, **Structure and function of melanins**, PanAmerican Society for Pigment Cell Research, Annual Meeting, April 1989, Bethesda, MD

Co-chairman, platform session, **Photobiology of melanin pigmentation**, European Society for Pigment Cell Research, Meeting, June 1989, Uppsala, SWEDEN

Poster Presentation: **HZ HILL**, J Bargellini, Z Trizna and GJ Hill. "Induction of double strand breaks by low LET ionizing radiation in DNA of mouse melanoma cells varying in intracellular melanin." NJ Commission on Cancer Research 4th Annual Workshop on Cancer Research in New Jersey, October 1990, Princeton. NJ

Poster Presentation: GJ Hill, M Ali, U Schlehaider, **HZ HILL**. "Characteristics of a radiation rescue factor (RF) produced by S91 mouse melanoma cells in vitro". Society of Surgical Oncology Annual Meeting New York, NY, Mar 17, 1992

UMDNJ Presenter for June 8, 1993 Cancer Briefing for media representatives

Ad hoc grant reviewer for NSF (2 grants); Veterans' Administration Merit Review, 1993

Reviewer for promotion to tenure of Dr. Thomas Hei, Department of Radiobiology, Columbia University, 1993

Reviewer for promotion to tenure of Dr. Patricia Gallagher, Department of Biochemistry, West Virginia University, 1993

Poster Presentation: J Cosmi, GJ Hill, **HZ Hill**, K Cieszka. "Solar induced DNA-protein cross-links in pigmented melanoma cells". Annual Workshop on Cancer Research in New Jersey, November, 1993, Piscataway, NJ.

Co-Chairman platform session, Annual Meeting American Society for Photobiology 'Photobiology of the dermis' June 1994

Ad hoc member of the American Cancer Society Advisory Committee on Cell Biology, Jan 22-23, 1995.

Member of the Task Force for New Item Development (Board questions on Radiation Biology) for the American Board of Radiology, 1994-1996.

Captain of judges for medical science projects at the North Jersey Science Fair, Morristown, NJ March, 1995.

Co-Chairman Contributed Papers Session 'UV Photobiology', Annual Meeting American Society for Photobiology June, 1995

Organizer and Co-Chairman 'Melanin' Symposium Annual Meeting American Society for Photobiology June, 1996

Co-Chairman, afternoon session. 'The Melanocyte' Fondation Rene Touraine, Scientific Meeting, Paris, France, Oct, 1996.

Co-Chairman, Workshop on Extracutaneous Melanin, Melanocytes and Melanogenesis, XVIth International Pigment Cell Conference, Anaheim, CA, Oct, 1996

Poster Presentation, HZ Hill, H Tang, S Zhou, HC Silva, W Li and F Kueppers. Immunological inactivation of an autocrine multitherapy resistance factor (MTRF) from melanoma. 45th Annual Meeting of the Radiation Research Society, Providence, RI, May, 1997.

Co-Chairman, Minisymposium on Photobiology and biophsyics of melanin and melanocytes, 7th Annual Meeting of the PanAmerican Society for Pigment Cell Research, Providence, RI, June, 1997

Co-Chairman, Platform Session on Environmental Photobiology and UVR Effects, 25th Annual Meeting of the American Society for Photobiology, St. Louis, MO, July, 1997.

Poster Presentation, HZ HILL and H Tang. Western blot analysis of serum-free conditioned medium (SFCM) that rescues cultured melanoma cells from radiation and chemotherapy. 46th Ann Meeting Radiation Research Soc. Louisville, KY, April, 1998

Representative for Brandeis University at the inauguration of Stuart D. Cook, M.D. as President of the University of Medicine and Dentistry of New Jersey, April 9, 1999.

Co-Chairman, Platform Session on Environmental Photobiology/Sunscreens, 29th Annual Meeting of the American Society for Photobiology, Chicago, IL July 10, 2001.

Co-Chairman, Platform Session of Contributed Papers, American Society for Photobiology, Baltimore, MD July 9, 2003.

WORKSHOPS AND GRADUATE EDUCATION:

Tumor Biology, Harvard Medical School, November 15-19, 1976

ICN-UCLA Symposia: DNA Repair Mechanisms, 1978; DNA Replication and Recombination, 1980; Mechanisms of Chemical Carcinogenesis, 1981; Rational Basis for Chemotherapy, 1982, Cellular Responses to DNA Damage, 1983

UCLA Symposium: Mechanisms and Consequences of DNA Damage Processing, 1988; Genetic Mechanisms in Carcinogenesis and Tumor Progression, 1989

Gordon Conferences: Mutagenesis: Biological and Chemical Mechanisms, 1980, 1982; Chemotherapy of Experimental and Clinical Cancer, 1980, 1982, 1983, 1986

Waters Associates LC Short Course, January 1984

New Jersey Commission on Cancer Research Workshop on Cancer Research in New Jersey, 1986, 1987, 1989

UCLA Symposium: Mechanisms and consequences of DNA damage processing, Taos, NM, January 1988; Genetic mechanisms in carcinogenesis and tumor progression, Keystone, CO, January 1989

NIH Consensus Conference: Sunlight, UV and skin, Bethesda, MD, May 1989

UMDNJ-University Libraries: Medical Literature Searching Using Grateful Med Software, Newark, NJ, June 1990

Rutgers University: Recombinant DNA Techniques: An Introductory Laboratory Course, New Brunswick, NJ, June 1990
Waters Associates: Essentials in Bioresearch, Seminar, June 14, 1991
Sepracor Inc: Purification of Protein Biologics, One Day Workshop, June 14, 1991
Keystone Symposium: Melanoma and Diseases of the Neural Crest, Taos, NM, Feb 1-8, 1992
Keystone Symposium on the Molecular Biology of Aging, Lake Tahoe, CA, March, 1993
Annual Workshop on Cancer Research in New Jersey, November 1993-96
International Workshop on the Tumor Microenvironment: An important paradigm in cancer etiology and treatment, Edgartown, MA, April, 1997.
Pharmacia Biotech: Electrophoresis Seminar, Somerville, NJ, May, 1997.
Photoshop Seminar, Freehold, NJ, July, 2003.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Roger W. Howell, Ph.D.	Associate Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Massachusetts, Amherst, MA	B.S.	1982	Physics
University of Massachusetts, Amherst, MA	Ph.D.	1987	Physics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

1995- **Associate Professor**, UMDNJ - New Jersey Medical School
1989-1995 **Assistant Professor**, UMDNJ - New Jersey Medical School
1987-1989 **Instructor**, UMDNJ - New Jersey Medical School

Awards:

1995 Outstanding Dosimetry Manuscript Award by the Journal of Nuclear Medicine. S. Murty Goddu, R.W. Howell, D.V. Rao. "A generalized approach to absorbed dose calculations for dynamic tumor and organ masses". J. Nucl. Med. 36: 1923-1927 (1995).

Research Projects Ongoing or Completed During the Last 3 Years:

July 1991-June 1997. USPHS Grant No. R29 CA54891.
Principal Investigator: Roger W. Howell, Ph.D.
Agency: National Cancer Institute

The long-term objective of this project was to investigate the biological effects of incorporated alpha particle emitters as they relate to radon exposure. Experiments were carried out in vivo in the mouse testes and in vitro in cell culture models. Radionuclides emitting alpha particles with energies ranging from 3.2 to 8.8 MeV were examined and various subcellular distributions of the radionuclides were studied. While energy plays an important role in the response, the subcellular distribution did not.

1998. NE Hazardous Substance Research Center Subcontract No. 991653.
Principal Investigator: Roger W. Howell, Ph.D.
Agency: U.S. Department of Energy

The long-term objective of this project was to provide educational assistance to residents of the city of Bloomfield, NJ on risk associated with exposure to thorium and uranium industrial waste products. In addition, methods of remediation and remediation assessment were also covered. Finally, the remediation process used by Westinghouse was also examined in detail.

Special Professional Service:

Society of Nuclear Medicine Medical Internal Radiation Dose Committee (MIRD), July 1992 - present.
International Commission on Radiation Units and Measurements (ICRU). Report Committee on Conceptual Basis for Dose Specification in Nuclear Medicine. 1998 - present.
Program Committee, Second International Symposium on Biophysical Aspects of Auger Processes, July 5-6, 1991, Univ. of Massachusetts, Amherst, MA.

Scientific Program Sub-Chair, Dosimetry/Radiobiology. 1993 and 1994 Annual Meetings of the Society of Nuclear Medicine.

Am. Assoc. Physicists in Medicine Task Group on Auger Electron Dosimetry, June 1989 - June 1994

Program Committee, 1991-1994, 1998 Annual Meeting of the Society of Nuclear Medicine.

Program Committee, 1993-1995 , Annual Meeting of the American Association of Physicists in Medicine.

Publications: 65 articles, 1 edited book, 1 book, 2 reports

Selected Bibliography:

1. R.W. Howell, D.V. Rao, and K.S.R. Sastry. Macroscopic dosimetry for radioimmunotherapy: Non-uniform activity distributions in solid tumors. Med. Phys. 16:66-74 (1989).
2. D.V. Rao, V.R. Narra, R.W. Howell, V.K. Lanka, K.S.R. Sastry. Induction of spermhead abnormalities by incorporated radionuclides: Dependence on subcellular distribution, type of radiation, dose rate, and presence of radioprotectors. Radiat. Res. 125:89-97 (1991).
3. R.W. Howell, D.V. Rao, D.Y. Hou, V.R. Narra, K.S.R. Sastry. The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. Radiat. Res. 128:282-292 (1991).
4. R.W. Howell, V.R. Narra, and D.V. Rao. Absorbed dose calculations for rapidly growing tumors. J. Nucl. Med., 33:277-281 (1992).
5. V.R. Narra, R.W. Howell, R. S. Harapanhalli, K. S. R. Sastry, and D. V. Rao, Radiotoxicity of some I-123, I-125, and I-131 labeled compounds in mouse testis: Implications for radiopharmaceutical design. J. Nucl. Med. 33:2196-2201 (1992).
6. R.W. Howell, Radiation spectra for Auger-electron emitting radionuclides: Report No. 2 of AAPM Nuclear Medicine Task Group No. 6. Med. Phys. 19, 1371-1383 (1992).
7. R.W. Howell, V. R. Narra, K. S. R. Sastry, and D. V. Rao, On the equivalent dose for Auger electron emitters. Radiat. Res. 134:71-78 (1993).
8. R.W. Howell, M.T. Azure, V.R. Narra, and D.V. Rao. Relative biological effectiveness of alpha-particle emitters at low doses. Radiat. Res. 137:352-360 (1994).
9. S. M. Goddu, R. W. Howell, and D. V. Rao, Cellular dosimetry: Absorbed fractions for monoenergetic electron (0.1 keV - 1 MeV) and alpha particle (3 - 10 MeV) sources, and S-values for radionuclides, distributed uniformly in different cell compartments. J. Nucl. Med. 35:303-316 (1994).
10. S. M. Goddu, D. V. Rao, and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation, and subcellular distribution of radionuclides. J. Nucl. Med. 35:521-530 (1994).
11. M. T. Azure, R. D. Archer, K. S. R. Sastry, D. V. Rao, and R. W. Howell, Biologic effect of ^{212}Pb localized in the nucleus of mammalian cells: Role of recoil energy in the radiotoxicity of internal alpha emitters. Radiat. Res. 140, 276-283 (1994).
12. R. W. Howell, A. I. Kassis, S. J. Adelstein, D. V. Rao, H. A. Wright, R. N. Hamm, J. E. Turner, and K. S. R. Sastry, Radiotoxicity of ^{195m}Pt labeled trans-platinum (II) in mammalian cells. Radiat. Res. 140, 55-62 (1994).
13. R. W. Howell, S. M. Goddu, and D. V. Rao, Application of the linear-quadratic model to radioimmunotherapy: Further support for the advantage of longer-lived radionuclides. J. Nucl. Med. 35, 1861-1869, (1994).
14. S. M. Goddu, R. W. Howell, and D. V. Rao, A generalized approach to absorbed dose calculations for dynamic tumor and organ masses. J. Nucl. Med. 36, 1923-1927 (1995).
15. S. M. Goddu, R. W. Howell, and D. V. Rao, A generalized approach to absorbed dose calculations for dynamic tumor and organ masses. J. Nucl. Med. 36, 1923-1927 (1995).
16. R. S. Harapanhalli, V. Yaghmai, D. Giuliani, R. W. Howell, and D. V. Rao, Antioxidant effects of vitamin C in mice following X-irradiation. *Research Communications in Molecular Pathology and Pharmacology* **94,** 271-287 (1996).

17. S. M. Goddu, R. W. Howell, and D. V. Rao, Calculation of equivalent dose for Auger electron emitting radionuclides distributed in human organs. *Acta Oncologica* **35,** 909-916 (1996).
18. S. M. Goddu, V. R. Narra, R. S. Harapanhalli, R. W. Howell, and D. V. Rao, Radioprotection by DMSO against the biological effects of incorporated radionuclides in vivo. *Acta Oncologica* **35,** 901-907 (1996).
19. R. W. Howell, and D. V. Rao, Auger electron emitters: Equivalent dose, chemical protection against their biological effects and use in cancer treatment. In *Radiation Research 1895-1995* (U. Hagen, D. Harder, H. Jung, and C. Streffer, Eds.), pp. 82-85. Universitätsdruckerei H. Stürtz AG, Würzburg, 1996.
20. V. R. Narra, R. W. Howell, S. M. Goddu, and D. V. Rao, Effects of a 1.5-Tesla magnetic field on spermatogenesis and embryogenesis in mice. *Investigative Radiology* **31,** 586-590 (1996).
21. R. S. Harapanhalli, L. W. McLaughlin, R. W. Howell, D. V. Rao, S. J. Adelstein, and A. I. Kassis, $^{125}I/^{127}I$-IodoHoechst 33342: Synthesis, DNA binding, and biodistribution studies. *J. Med. Chem.* **39,** 4804-4809 (1996).
22. R. W. Howell, S. M. Goddu, and D. V. Rao, Design and performance characteristics of an experimental Cs-137 irradiator to simulate internal radionuclide dose rate patterns. *J. Nucl. Med.* **38,** 727-731 (1997).
23. R. W. Howell, S. M. Goddu, V. R. Narra, D. R. Fisher, R. E. Schenter, and D. V. Rao, Radiotoxicity of gadolinium-148 and radium-223 in mouse testes: Relative biological effectiveness of alpha particle emitters *in vivo*. *Radiat. Res.* **147,** 342-348 (1997).
24. S. M. Goddu, R. W. Howell, L. G. Bouchet, W. E. Bolch, and D. V. Rao, *MIRD Cellular S values: Self-absorbed dose per unit cumulated activity for selected radionuclides and monoenergetic electron and alpha particle emitters incorporated into different cell compartments*. Society of Nuclear Medicine, Reston, VA, 1997.
25. S. J. Adelstein, R. W. Howell, J. L. Humm, G. M. Makrigiorgos, and B. W. Wessels, On the conceptual basis for dose quantities in nuclear medicine. *ICRU News* **1,** 4-10 (1998).
26. R. W. Howell, S. M. Goddu, A. Bishayee, and D. V. Rao, Radioprotection against lethal damage caused by chronic irradiation with radionuclides in vitro. *Radiat. Res.* **150,** 391-399 (1998).
27. S. M. Goddu, R. W. Howell, D. C. Giuliani, and D. V. Rao, Biological dosimetry of bone marrow for incorporated ^{90}Y. *J. Nucl. Med.* **39,** 547-551 (1998).
28. R. W. Howell, S. M. Goddu, and D. V. Rao, Proliferation and the advantage of longer-lived radionuclides in radioimmunotherapy. *Med. Phys.* **25,** 37-42 (1998).
29. W. E. Bolch, L. G. Bouchet, J. S. Robertson, B. W. Wessels, J. A. Siegel, R. W. Howell, A. K. Erdi, B. Aydogan, S. Costes, and E. E. Watson, MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions - radionuclide S values at the voxel level. *J. Nucl. Med.* **40,** 11S-36S (1999).
30. R. W. Howell, B. W. Wessels, and R. Loevinger, The MIRD Perspective 1999. *J. Nucl. Med.* **40,** 3S-10S (1999).
31. A. Bishayee, D. V. Rao, and R. W. Howell, RAPID COMMUNICATION: Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiat. Res.* **152,** 88-97 (1999).
32. L. G. Bouchet, W. E. Bolch, R. W. Howell, and D. V. Rao, S values for radionuclides localized within the skeleton. *J. Nucl. Med.* In press
33. L. G. Bouchet, W. E. Bolch, S. M. Goddu, R. W. Howell, and D. V. Rao, Considerations in the selection of radiopharmaceuticals for palliation of bone pain from metastatic osseous lesions. *J. Nucl. Med.* In press.
34. S. M. Goddu, A. Bishayee, L. G. Bouchet, W. E. Bolch, D. V. Rao, and R. W. Howell, Marrow toxicity of ^{33}P- versus ^{32}P-orthophosphate: Implications for therapy of bone pain and bone metastases. *J. Nucl. Med.* In press.
35. W. E. Bolch, L. G. Bouchet, J. S. Robertson, B. W. Wessels, J. A. Siegel, R. W. Howell, A. K. Erdi, B. Aydogan, S. Costes, and E. E. Watson, MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions - radionuclide S values at the voxel level. *J. Nucl. Med.* **40:**1, 11S-36S (1999).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Anupam Bishayee, Ph.D.	Research & Teaching Specialist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
Jadavpur University, Calcutta, India	B.Pharm.	1989	Pharm. Tech.
Jadavpur University, Calcutta, India	M.Pharm.	1991	Biochem. Pharmacol.
Jadavpur University, Calcutta, India	Ph.D.	1996	Tumor Biol.

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Ph.D. Thesis: Biological and biochemical role vanadium in the chemoprevention of neoplastic transformation against chemically-induced hepatocarcinogenesis in rats.

Professional Experience:

Feb. 1997-present Research & Teaching Specialist, UMDNJ - New Jersey Medical School

Awards:

1991 Award of Senior Research Fellowship from CSIR, India
1994 "Young Scientist" Award from National Science Council, Canada
1995 Award of Research Associateship from CSIR, India
1995 "Younger Scientist" Award from Indian Chemical Society
1999 "Young Investigator" Award from Indo-American Society of Nuclear Medicine, Los Angles

Bibliography:

A. Chapters or articles in books

1. Chatterjee, M. and Bishayee, A.: Vanadium - a new tool for cancer prevention. In, Vanadium in the Environment, Part 2: Health Effects (edited by Nriagu, J.O.). John Wiley and Sons Inc., New York, pp. 347-390 (1998)

2. Bishayee, A. and Chatterjee, M.: Antitumour potential of vanadium against chemically induced hepatocarcinogenesis : reflection in hepatic drug detoxification. In, Proceedings of the XVIth International Cancer Congress (edited by Rao, R.S., Deo, M.G. and Sanghvi, L.D.), Monduzzi Editore S.p.A., Bologna, pp. 3071-3076 (1994)

B. Articles

Goddu, S.M., **Bishayee, A.,** Bouchet, L.G., Bloch, W.E., Rao, D.V., Howell, R.W.: Marrow toxicity of ^{33}P- versus ^{32}P-orthophosphate: implications for therapy of bone pain and bone metastases. *Journal of Nuclear Medicine* - accepted (1999)

EXHIBIT 3

2. **Bishayee, A.,** Beguinot. L., Bishayee, S.: Conformational analysis of the phosphorylated epidermal growth factor receptor. *Bioscience Reports - in press* (1999)
3. **Bishayee, A.,** Roy, S., Chatterjee, M.: Characterization of selective induction and alteration of xenobiotic biotransforming enzymes by vanadium during diethylnitrosamine-induced chemical rat liver carcinogenesis. *Oncology Research* 11, 41-53 (1999)
4. **Bishayee, A.,** Rao, D.V., Howell, R.W.: Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using novel three-dimensional tissue culture model. *Radiation Research.* 152, 88-97 (1999)
5. **Bishayee, A.,** Beguinot, L., Bishayee, S.: Phosphorylation of tyrosine 992, 1068, and 1086 is required for conformational change of the human epidermal growth factor receptor C-terminal tail. *Molecular Biology of the Cell, 10, 525-536* (1999)
6. Howell, R.W., Goddu, S.M., **Bishayee, A.,** Rao, DV.: Radioprotection against lethal damage caused by chronic irradiation with radionuclides *in vitro. Radiation Research,* 150, 391-399 (1998)
7. **Bishayee, A.,** Kulkarni, S.P., Roy, K., Chatterjee, M. : Alterations in total iron, zinc and calcium levels and its influence on the hepatic activities of gamma-glutamyl transferase and glucose-6-phosphatase in the host bearing transplantable murine lymphoma. *Cancer Investigation,* 16, 231-236 (1998)
8. Sarkar, A., Basak, R., **Bishayee, A.,** Basak, J. and Chatterjee, M.: β-Carotene inhibits rat liver chromosomal aberrations and DNA chain break after a single injection of diethylnitrosamine. *British Journal of Cancer,* 76, 855-861 (1997)
9. **Bishayee, A.,** Karmakar, R., Mandal, A., Kundu, S.N. and Chatterjee, M.: Vanadium-mediated chemoprotection against chemical rat hepatocarcinogenesis : reflection in haematological and histological characteristics. *European Journal of Cancer Prevention,* 6, 58-70 (1997)
10. **Bishayee, A.,** Banik, S., Marimuthu, P. and Chatterjee, M.: Vanadium-mediated suppression of diethylnitrosamine-induced chromosomal aberrations in rat hepatocytes and its correlation with induction of hepatic glutathione and glutathione S-transferase. *International Journal of Oncology,* 10, 413-423 (1997)
11. Banerjee, K.K., **Bishayee, A.,** Marimuthu, P.: Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. *Journal of Occupational and Environmental Medicine,* 39, 258-260 (1997)
12. Mandal, A., **Bishayee, A.** and Chatterjee, M.: *Trianthema portulacastrum* affords antihepatotoxic activity against carbon tetrachloride-induced chronic liver damage in mice : reflection in subcellular levels. *Phytotherapy Research,* 11, 216-221 (1997)
13. **Bishayee, A.,** Mandal, A. and Chatterjee, M.: Prevention of alcohol carbon tetrachloride-induced signs of early hepatotoxicity in mice by *Trianthema portulacastrum* L. *Phytomedicine,* 3, 155-161 (1996)
14. **Bishayee, A.** and Chatterjee, M.: Inhibitory effect of vanadium on rat liver carcinogenesis initiated with diethylnitrosamine and promoted by phenobarbital. *British Journal of Cancer,* 71, 1214-1220 (1995)
15. **Bishayee, A.** and Chatterjee, M.: Inhibition of altered liver cell foci and persistent nodule growth by vanadium during diethylnitrosamine-induced hepatocarcinogenesis in rats. *Anticancer Research,* 15, 455-462 (1995)
16. Sarkar, A., **Bishayee, A.** and Chatterjee, M.: Beta-carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepatocarcinogenesis. *Cancer Biochemistry Biophysics,* 15, 111-125 (1995)
17. **Bishayee, A.** and Chatterjee, M.: Time course effects of vanadium supplement on cytosolic reduced glutathione level and glutathione S-transferase activity. *Biological Trace Element Research,* 48, 275-285 (1995)
18. **Bishayee, A.,** Sarkar, A. and Chatterjee, M.: Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. *Journal of Ethnopharmacology,* 47, 69-74 (1995)
19. **Bishayee, A.** and Chatterjee, M.: Mechanism of anti-stress activity of *Mikania cordata* root extract in albino mice. *International Journal of Pharmacognosy,* 33, 215-221 (1995)

20. **Bishayee, A.,** Banerjee, K.K. and Chatterjee, M.: Alterations in hepatic drug metabolizing enzyme activities following administration of human placental extract in rats. *European Review for Medical and Pharmacological Sciences,* 17, 19-16 (1995)
21. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Effects of human placental extract on brain monoamines and monoamine oxidase activity in rats. *Tohoku Journal of Experimental Medicine,* 176, 17-24 (1995)
22. **Bishayee, A.** and Chatterjee, M.: Anticarcinogenic biological response of *Mikania cordata* : reflections in hepatic biotransformation systems. *Cancer Letters,* 81, 193-206 (1994)
23. **Bishayee, A.** and Chatterjee, M.: Protective effects of *Mikania cordata* root extract against physical and chemical factors-induced gastric erosions in experimental animals. *Planta Medica,* 60, 110-113 (1994)
24. **Bishayee, A.** and Chatterjee, M.: Increased lipid peroxidation in tissues of catfish *Clarias batracnus* following vanadium treatment : *in vivo* and *in vitro* evaluation. *Journal of Inorganic Biochemistry,* 54, 277-284 (1994)
25. **Bishayee, A.** and Chatterjee, M.: Anti-stress potential of *Mikania cordata* root extract in mice. *International Journal of Pharmacognosy,* 32, 126-134 (1994)
26. Banerjee, K.K., **Bishayee, A.,** Banik, S. and Chatterjee, M.: Hepatotoxicity of human placental extract in rats : a biochemical evaluation. *Nihon University Journal of Medicine,* 36, 197-206 (1994)
27. **Bishayee, A.** and Chatterjee, M.: Hypolipidaemic and anti-atherosclerotic effects of oral *Gymnema sylvestre* R. Br. leaf extract in albino rats fed on high fat diet. *Phytotherapy Research,* 8, 118-120 (1994)
28. **Bishayee, A.** and Chatterjee, M.: Dose-related enhancement of cytosolic glutathione S-transferase activity and glutathione content in liver and extrahepatic tissues in mice with *Mikania cordata* root extract : probable involvement in chemical carcinogenesis. *Australian Journal of Medical Herbalism,* 6, 9-13 (1994)
29. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Elevated lipid peroxidation, decreased glutathione levels and changes in glutathione-related enzymes in rats treated with human placental extract. *Acta Medica Okayama,* 47, 223-227 (1993)
30. **Bishayee, A.** and Chatterjee, M.: Selective enhancement of glutathione S-transferase activity in liver and extrahepatic tissues of rat following oral administration of vanadate. *Acta Physiologica et Pharmacologica Bulgarica,* 19, 83-89 (1993)
31. **Bishayee, A.** and Chatterjee, M.: Carrot aqueous extract protection against hepatic oxidative stress and lipid peroxidation induced by acute carbon tetrachloride intoxication in mice. *Fitoterapia,* 64, 261-265 (1993)
32. Mandal, P.K., **Bishayee, A.** and Chatterjee, M.: Stimulation of tissue repair by *Mikania cordata* root extract in carbon tetrachloride-induced liver injury in mice. *Phytotherapy Research,* 7, 103-105 (1993)
3. Mandal, P.K., **Bishayee, A.** and Chatterjee, M.: Stimulation of hepatic protein synthesis in response to *Mikania cordata* root extract in carbon tetrachloride-induced hepatotoxicity in mice. *Italian Journal of Biochemistry,* 41, 345-351 (1992)
34. Mandal, P.K., **Bishayee, A.,** Mukherjee, J.R. and Chatterjee, M.: *Mikania cordata* root extract in the inhibition of lipid peroxidation and reduction of enzyme leakage in mice with carbon tetrachloride-induced liver damage. *Phytotherapy Research,* 6, 227-229 (1992)
35. Mandal, P.K., **Bishayee, A.,** Mukherjee, J.R. and Chatterjee, M.: Effect of *Mikania cordata* root extract on lipid metabolism in carbon tetrachloride-induced fatty liver in mice. *Fitoterapia,* 63, 160-162 (1992)
36. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Anti-inflammatory effect of human placental extract : a biochemical mechanistic approach. *European Review for Medical and Pharmacological Sciences,* 14, 361-366 (1992)

AA

Form Approved Through 02/28/01
OMB No. 0925-0001

Department of Health and Human Services
Public Health Service

Grant Application

Follow instructions carefully.
Do not exceed character length restrictions indicated on sample.

LEAVE BLANK-FOR PHS USE ONLY.

Type	Activity	Number
Review Group	Formerly	
Council/Board *(Month, Year)*		Date Received

1. TITLE OF PROJECT *(Do not exceed 56 characters, including spaces and punctuation.)*
Effects of nonuniform distributions of radioactivity

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT ☒ NO ☐ YES *(If "Yes," state number and title)*
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR New Investigator ☐ YES

3a. NAME *(Last, first, middle)*: Howell, Roger W.

3b. DEGREE(S): Ph.D.

3c. SOCIAL SECURITY NO. *Provide on Form Page KK.*

3d. POSITION TITLE: Associate Professor

3e. MAILING ADDRESS *(Street, city, state, zip code)*:
Department of Radiology, MSB F-451
UMDNJ - New Jersey Medical School
185 S. Orange Ave.
Newark, NJ 07103

3f. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Radiology

3g. MAJOR SUBDIVISION: New Jersey Medical School

3h. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 973-972-5067
FAX: 973-972-6474

E-MAIL ADDRESS: rhowell@umdnj.edu

4. HUMAN SUBJECTS ☒ No ☐ Yes
4a. If "Yes," Exemption no. or IRB approval date
Full IRB or Expedited Review
4b. Assurance of compliance No. M1466-01NR

5. VERTEBRATE ANIMALS ☒ No ☐ Yes
5a. If "Yes," IACUC approval date
5b. Animal welfare assurance no. A3158-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year-MM/DD/YY)*
From: 07/01/00 Through: 06/30/05

7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD
7a. Direct Costs ($): 175,000.
7b. Total Costs ($): 259,075.

8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT
8a. Direct Costs ($): 875,000.
8b. Total Costs ($): 1,358,075.

9. APPLICANT ORGANIZATION
Name: UMDNJ - New Jersey Medical School
Address: 185 South Orange Avenue
Newark, NJ 07103-2714

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
Forprofit: → ☐ General ☐ Small Business

11. ORGANIZATIONAL COMPONENT CODE: 01

12. ENTITY IDENTIFICATION NUMBER: 1221775306A2
DUNS NO. *(if available)*:
Congressional District: 10

13. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Mr. Richard Wagner
Title: Manager, Grants and Contracts
Address: 30 Bergen Street
Newark, NJ 07103-3000

Telephone: (973)972-6456
FAX: (973)972-3425
E-Mail: grants_newark@umdnj.edu

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Neil S. Cherniack, M.D.
Title: Director of Research & Clinical Affairs
Address: UMDNJ - New Jersey Medical School
185 South Orange Avenue
Newark, NJ 07103-2714

Phone: (973)972-4568
FAX: (973)972-3585
E-Mail: cherniac@umdnj.edu

15. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE:
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*
R G Howell
DATE: 10/21/99

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE:
I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 14. *(In ink. "Per" signature not acceptable.)*
DATE: 10/21/99

EXHIBIT
tabbies
4

Principal Investigator/Program Director *(Last, first, middle)*: HOWELL, ROGER W

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

There are numerous factors that determine the biological response of tissues that contain radioactivity such as radiosensitivity, distribution of radioactivity, type and number of radiations emitted by the radionuclide, biokinetics of the radionuclide, repair time, etc. Traditionally, the mean absorbed dose to the tissue is calculated to correlate the biological response with mean absorbed dose. However, nonuniform activity distributions in tissue at the multicellular and subcellular levels result in nonuniform doses and therefore have made it difficult to adequately correlate biological response with mean absorbed dose. This is an important problem in diagnostic and therapeutic nuclear medicine. In the case of diagnosis, the risk of the radiation insult can in principle be drastically underestimated and potentially lead to increased risk of inducing cancer. In contrast, patients can be over- or under-treated in radionuclide therapy of cancer. Over-treatment or under-treatment in radionuclide therapy of cancer can have very adverse consequences in the final outcome for the patient. While calculation of absorbed dose at the cellular level has been advocated as a means to address this problem, this has largely remained a theoretical exercise. We hypothesize that the biological response of tissues containing incorporated radionuclides can be correlated with absorbed dose when calculated at the cellular level. To test our hypothesis, a novel in vitro multicellular cluster model will be used which allows tight control over variables. Multicellular clusters will be assembled with mammalian cells containing radioactivity and the cell survival fraction as a function of cluster activity will be determined for several different radiopharmaceuticals which emit alpha particles, beta particles, or Auger electrons. Different percentages of the cells will be labeled with the different radiochemicals to ascertain the impact of nonuniform distributions of radioactivity at the cellular and subcellular levels. By controlling the percentage of cells labeled, this model will also be used to ascertain the role of bystander effects in the biological effects of incorporated radioactivity. These data and cellular dosimetry calculations will be used to develop a theoretical model to predict response based on cellular absorbed dose and bystander effects. The outcome of this research is expected to have a major impact on understanding and predicting the biological response of tumor and normal tissue to nonuniform distributions of radioactivity.

PERFORMANCE SITE(S) *(organization, city, state)*

UMDNJ - New Jersey Medical School
Newark, NJ

KEY PERSONNEL. See instructions on page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

Name	Organization	Role on Project
Roger W. Howell, Ph.D.	UMDNJ - New Jersey Medical School, Radiology	Principal Investigator
Helene Z. Hill, Ph.D.	UMDNJ – New Jersey Medical School, Radiology	Co-Investigator
Dandamudi V. Rao, Ph.D.	UMDNJ - New Jersey Medical School, Radiology	Co-Investigator
Anupam Bishayee, Ph.D.	UMDNJ - New Jersey Medical School, Radiology	Res. Teaching Specialist

RESEARCH GRANT

TABLE OF CONTENTS

Budget Justification

Total Direct Costs for Entire Period of Support: $875,000

Initial Budget Period	Second Year of Support	Third Year of Support	Fourth Year of Support	Fifth Year of Support
$175,000	$175,000	$175,000	$175,000	$175,000

Personnel:

Roger W. Howell, Ph.D., Principal Investigator, (25% effort) will direct the project as a whole, supervise the experiments that are proposed and ensure that the Specific Aims are accomplished in a timely fashion. Dr. Howell has over a decade of experience with experimental and theoretical dosimetry of radionuclides.

Helene Z. Hill, Ph.D., Co-Investigator, (5% effort) will assist in the design and implementation of the HGPRT mutation and comet assays. She has extensive experience in radiobiology and has spearheaded the acquisition of the preliminary HGPRT mutation data.

Dandamudi V. Rao, Ph.D., Co-Investigator, (5% effort) will assist in experimental design, interpretation of the results, and manuscript preparation. He has more than two decades of experience with radiobiological models and dosimetry of radionuclides. *No salary support* is requested for Dr. Rao.

Anupam Bishayee, Ph.D., Research Teaching Specialist V, (100% effort) has three years of experience with the assays to be performed in this project. He will use this experience to immediately begin carrying out the day to day experiments described in the project. He will be responsible for generation of biokinetics, survival, and mutation data, as well as their analyses. He will also ensure that all necessary supplies are maintained.

Post-doctoral fellow, (100% effort) will be responsible for the computer coding required for the theoretical modeling calculations. This will be carried out under the guidance of Dr. Howell. He will also be responsible for the radiochemical synthesis and purification. Finally, he will also carry out some of the V79 cell experiments due to the large amount of data that needs to be collected.

Equipment:

Packard Automatic Gamma Counter: A Packard Cobra Model 5003 Automatic Gamma Counter is requested in the budget ($25,000). The instrument is required for the extensive radiation quantification measurements that are an integral part of the project. We currently use a NaI well-counter with Canberra Model 10 portable multichannel analyzer to assay ^{131}I and ^{125}I. This instrument requires that samples be counted one at a time and it is 15-20 years old with numerous keypad operational problems. The proposed studies involve counting hundreds of tubes per week and therefore the automatic aspect of the requested instrument is essential.

Personal Computer: One high speed personal computer to support the theoretical calculations ($2,500).

Increase in Budget Over First Submission:

The direct costs requested for this revised grant proposal are increased primarily because of experiments added at the request of the reviewers. Fluorescent activated cell sorting adds an important dimension to the work. We anticipate 8 hours of sorting per week at an annual cost of about $12,500. To accommodate the sorting experiments and the reviewers request for additional assays, we have increased the requested budget period from four years to five years. Finally, we have added Dr. Hill to help provide support for the additional assays, namely mutagenesis and comet assays.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Roger W. Howell, Ph.D.	Associate Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Massachusetts, Amherst, MA	B.S.	1982	Physics
University of Massachusetts, Amherst, MA	Ph.D.	1987	Physics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

1995- **Associate Professor**, UMDNJ - New Jersey Medical School
1989-1995 **Assistant Professor**, UMDNJ - New Jersey Medical School
1987-1989 **Instructor**, UMDNJ - New Jersey Medical School

Awards:

1995 Outstanding Dosimetry Manuscript Award by the Journal of Nuclear Medicine. S. Murty Goddu, R.W. Howell, D.V. Rao. "A generalized approach to absorbed dose calculations for dynamic tumor and organ masses". J. Nucl. Med. 36: 1923-1927 (1995).

Research Projects Ongoing or Completed During the Last 3 Years:

July 1991-June 1997. USPHS Grant No. R29 CA54891.
Principal Investigator: Roger W. Howell, Ph.D.
Agency: National Cancer Institute
The long-term objective of this project was to investigate the biological effects of incorporated alpha particle emitters as they relate to radon exposure. Experiments were carried out in vivo in the mouse testes and in vitro in cell culture models. Radionuclides emitting alpha particles with energies ranging from 3.2 to 8.8 MeV were examined and various subcellular distributions of the radionuclides were studied. While energy plays an important role in the response, the subcellular distribution did not.

1998. NE Hazardous Substance Research Center Subcontract No. 991653.
Principal Investigator: Roger W. Howell, Ph.D.
Agency: U.S. Department of Energy
The long-term objective of this project was to provide educational assistance to residents of the city of Bloomfield, NJ on risk associated with exposure to thorium and uranium industrial waste products. In addition, methods of remediation and remediation assessment were also covered. Finally, the remediation process used by Westinghouse was also examined in detail.

Special Professional Service:

Society of Nuclear Medicine Medical Internal Radiation Dose Committee (MIRD), July 1992 - present.
International Commission on Radiation Units and Measurements (ICRU). Report Committee on Conceptual Basis for Dose Specification in Nuclear Medicine. 1998 - present.
Program Committee, Second International Symposium on Biophysical Aspects of Auger Processes, July 5-6, 1991, Univ. of Massachusetts, Amherst, MA.

Scientific Program Sub-Chair, Dosimetry/Radiobiology. 1993 and 1994 Annual Meetings of the Society of Nuclear Medicine.

Am. Assoc. Physicists in Medicine Task Group on Auger Electron Dosimetry, June 1989 - June 1994

Program Committee, 1991-1994, 1998 Annual Meeting of the Society of Nuclear Medicine.

Program Committee, 1993-1995 , Annual Meeting of the American Association of Physicists in Medicine.

Publications: 65 articles, 1 edited book, 1 book, 2 reports

Selected Bibliography:

1. R.W. Howell, D.V. Rao, and K.S.R. Sastry. Macroscopic dosimetry for radioimmunotherapy: Non-uniform activity distributions in solid tumors. Med. Phys. 16:66-74 (1989).
2. D.V. Rao, V.R. Narra, R.W. Howell, V.K. Lanka, K.S.R. Sastry. Induction of spermhead abnormalities by incorporated radionuclides: Dependence on subcellular distribution, type of radiation, dose rate, and presence of radioprotectors. Radiat. Res. 125:89-97 (1991).
3. R.W. Howell, D.V. Rao, D.Y. Hou, V.R. Narra, K.S.R. Sastry. The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. Radiat. Res. 128:282-292 (1991).
4. R.W. Howell, V.R. Narra, and D.V. Rao. Absorbed dose calculations for rapidly growing tumors. J. Nucl. Med., 33:277-281 (1992).
5. V.R. Narra, R.W. Howell, R. S. Harapanhalli, K. S. R. Sastry, and D. V. Rao, Radiotoxicity of some I-123, I-125, and I-131 labeled compounds in mouse testis: Implications for radiopharmaceutical design. J. Nucl. Med. 33:2196-2201 (1992).
6. R.W. Howell, Radiation spectra for Auger-electron emitting radionuclides: Report No. 2 of AAPM Nuclear Medicine Task Group No. 6. Med. Phys. 19, 1371-1383 (1992).
7. R.W. Howell, V. R. Narra, K. S. R. Sastry, and D. V. Rao, On the equivalent dose for Auger electron emitters. Radiat. Res. 134:71-78 (1993).
8. R.W. Howell, M.T. Azure, V.R. Narra, and D.V. Rao. Relative biological effectiveness of alpha-particle emitters at low doses. Radiat. Res. 137:352-360 (1994).
9. S. M. Goddu, R. W. Howell, and D. V. Rao, Cellular dosimetry: Absorbed fractions for monoenergetic electron (0.1 keV - 1 MeV) and alpha particle (3 - 10 MeV) sources, and S-values for radionuclides, distributed uniformly in different cell compartments. J. Nucl. Med. 35:303-316 (1994).
10. S. M. Goddu, D. V. Rao, and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation, and subcellular distribution of radionuclides. J. Nucl. Med. 35:521-530 (1994).
11. M. T. Azure, R. D. Archer, K. S. R. Sastry, D. V. Rao, and R. W. Howell, Biologic effect of ^{212}Pb localized in the nucleus of mammalian cells: Role of recoil energy in the radiotoxicity of internal alpha emitters. Radiat. Res. 140, 276-283 (1994).
12. R. W. Howell, A. I. Kassis, S. J. Adelstein, D. V. Rao, H. A. Wright, R. N. Hamm, J. E. Turner, and K. S. R. Sastry, Radiotoxicity of ^{195m}Pt labeled trans-platinum (II) in mammalian cells. Radiat. Res. 140, 55-62 (1994).
13. R. W. Howell, S. M. Goddu, and D. V. Rao, Application of the linear-quadratic model to radioimmunotherapy: Further support for the advantage of longer-lived radionuclides. J. Nucl. Med. 35, 1861-1869, (1994).
14. S. M. Goddu, R. W. Howell, and D. V. Rao, A generalized approach to absorbed dose calculations for dynamic tumor and organ masses. J. Nucl. Med. 36, 1923-1927 (1995).
15. S. M. Goddu, R. W. Howell, and D. V. Rao, A generalized approach to absorbed dose calculations for dynamic tumor and organ masses. J. Nucl. Med. 36, 1923-1927 (1995).
16. R. S. Harapanhalli, V. Yaghmai, D. Giuliani, R. W. Howell, and D. V. Rao, Antioxidant effects of vitamin C in mice following X-irradiation. *Research Communications in Molecular Pathology and Pharmacology* **94,** 271-287 (1996).

17. S. M. Goddu, R. W. Howell, and D. V. Rao, Calculation of equivalent dose for Auger electron emitting radionuclides distributed in human organs. *Acta Oncologica* **35,** 909-916 (1996).
18. S. M. Goddu, V. R. Narra, R. S. Harapanhalli, R. W. Howell, and D. V. Rao, Radioprotection by DMSO against the biological effects of incorporated radionuclides in vivo. *Acta Oncologica* **35,** 901-907 (1996).
19. R. W. Howell, and D. V. Rao, Auger electron emitters: Equivalent dose, chemical protection against their biological effects and use in cancer treatment. In *Radiation Research 1895-1995* (U. Hagen, D. Harder, H. Jung, and C. Streffer, Eds.), pp. 82-85. Universitätsdruckerei H. Stürtz AG, Würzburg, 1996.
20. V. R. Narra, R. W. Howell, S. M. Goddu, and D. V. Rao, Effects of a 1.5-Tesla magnetic field on spermatogenesis and embryogenesis in mice. *Investigative Radiology* **31,** 586-590 (1996).
21. R. S. Harapanhalli, L. W. McLaughlin, R. W. Howell, D. V. Rao, S. J. Adelstein, and A. I. Kassis, $^{125}I/^{127}I$-IodoHoechst 33342: Synthesis, DNA binding, and biodistribution studies. *J. Med. Chem.* **39,** 4804-4809 (1996).
22. R. W. Howell, S. M. Goddu, and D. V. Rao, Design and performance characteristics of an experimental Cs-137 irradiator to simulate internal radionuclide dose rate patterns. *J. Nucl. Med.* **38,** 727-731 (1997).
23. R. W. Howell, S. M. Goddu, V. R. Narra, D. R. Fisher, R. E. Schenter, and D. V. Rao, Radiotoxicity of gadolinium-148 and radium-223 in mouse testes: Relative biological effectiveness of alpha particle emitters *in vivo*. *Radiat. Res.* **147,** 342-348 (1997).
24. S. M. Goddu, R. W. Howell, L. G. Bouchet, W. E. Bolch, and D. V. Rao, *MIRD Cellular S values: Self-absorbed dose per unit cumulated activity for selected radionuclides and monoenergetic electron and alpha particle emitters incorporated into different cell compartments*. Society of Nuclear Medicine, Reston, VA, 1997.
25. S. J. Adelstein, R. W. Howell, J. L. Humm, G. M. Makrigiorgos, and B. W. Wessels, On the conceptual basis for dose quantities in nuclear medicine. *ICRU News* **1,** 4-10 (1998).
26. R. W. Howell, S. M. Goddu, A. Bishayee, and D. V. Rao, Radioprotection against lethal damage caused by chronic irradiation with radionuclides in vitro. *Radiat. Res.* **150,** 391-399 (1998).
27. S. M. Goddu, R. W. Howell, D. C. Giuliani, and D. V. Rao, Biological dosimetry of bone marrow for incorporated ^{90}Y. *J. Nucl. Med.* **39,** 547-551 (1998).
28. R. W. Howell, S. M. Goddu, and D. V. Rao, Proliferation and the advantage of longer-lived radionuclides in radioimmunotherapy. *Med. Phys.* **25,** 37-42 (1998).
29. W. E. Bolch, L. G. Bouchet, J. S. Robertson, B. W. Wessels, J. A. Siegel, R. W. Howell, A. K. Erdi, B. Aydogan, S. Costes, and E. E. Watson, MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions - radionuclide S values at the voxel level. *J. Nucl. Med.* **40,** 11S-36S (1999).
30. R. W. Howell, B. W. Wessels, and R. Loevinger, The MIRD Perspective 1999. *J. Nucl. Med.* **40,** 3S-10S (1999).
31. A. Bishayee, D. V. Rao, and R. W. Howell, RAPID COMMUNICATION: Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiat. Res.* **152,** 88-97 (1999).
32. L. G. Bouchet, W. E. Bolch, R. W. Howell, and D. V. Rao, S values for radionuclides localized within the skeleton. *J. Nucl. Med.* In press
33. L. G. Bouchet, W. E. Bolch, S. M. Goddu, R. W. Howell, and D. V. Rao, Considerations in the selection of radiopharmaceuticals for palliation of bone pain from metastatic osseous lesions. *J. Nucl. Med.* In press.
34. S. M. Goddu, A. Bishayee, L. G. Bouchet, W. E. Bolch, D. V. Rao, and R. W. Howell, Marrow toxicity of ^{33}P- versus ^{32}P-orthophosphate: Implications for therapy of bone pain and bone metastases. *J. Nucl. Med.* In press.
35. W. E. Bolch, L. G. Bouchet, J. S. Robertson, B. W. Wessels, J. A. Siegel, R. W. Howell, A. K. Erdi, B. Aydogan, S. Costes, and E. E. Watson, MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions - radionuclide S values at the voxel level. *J. Nucl. Med.* **40:**1, 11S-36S (1999).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Helene Z. Hill, Ph.D.	Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
Smith College, Northampton, MA	BA	1950	Pre-Med/French
Brandeis University, Waltham, MA	PhD	1964	Biology
Harvard Medical School, Boston, MA	Post-doc	1964-66	Protein Synthesis
University of Colorado Medical Center, Denver, CO	Post-doc	1966-67	Medical Genetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

MAJOR UNIVERSITY APPOINTMENTS

1967-72 Assistant Professor of Biophysics and Genetics University of Colorado School of Medicine, Denver, CO
1973-76 Associate Professor of Radiology, Section of Cancer Biology Washington University School of Medicine, St. Louis, MO
1976-81 Associate Professor of Biochemistry, Marshall University School of Medicine, Huntington, WV
1981 Professor of Biochemistry, Marshall University School of Medicine, Huntington, WV
1981- Professor of Radiology, UMDNJ-New Jersey Medical School, Newark, NJ
1981- Professor of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School
1991- Professor of Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School

HONORS AND AWARDS

First recipient, Life Achievement Award, The Baldwin School, Bryn Mawr, PA, 1991
Listed in "American Men and Women in Science," "Who's Who in the East", "Who's Who of American women", "Who's Who Directory of Professionals and Resources in Cancer", "Who's Who in Science and Engineering"
Smith College Medal, February, 1997
Gallo Award for Outstanding Research, presented at the 1998 Annual Retreat on Cancer Research in New Jersey, Princeton, NJ May, 1998

RESEARCH PROJECTS ONGOING OR COMPLETED DURING THE LAST 3 YEARS:

USPHS-NCI: Photoprotection and Photosensitization of DNA by Melanin, 1992-1996 The goal of these studies was to define the role of melanin in the carcinogenesis of melanoma and in the photoprotection of the skin.
Foundation of the UMDNJ: Autocrine rescue of tumors from death by radiation, 1996-1998 The goal of this project was to determine the biological properties of an extracellular factor that rescued melanoma cells from death by both ionizing radiation and chemotherapy.
Dean's Bridging Fund, 1997-1999 The aim was to further characterize the rescue factor in order to isolate the protein.
With George J. Hill, M.D. Elsa U. Pardee Foundation: Studies of a new multi-therapy resistance factor, 1993-1999. This grant provided additional support for the isolation and characterization of the rescue factor from melanoma.
Fellowships sponsored:
NJ Commission for Cancer Research: Fellow: H. Colleen Silva, M.D., Chief of Surgical Oncology, Jersey City Medical Center, Jersey City, NJ Clinical relevance of a multi-therapy resistance factor 1994-1996
Society for Surgical Oncology: Summer Fellowship for Medical Students awarded to Mauricio Zapiach, NJMS '97 Role of a melanoma multi-therapy resistance factor in apoptosis inhibition, summer, 1996.
NJ Commission for Cancer Research: Summer Fellowship for Medical Students awarded to Joseph Grossman NJMS '98. Role of a melanoma multi-therapy resistance factor in apoptosis, summer, 1997.
Cancer Education Institutional Grant: Summer Fellowship for Undergraduate Students awarded to Cynthia Quainoo, Smith College '99; Summer, 1996, 1997; Fellowships for Medical Students, awarded to Gayatri Rao NJMS '02 and David Muccino NJMS '02, summer, 1998; Stephen Cohen NJMS '02, summer, 1998.

PUBLICATIONS (from a total of 64 publications, 8 chapters or reviews and 155 abstracts)

HZ HILL and RB Setlow. Post-replication repair in 3 murine melanomas, EMT6 and a normal mouse lung fibroblast line. Cancer Research 40:1867-72, 1980

HZ HILL and RB Setlow. Comparative action spectra for pyrimidine dimer formation in Cloudman S91 mouse melanoma and EMT6 mouse mammary carcinoma cells. Photochemistry and Photobiology 35:681-84, 1982.

HZ HILL and GJ Hill. Effect of scheduling of combinations of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide and 1-(2-chloro- ethyl)-3-(4-methylcyclohexyl)-1-nitrosourea on the Harding-Passey and the Cloudman S91 mouse melanomas. Cancer Research 42:838-42, 1982.

GJ Hill and HZ HILL. Cyclophosphamide activity against B16 melanoma in a rapid in vitro test system. J Surg Oncol 24:73-78, 1983.

GJ Hill, ET Krementz and HZ HILL. DTIC and combination therapy for melanoma: IV. Late results after complete response to chemotherapy (COG protocols 7130, 7131 and 7131A). Cancer 53:1299-1305, 1084.

S. Raina, HZ HILL, GJ Hill and BF Rush Jr. Scheduling of combination chemotherapy for a murine melanoma with the sub-renal capsular assay. J Surg Oncol 26:51-52, 1984.

HZ HILL and GJ Hill. In vitro activation of cyclophosphamide for an in vitro assay system. J Surg Oncol 26:225-29, 1984.

BH Fadem and HZ HILL. The gray opossum (Monodelphis domestica): a marsupial model for xenogeneic neoplasms. Cancer Letters 27:233-38, 1985.

DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and HZ HILL. In vivo effects of iron-55 and iron-59 on mouse testes: Biophysical dosimetry of Auger-electrons. J Nuclear Medicine 26:1456-65, 1985.

DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and HZ HILL. Radiobiological effects of Auger electron emitters in vivo: Spermatogenesis in mice as an experimental model. Radiat Prot Dosimetry 13:245-49, 1985.

KA Zirvi, GJ Hill and HZ HILL. Comparative studies of chemotherapy of human tumor cells in vitro by 3HThd uptake inhibition and soft agar clonogenic assay. J Surg Oncol 31:123-26, 1986.

MA Lea, A Luke, O Velezquez, L Carpenter, CF Martinson, HZ HILL and GJ Hill. Effects of sodium cyanate in mice bearing B16 melanoma. Cancer Chemother and Pharmacol 17:231-35, 1986.

KS Dasmahapatra, HZ HILL, A Dasmahapatra and S Suarez. Evaluation of adenosine deaminase activity in patients with head and neck cancer. J Surg Research 40:368-73, 1986.

DV Rao, KSR Sastry, GF Govelitz, HE Grimmond and HZ HILL. In vivo effects of iron-55 and iron-59 on mouse testes: Efficacy of Auger electrons vs beta rays. Radiat Prot Dosimetry, 1986.

GJ Hill, KA Zirvi, HZ HILL and S Raina. Chemosensitivity testing with antineoplastic agents. J Med Soc NJ 84:437-41, 1987.

HZ HILL, M Ohanian, GJ Hill and R Winne. The use of the 90Sr applicator for intraoperative radiation therapy in a mouse tumor model. J Surg Oncol 34:264-67, 1987.

HZ HILL, CA Huselton, B Pilas and GJ Hill. The ability of melanins to protect against the radiolysis of thymine and thymidine. Pigment Cell Research 1:81-86, 1987.

HZ HILL and GJ Hill. Eumelanin causes strand breaks and kills cells. Pigment Cell Research 1:163-70, 1987.

BH Fadem, HZ HILL, CA Huselton and GJ Hill. Transplantation, growth and regression of mouse melanoma xenografts in neonatal marsupials. Cancer Investigation 6:403-8-1988.

HZ HILL, JG Peak and MJ Peak. Induction of DNA-protein crosslinks in melanotic Cloudman S91 mouse melanoma cells and EMT6 mouse mammary carcinoma cells by monochromatic 254 and 405 nm light. Pigment Cell Research 2:427-30, 1989.

CA Huselton and HZ HILL. Melanin photosensitizes UVC DNA damage in pigmented cells. Environmental and Molecular Mutagenesis 16:37-43, 1990.

HZ HILL and GJ Hill. Irradiation of cells attached or suspended by rubber-policeman or by trypsin influences the extent of ionizing radiation-induced DNA strand breaks. Radiation Research 125:343-345, 1991.

GP Studzinski, KB Reddy, HZ HILL and AK Bhandal. Potentiation of ara-C cytotoxicity to HL 60 cells by 1,25$(OH)_2$ vitamin D_3 correlates with reduced rate of maturation of DNA relication intermediates. Cancer Research 51: 3451-3455, 1991.

HZ HILL, KN Cathcart, J Bargellini, Z Trizna, GJ Hill, KU Schallreuter and JM Wood. Does melanin affect the low LET radiation response of Cloudman S91 mouse melanoma cell lines? Pigment Cell Research 4: 80-86, 1991.

HZ HILL, Z Trizna, M Ali and GJ Hill. A radiation resistance factor in cultured Cloudman mouse melanoma cells. Radiation Research 129: 43-47, 1992.

K Hubbard-Smith, HZ HILL and GJ Hill. Melanin both causes and prevents oxidative base damage in DNA: Quantitation by anti-thymine glycol antibody. Radiation Research 130: 160-165, 1992.

UK Schlehaider, GJ Hill and HZ HILL. Influence of an autocrine diffusible resistance factor on cell survival after exposure to therapeutic agents. Melanoma Research 3: 357-362, 1993.

U Schlehaider, HZ HILL, A Pashapour and GJ Hill. Influence of an autocrine multi-therapy resistance factor on radiation responses of melanoma cells. Melanoma Research 4:21-27, 1994

KU Schallreuter, KR Lemke, HZ HILL and JM Wood. Thioredoxin reductase induction coincides with melanin biosynthesis in brown and black guinea pigs and in murine melanoma cells J Invest Dermatol 103: 820-824, 1994.

KA Cieszka, HZ HILL, GJ Hill and PM Plonka. Growth and pigmentation in genetically related Cloudman S91 melanoma cell lines treated with 3-isobutyl-1-methyl-xanthine and β-melanocyte stimulating hormone. Experimental Dermatology, 4: 192-198,1995

HZ HILL, GJ Hill, K Cieszka, M Azure, I Chowdhary and RM Sayre. A multi-therapy resistance factor from melanoma reveals that killing by near UV is different from genotoxic agents. Photochemistry and Photobiology 61: 479-483, 1995.

RM Goodman and HZ HILL. Interference by cellular melanin with assay of DNA-protein crosslinks by the potassium dodecyl sulfate precipitation method. Pigment Cell Research 9:68-71, 1996.

GJ Hill, BF Rush, Jr, HZ HILL, LF Pliner, S Raina, S Cook, K Klein, C Gerne and R Slimowitz. Breast cancer in the inner city: Intensive efforts may be succeeding. J. Medicine 27: 135-151, 1996.

HZ HILL. The photobiology of melanin. Introduction. Photochemistry and Photobiology 65: 471, 1997.

W. Li and HZ HILL. Induced melanin reduces mutations and cell killing in mouse melanoma. Photochemistry and Photobiology 65:480-485, 1997. *Symposium-in-Print*, HZHILL, editor.

HZ HILL, GJ Hill, K Cieszka, PM Plonka, DL Mitchell, MF Meyenhofer, P. Xin and RE Boissy. Comparative action spectrum for ultraviolet light killing of mouse melanocytes from different genetic coat color backgrounds. Photochemistry and Photobiology 65: 983-989, 1997.

K. Cieszka, HZ HILL, P. Xin, M. Azure, GJ Hill, MF Meyenhofer, RE Boissy and DL Mitchell. Survival of Cloudman mouse melanoma cells after irradiation by solar wavelengths of light. Pigment Cell Research 10: 193-200, 1997.

HZ HILL, W. Li, P Xin and DL Mitchell. Melanin: A two edged sword? Pigment Cell Research 10: 158-161, 1997.

S Zhou, W Li, HZ Hill, GJ Hill, F Kueppers, WG McKenna, RJ Muschel, LJ Wolansky and HC Silva. Therapeutic resistance: characterization and inactivation by specific antiserum of a putative protein family produced by tumour cells. Melanoma Research 9: 40-50, 1999.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Dandamudi V. Rao, Ph.D.	Emeritus Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
University of Massachusetts, Amherst, MA	Ph.D.	1972	Physics
University of Massachusetts, Amherst, MA	M.S.	1970	Physics
Andhra University, Waltair, India	M.S.	1966	Nucl. Phys.
Andhra University, Waltair, India	B.S.	1964	Physics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

1998- Emeritus Professor of Radiology, UMDNJ, New Jersey Medical School, NJ
1987-98 Professor of Radiology, UMDNJ, New Jersey Medical School, NJ
1987-98 Director of Radiation Research, Dept. Radiology , UMDNJ, Newark, NJ
1978-87 Associate Professor of Radiology, UMDNJ, Newark, NJ
1974-78 Assistant Professor of Radiology, UMDNJ, Newark, NJ
1974-78 Director of Health Physics, UMDNJ, Newark, NJ
1972-74 Instructor of Radiology, Albert Einstein College of Medicine, Bronx, NY

Special Professional Service:

Program Chairman, 2nd International Symposium on Biophysical Aspects of Auger Processes, UMass, Amherst, July 5-6	1991
NIH Special Reviewer	1989-93
Chairman, American Association of Physicists in Medicine (AAPM), Task Group #6 : Dosimetry of Auger Emitters	1989-1994
Delegate, Nuclear Medicine Delegation to Peoples' Republic of China	1988
American College of Medical Physics, Task Group #3 on Nuclear Medicine Calibration and survey standards	1985-90
IAEA Nuclear Medicine Consultant to Kenya	1984
Member, Continuing Education Committee, AAPM	1982-84
Program Director, AAPM Summer School on Physics of Nuclear Medicine	1983
Member, Nuclear Medicine Committee, AAPM	1981-84
International Atomic Energy Agency (IAEA) Nucl. Med. Consultant to Ghana	1981-82

Grants & Honors :

USPHS Grant No. CA 32877, 1982-93 : Effects of low energy electrons from Auger processes.
NJ Cancer Comm. Grant No. 689-042, 1989-91 : Effects of alpha emitters in spermatogonial cells.
Swedish Medical Society Medal for research on effects of Auger electrons, 1989.
1995 Outstanding Manuscript Award by the Journal of Nuclear Medicine. S. Murty Goddu, R.W. Howell, D.V. Rao. J. Nucl. Med. 36: 1923-1927 (1995).

Books Published: Four (Authored 2 and edited 2)
Publications: 85 articles, 1 patent

Bibliography of Selected Relevant Publications:

1. D.V. Rao, G.F. Govelitz, and K.S.R. Sastry, Radiotoxicity of Tl-201 in mouse testes: Inadequacy of conventional dosimetry. *Journal of Nuclear Medicine* **24**, 145 (1983).
2. D.V. Rao, H.E. Grimmond, and K.S.R. Sastry, Biological dosimetry of Indium-Radiopharmaceuticals in mouse testis. *Invited Paper: Forum on Microdosimetry of radiopharmaceuticals*, Medical Research Council, London, 1985, *International Journal of Radiation Biology* **50**, 561 (1986).
3. D.V. Rao, K.S.R. Sastry, H.E. Grimmond, R.W. Howell, G.F. Govelitz, V.K. Lanka, and V.B. Mylavarapu, Cytotoxicity of some indium radiopharmaceuticals in mouse testes. *Journal of Nuclear Medicine* **29**, 375 (1988).
4. D.V. Rao, V.R. Narra, R.W. Howell, G.F. Govelitz, and K.S.R. Sastry, In vivo Radiotoxicity of DNA-incorporated I-125 compared with that of densely ionising Alpha-particles. *The Lancet.* **II**, No. 8664, 650. 1989.
5. D.V. Rao, V.R. Narra, R.W. Howell, and K.S.R. Sastry. Biological consequence of nuclear versus cytoplasmic decays of I-125: Cysteamine as a radioprotector against Auger cascades in vivo. *Radiation Research* **124**, 188 (1990).
6. D.V. Rao, V.R. Narra, R.W. Howell, V.K. Lanka, and K.S.R. Sastry, Induction of spermhead abnormalities by incorporated radionuclides: Dependence on subcellular distribution, type of radiation, dose rate, and presence of radioprotectors. *Radiation Research* **125**, 89 (1991).
7. V.R. Narra, R.W. Howell, R. S. Harapanhalli, K. S. R. Sastry, and D. V. Rao, Radiotoxicity of some I-123, I-125, and I-131 labeled compounds in mouse testis: Implications for radiopharmaceutical design. *J. Nucl. Med.* 33:2196-2201 (1992).
8. V. R. Narra, R.W. Howell, K. S. R. Sastry, and D. V. Rao, Vitamin C as a radioprotector against ^{131}I in vivo. *J. Nucl. Med.* 34: 637-640 (1993).
9. D. V. Rao and R.W. Howell, Time dose fractionation in radioimmunotherapy: Implications to selection of radionuclides. *J. Nucl. Med.* 34:1801-1810 (1993).
10. V. R. Narra, R. S. Harapanhalli, R. W. Howell, K. S. R. Sastry, and D. V. Rao, Vitamins as radioprotectors in vivo. I. Protection by Vitamin C against internal radionuclides in mouse testis: Implications to the mechanism of the Auger effect. *Radiat. Res.* 137:394-399 (1994).
11. S. M. Goddu, R. W. Howell, and D. V. Rao, Cellular dosimetry: Absorbed fractions for monoenergetic electron (0.1 keV - 1 MeV) and alpha particle (3 - 10 MeV) sources, and S-values for radionuclides, distributed uniformly in different cell compartments. *J. Nucl. Med.* 35:303-316 (1994).
12. S. M. Goddu, D. V. Rao, and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation, and subcellular distribution of radionuclides. *J. Nucl. Med.* 35:521-530 (1994).
13. R. S. Harapanhalli, V. R. Narra, V. Yaghmai, M. T. Azure, S. M. Goddu, R. W. Howell, and D. V. Rao. Vitamins as radioprotectors in vivo. II. Protection by vitamin A and soybean oil against radiation damage caused by internal radionuclides. *Radiat. Res.* 139, 115-122 (1994).
14. J. L. Humm, R. W. Howell, and D. V. Rao, Dosimetry of Auger electron emitting radionuclides: Report No. 3 of the AAPM Nuclear Medicine Task Group No. 6. *Med. Phys.* 21, 1901-1915 (1994).
15. R. W. Howell, S. M. Goddu, and D. V. Rao, Application of the linear-quadratic model to radioimmunotherapy: Further support for the advantage of longer-lived radionuclides. *J. Nucl. Med.* 35, 1861-1869 (1994).
16. D. V. Rao, B. Shepstone, H. G. Wilkins, and R. W. Howell, Testicular kinetics and dosimetry of Tl-201 in humans. J. Nucl. Med. 36, 607-609 (1995).
17. S. M. Goddu, R. W. Howell, and D. V. Rao, A generalized approach to absorbed dose calculations for dynamic tumor and organ masses. J. Nucl. Med. 36, 1923-1927 (1995).
18. V. R. Narra, R. S. Harapanhalli, S. M. Goddu, R. W. Howell, and D. V. Rao, Radioprotection against biological effects of internal radionuclides in vivo by S-(2-aminoethyl)isothiouronium bromide hydrobromide (AET). J. Nucl. Med. 36, 259-266 (1995).

19. V. R. Narra, R. W. Howell, S. M. Goddu, and D. V. Rao, Effects of a 1.5-Tesla magnetic field on spermatogenesis and embryogenesis in mice. Investigative Radiology 31, 586-590 (1996).
20. R. W. Howell, S. M. Goddu, and D. V. Rao, Design and performance characteristics of an experimental Cs-137 irradiator to simulate internal radionuclide dose rate patterns. J. Nucl. Med. 38, 727-731 (1997).
21. R. W. Howell, S. M. Goddu, V. R. Narra, D. R. Fisher, R. E. Schenter, and D. V. Rao, Radiotoxicity of gadolinium-148 and radium-223 in mouse testes: Relative biological effectiveness of alpha particle emitters in vivo. Radiat. Res. 147, 342-348 (1997).
22. R. W. Howell, S. M. Goddu, and D. V. Rao, Proliferation and the advantage of longer-lived radionuclides in radioimmunotherapy. Med. Phys. 25, 37-42 (1998).
23. S. M. Goddu, R. W. Howell, D. C. Giuliani, and D. V. Rao, Biological dosimetry of bone marrow for incorporated ^{90}Y. *J. Nucl. Med.* **39,** 547-551 (1998).
24. A. Bishayee, D. V. Rao, and R. W. Howell, RAPID COMMUNICATION: Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiat. Res.* **152,** 88-97 (1999).
25. L. G. Bouchet, W. E. Bolch, R. W. Howell, and D. V. Rao, S values for radionuclides localized within the skeleton. *J. Nucl. Med.* In press
26. L. G. Bouchet, W. E. Bolch, S. M. Goddu, R. W. Howell, and D. V. Rao, Considerations in the selection of radiopharmaceuticals for palliation of bone pain from metastatic osseous lesions. *J. Nucl. Med.* In press.
27. S. M. Goddu, A. Bishayee, L. G. Bouchet, W. E. Bolch, D. V. Rao, and R. W. Howell, Marrow toxicity of ^{33}P- versus ^{32}P-orthophosphate: Implications for therapy of bone pain and bone metastases. *J. Nucl. Med.* In press.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Anupam Bishayee, Ph.D.	Research & Teaching Specialist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR CONFERRED	FIELD OF STUDY
Jadavpur University, Calcutta, India	B.Pharm.	1989	Pharm. Tech.
Jadavpur University, Calcutta, India	M.Pharm.	1991	Biochem. Pharmacol.
Jadavpur University, Calcutta, India	Ph.D.	1996	Tumor Biol.

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Ph.D. Thesis: Biological and biochemical role vanadium in the chemoprevention of neoplastic transformation against chemically-induced hepatocarcinogenesis in rats.

Professional Experience:

Feb. 1997-present Research & Teaching Specialist, UMDNJ - New Jersey Medical School

Awards:

1991 Award of Senior Research Fellowship from CSIR, India
1994 "Young Scientist" Award from National Science Council, Canada
1995 Award of Research Associateship from CSIR, India
1995 "Younger Scientist" Award from Indian Chemical Society
1999 "Young Investigator" Award from Indo-American Society of Nuclear Medicine, Los Angles

Bibliography:

A. Chapters or articles in books

1. Chatterjee, M. and Bishayee, A.: Vanadium - a new tool for cancer prevention. In, Vanadium in the Environment, Part 2: Health Effects (edited by Nriagu, J.O.). John Wiley and Sons Inc., New York, pp. 347-390 (1998)

2. Bishayee, A. and Chatterjee, M.: Antitumour potential of vanadium against chemically induced hepatocarcinogenesis : reflection in hepatic drug detoxification. In, Proceedings of the XVIth International Cancer Congress (edited by Rao, R.S., Deo, M.G. and Sanghvi, L.D.), Monduzzi Editore S.p.A., Bologna, pp. 3071-3076 (1994)

B. Articles

Goddu, S.M., **Bishayee, A.,** Bouchet, L.G., Bloch, W.E., Rao, D.V., Howell, R.W.: Marrow toxicity of ^{33}P- versus ^{32}P-orthophosphate: implications for therapy of bone pain and bone metastases. *Journal of Nuclear Medicine* - accepted (1999)

2. **Bishayee, A.,** Beguinot. L., Bishayee, S.: Conformational analysis of the phosphorylated epidermal growth factor receptor. *Bioscience Reports - in press* (1999)
3. **Bishayee, A.,** Roy, S., Chatterjee, M.: Characterization of selective induction and alteration of xenobiotic biotransforming enzymes by vanadium during diethylnitrosamine-induced chemical rat liver carcinogenesis. *Oncology Research* 11, 41-53 (1999)
4. **Bishayee, A.,** Rao, D.V., Howell, R.W.: Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiation Research.* 152, 88-97 (1999)
5. **Bishayee, A.,** Beguinot, L., Bishayee, S.: Phosphorylation of tyrosine 992, 1068, and 1086 is required for conformational change of the human epidermal growth factor receptor C-terminal tail. *Molecular Biology of the Cell, 10, 525-536* (1999)
6. Howell, R.W., Goddu, S.M., **Bishayee, A.,** Rao, DV.: Radioprotection against lethal damage caused by chronic irradiation with radionuclides *in vitro. Radiation Research,* 150, 391-399 (1998)
7. **Bishayee, A.,** Kulkarni, S.P., Roy, K., Chatterjee, M. : Alterations in total iron, zinc and calcium levels and its influence on the hepatic activities of gamma-glutamyl transferase and glucose-6-phosphatase in the host bearing transplantable murine lymphoma. *Cancer Investigation,* 16, 231-236 (1998)
8. Sarkar, A., Basak, R., **Bishayee, A.,** Basak, J. and Chatterjee, M.: β-Carotene inhibits rat liver chromosomal aberrations and DNA chain break after a single injection of diethylnitrosamine. *British Journal of Cancer,* 76, 855-861 (1997)
9. **Bishayee, A.,** Karmakar, R., Mandal, A., Kundu, S.N. and Chatterjee, M.: Vanadium-mediated chemoprotection against chemical rat hepatocarcinogenesis : reflection in haematological and histological characteristics. *European Journal of Cancer Prevention,* 6, 58-70 (1997)
10. **Bishayee, A.,** Banik, S., Marimuthu, P. and Chatterjee, M.: Vanadium-mediated suppression of diethylnitrosamine-induced chromosomal aberrations in rat hepatocytes and its correlation with induction of hepatic glutathione and glutathione S-transferase. *International Journal of Oncology,* 10, 413-423 (1997)
11. Banerjee, K.K., **Bishayee, A.,** Marimuthu, P.: Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. *Journal of Occupational and Environmental Medicine,* 39, 258-260 (1997)
12. Mandal, A., **Bishayee, A.** and Chatterjee, M.: *Trianthema portulacastrum* affords antihepatotoxic activity against carbon tetrachloride-induced chronic liver damage in mice : reflection in subcellular levels. *Phytotherapy Research,* 11, 216-221 (1997)
13. **Bishayee, A.,** Mandal, A. and Chatterjee, M.: Prevention of alcohol carbon tetrachloride-induced signs of early hepatotoxicity in mice by *Trianthema portulacastrum* L. *Phytomedicine,* 3, 155-161 (1996)
14. **Bishayee, A.** and Chatterjee, M.: Inhibitory effect of vanadium on rat liver carcinogenesis initiated with diethylnitrosamine and promoted by phenobarbital. *British Journal of Cancer,* 71, 1214-1220 (1995)
15. **Bishayee, A.** and Chatterjee, M.: Inhibition of altered liver cell foci and persistent nodule growth by vanadium during diethylnitrosamine-induced hepatocarcinogenesis in rats. *Anticancer Research,* 15, 455-462 (1995)
16. Sarkar, A., **Bishayee, A.** and Chatterjee, M.: Beta-carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepatocarcinogenesis. *Cancer Biochemistry Biophysics,* 15, 111-125 (1995)
17. **Bishayee, A.** and Chatterjee, M.: Time course effects of vanadium supplement on cytosolic reduced glutathione level and glutathione S-transferase activity. *Biological Trace Element Research,* 48, 275-285 (1995)
18. **Bishayee, A.,** Sarkar, A. and Chatterjee, M.: Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. *Journal of Ethnopharmacology,* 47, 69-74 (1995)
19. **Bishayee, A.** and Chatterjee, M.: Mechanism of anti-stress activity of *Mikania cordata* root extract in albino mice. *International Journal of Pharmacognosy,* 33, 215-221 (1995)

20. **Bishayee, A.,** Banerjee, K.K. and Chatterjee, M.: Alterations in hepatic drug metabolizing enzyme activities following administration of human placental extract in rats. *European Review for Medical and Pharmacological Sciences,* 17, 19-16 (1995)
21. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Effects of human placental extract on brain monoamines and monoamine oxidase activity in rats. *Tohoku Journal of Experimental Medicine,* 176, 17-24 (1995)
22. **Bishayee, A.** and Chatterjee, M.: Anticarcinogenic biological response of *Mikania cordata* : reflections in hepatic biotransformation systems. *Cancer Letters,* 81, 193-206 (1994)
23. **Bishayee, A.** and Chatterjee, M.: Protective effects of *Mikania cordata* root extract against physical and chemical factors-induced gastric erosions in experimental animals. *Planta Medica,* 60, 110-113 (1994)
24. **Bishayee, A.** and Chatterjee, M.: Increased lipid peroxidation in tissues of catfish *Clarias batracnus* following vanadium treatment : *in vivo* and *in vitro* evaluation. *Journal of Inorganic Biochemistry,* 54, 277-284 (1994)
25. **Bishayee, A.** and Chatterjee, M.: Anti-stress potential of *Mikania cordata* root extract in mice. *International Journal of Pharmacognosy,* 32, 126-134 (1994)
26. Banerjee, K.K., **Bishayee, A.,** Banik, S. and Chatterjee, M.: Hepatotoxicity of human placental extract in rats : a biochemical evaluation. *Nihon University Journal of Medicine,* 36, 197-206 (1994)
27. **Bishayee, A.** and Chatterjee, M.: Hypolipidaemic and anti-atherosclerotic effects of oral *Gymnema sylvestre* R. Br. leaf extract in albino rats fed on high fat diet. *Phytotherapy Research,* 8, 118-120 (1994)
28. **Bishayee, A.** and Chatterjee, M.: Dose-related enhancement of cytosolic glutathione S-transferase activity and glutathione content in liver and extrahepatic tissues in mice with *Mikania cordata* root extract : probable involvement in chemical carcinogenesis. *Australian Journal of Medical Herbalism,* 6, 9-13 (1994)
29. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Elevated lipid peroxidation, decreased glutathione levels and changes in glutathione-related enzymes in rats treated with human placental extract. *Acta Medica Okayama,* 47, 223-227 (1993)
30. **Bishayee, A.** and Chatterjee, M.: Selective enhancement of glutathione S-transferase activity in liver and extrahepatic tissues of rat following oral administration of vanadate. *Acta Physiologica et Pharmacologica Bulgarica,* 19, 83-89 (1993)
31. **Bishayee, A.** and Chatterjee, M.: Carrot aqueous extract protection against hepatic oxidative stress and lipid peroxidation induced by acute carbon tetrachloride intoxication in mice. *Fitoterapia,* 64, 261-265 (1993)
32. Mandal, P.K., **Bishayee, A.** and Chatterjee, M.: Stimulation of tissue repair by *Mikania cordata* root extract in carbon tetrachloride-induced liver injury in mice. *Phytotherapy Research,* 7, 103-105 (1993)
3. Mandal, P.K., **Bishayee, A.** and Chatterjee, M.: Stimulation of hepatic protein synthesis in response to *Mikania cordata* root extract in carbon tetrachloride-induced hepatotoxicity in mice. *Italian Journal of Biochemistry,* 41, 345-351 (1992)
34. Mandal, P.K., **Bishayee, A.,** Mukherjee, J.R. and Chatterjee, M.: *Mikania cordata* root extract in the inhibition of lipid peroxidation and reduction of enzyme leakage in mice with carbon tetrachloride-induced liver damage. *Phytotherapy Research,* 6, 227-229 (1992)
35. Mandal, P.K., **Bishayee, A.,** Mukherjee, J.R. and Chatterjee, M.: Effect of *Mikania cordata* root extract on lipid metabolism in carbon tetrachloride-induced fatty liver in mice. *Fitoterapia,* 63, 160-162 (1992)
36. Banerjee, K.K., **Bishayee, A.** and Chatterjee, M.: Anti-inflammatory effect of human placental extract : a biochemical mechanistic approach. *European Review for Medical and Pharmacological Sciences,* 14, 361-366 (1992)

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other", identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: Three adjoining laboratories with four fume hoods, four sinks, freezer, refrigerator, and stainless steel countertops: 25'x15', 15'x10', 15'x10'.

Clinical:

Animal:

Computer: Two personal computers for word processing (RWH, DVR). One high-speed HP minicomputer housed in UMDNJ Academic Computing for computational purposes.

Office: Office adjacent to laboratories: 12'x12' (RWH), 12'x10' (HZH).

Other: Office of Radiation Safety Services. UMDNJ has a fully staffed radiation safety program that will oversee the radiological safety aspects of this project.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Equipment in the Radiation Research Laboratory: Canberra NaI and HpGe detectors with multichannel analyzers, Beckman Model LS3800 liquid scintillation counter, Nuclear Associates Comp-U-Cal dose calibrator, Coulter Model ZM cell counter, NuAire LabGard laminar flow hood for cell culture, Queue CO_2 incubator, Beckman refrigerated centrifuge, Nikon Labophot II upright microscope with camera and video, Olympus CK inverted microscope, Olympus dissecting microscope, Mettler H54 balance, Kinematic GmbH tissue homogenizer, Virtis Unitrap II lyophilizer, J.L. Shepherd Model 28-8 low-dose-rate irradiator equipped with variable mercury attenuator system, Thomson-Nielson MOSFET dosimeter system, Waters 501 HPLC Pumps, Waters 660 Solvent Programmer, Waters 440 Absorbance Detector, Pharmacia Fraction Collector FRAC-100, Spectra Physics Data Jet Integrator.

Confocal microscope: A Zeiss Laser Scan Microscope LSM 410 invert equipped with ArKr laser (488/568/467 nm) is housed in an adjacent laboratory on our floor in the NJMS Department of Pediatrics. This instrument is available at a cost of $2,500 per annum.

Fluorescent activated cell sorter. Beckton-Dickinson FACStar dual laser and FACSCalibur cell sorters are housed in an adjacent laboratory in the NJMS Department of Pediatrics. This instruments are available at $30/h.

High Dose Rate Cs-137 irradiator. A J.L. Shepherd Mark I Cs-137 gamma irradiator is located in Radiation Safety Services of our building. This instrument is available for unlimited use at a cost of $250 per annum.

Research Plan

Introduction to Revised Application *(Not to exceed 3 pages)*

This application represents a major revision of our original submission of February 1999 entitled *Effects of Nonuniform Distributions of Radioactivity* that was assigned Application number 1 RO1 CA83838-01. In the Radiation Study Section SUMMARY STATEMENT, both reviewers of the original application were enthusiastic in that the work proposed was both innovative, original, and ultimately useful in clinical use of radionuclides. However, they expressed some reservations regarding the selection of a single biological endpoint, lack of details regarding the theoretical modeling, and some aspects of the experimental model. Accordingly, they asked a number of thought-provoking questions and made a number of excellent suggestions to improve the research plan. These concerns have been addressed and folded into the research plan of the revised application. Substantial revisions are denoted by a vertical line along the right margin. For ease of review, responses to each of the questions and suggestions are given below in the order that they appear. The location in the revised application where they have been addressed is provided in each case.

1. Critique #1 (pg. 2, para 3, last line of SUMMARY STATEMENT). A question has been raised regarding the biological consequence of a small amount of compound in the wrong subcellular area. Actually, there is no "wrong" subcellular area as long as the distribution of the radiochemical is measured so that it can be folded into the dosimetry calculations. The distribution of radiochemical between more than one subcellular compartment is expected and is a phenomenon that we have dealt with routinely in our work on dosimetry and biological response of mammalian cells to incorporated radionuclides (*1-3*). Methods to handle this are now described more explicitly in Section D.4c by equations D.4 and D.5.
2. Critique #1 (pg. 2, last sentence). Fluorescent activated cell sorting techniques have been used to obtain preliminary data that show functional gap junctional intercellular communication in our multicellular model (see Section C.2g). In addition, we have acquired extensive preliminary data on the use of lindane in our model. These data are provided in Sections C2.e and C2.f.
3. Critique #1 (pg. 3, paras 1 and 3; pg. 4, para 3). This reviewer has expressed concerns over the theoretical model used in the analysis of the data. These concerns appear to focus mainly on that fact that "individual energy depositions from these radiochemicals can vary by factors of four or five decades for the same mean energy deposited in subcellular structures", and that "microdosimetric theories can predict sizable synergistic effects, even for the alpha/beta model being used in the proposed study". Indeed, energy depositions in critical targets can vary by these magnitudes, most notably in the case of Auger electron emitters. We have carried out extensive theoretical modeling in this regard for Auger electron emitters and alpha emitters (*4-8*). In fact, absorbed doses as high as 10^7 Gy may be found in the immediate vicinity (< 1 nm) of the decay site of Auger electron emitters (*4*). Although enlightening in terms of providing an explanation for the high-LET type effects of Auger emitters when incorporated into DNA, we have not found this level of theoretical detail to be helpful in terms of dose response relationships for endpoints such as survival. Rather, cellular dosimetry offers a tractable approach that provides good dose response relationships for in vitro cell culture experiments (*1, 3, 9, 10*). Our positive experience with cellular dosimetry in cell suspensions gives us confidence that it can be adapted to the case of multicellular dosimetry. Nearly all of our cell suspension studies have involved mixed radiation fields, a natural course in the study of the radiotoxicity of incorporated radionuclides. This has not been a stumbling block with respect to cellular dosimetry which gives us confidence that it can be tackled in the multicellular environment. While our cellular dosimetry approach utilizes the MIRD Schema, it is <u>not</u> simply a conventional calculation of the mean absorbed dose to the cluster. Our data in Fig. 2 clearly indicate that the mean cluster dose would not suffice. We recognize that the sparse details provided in the original submission were insufficient to convey the cellular dosimetry approach. Therefore, we have added considerable detail to the modeling described in Section D.4. It is likely that our approach (and any

approach for that matter) will require modification before it is able to fully accommodate the complex nature of the biological response to nonuniform distributions of radioactivity. This will be done continuously as the experimental data is collected. We recognize that there are numerous approaches to solving this problem, therefore, the best overall approach is one that considers many possible solutions to the problem. To achieve this, we will post our protocols, raw data, graphs, and curve fits on an advertised web site at the New Jersey Medical School (see end of Section D.4d). This will open the analysis to all scientists who are interested so that scientific progress in this field can be accelerated. We have already had requests from theoretical microdosimetrists to provide data for analysis and we have obliged when possible (e.g. Ref. (*11*)).

4. Critique #1 (pg. 3, para 2; pg. 4, para 3). The reviewer has indicated that the original proposal was too narrow in scope with respect to the use of a single biological endpoint (survival) and cell line (V79). This deserved criticism is well taken and consequently we have made considerable expansions in these regards. First, we have added a mutation assay (HGPRT) and comet assay to address the issue of biological endpoints. Prof. Helene Z. Hill, an experienced radiobiologist of note, has been added as a coinvestigator to aid in the design and implementation of these new assays. She spearheaded the acquisition of our preliminary mutation data presented in Section C.2i. Second, we have added a series of rat liver cell lines which have been provided by our new consultant Prof. James E. Trosko, a leading expert in the field of gap junctional intercellular communication. This series includes both gap junctional competent and incompetent cell lines, which provide considerable depth to our exploration of bystander effects in that they allow one to examine radiation effects in the absence of any bystander effects. The new cell lines are described in Section D.2b, and their experimental application is described in Sections D.3a, D.3b, D.3e.1, and D.3e.2. The addition of these new assays and cell lines have prompted our request for a fifth year of funding which is essential to complete the extensive array of studies proposed.

5. Critique #2 (pg. 5, para 2, lines 19-22; pg. 6, para 2, lines 20-24). Concern has been expressed regarding the capacity of the cells in the cluster to form normal cell-to-cell contacts (e.g. gap junctions). We have carried out new experiments in the cluster system that show functional gap junctions are indeed formed under our experimental conditions. These experiments utilize specialized fluorescent dyes and fluorescent activated cell sorting techniques that have successfully been used by other investigators to establish the presence of functional gap junctional intercellular communication. These preliminary studies are described in Section C.2g.

6. Critique #2 (pg. 5, para 2, lines 23-29). A question has been raised about the presence of hypoxia in the clusters, in particular, whether only some of the cells in the pellet were hypoxic which would confound interpretation of the experimental results. The reviewer has suggested carrying out monolayer experiments to study this possibility. Instead we have implemented a simple and novel technique that involves a comparison of the dose response of intact clusters compared to clusters that have been disassembled immediately prior to irradiation with external gamma rays (Section 2c.i). These preliminary studies suggest that some hypoxia is present, however, the limited hypoxia is uniform throughout the cluster. Therefore, we do not anticipate that this will impede our ability to interpret the data.

7. Critique #2 (pg. 5, para 2, lines 29-40). This question concerns the dose delivered to the cells during the colony formation period and whether this has been accounted for in our analysis. The answer to these questions are both yes, and we apologize for not describing them in more detail in the original proposal. Our revised proposal does a much better job of describing exactly how this is folded into the calculations (Section D.4c). The decays that occur in the cells during both the period of uptake of radioactivity as well as the colony forming period are accounted for in the determination of the cumulated activity in the labeled cells. However, these decays only affect the absorbed dose to the labeled cells and do not impact bystander effects to the unlabeled cells or the dose delivered to unlabeled cells from decays. Only decays during the 72 h cluster period impact the bystander response. This is specifically accounted for in the calculation of cumulated activity in the labeled cells as per Eqs. D.6 and D.7. These are used in turn to calculate self- and cross-doses in Eqs. D.4 and D.5.

8. Critique #2 (pg. 6, para 1, last 5 lines). The V79 cell diameters were correctly listed as 10 μm and erroneously as 5 μm. As described in Section D.3g, we plan to experimentally measure these diameters and the packing density *in situ* using confocal microscopy techniques. These measurements will be performed for each cell line and folded into the theoretical modeling.

9. Critique #2 (pg. 6, para 2, lines 11-14). The reviewer offered an excellent suggestion of separating the labeled and unlabeled cells to separately determine their biological response. We have acquired preliminary data to demonstrate that we can now separate the labeled and unlabeled cells in the cluster using fluorescent activated cell sorting techniques. This approach along with our preliminary data is given in Section C.2j. Accordingly, experiments are now proposed that determine the biological response of each subpopulation separately, as well as obtaining survival data on the total combined population. These are folded into the Research Design and Methods under Section D.3d and D.3f.

10. Critique #2 (pg. 6, para 2, lines 14-23). These concerns regarding formation of gap junctions and interpretation of the lindane results are discussed above in Items 2 and 5.

11. Critique #2 (pg. 6, para 2, last 4 lines). The reviewer has stated that it is unclear how the effects of DMSO on bystander cells will be separated out from that on radiolabeled cells when DMSO is added to the cluster. Indeed, changes in survival fraction or mutation frequency in a cluster containing a mixture of labeled and unlabeled cells could be due to mitigation of radiation effects and/or bystander effects. In the case of ^{3}HTdR, if DMSO only mitigated radiation effects, only the first component of the two-component exponential curve would be affected and one would expect an upward shift of the entire second component with no change in the slope (Eq. C.1). In contrast, mitigation of the bystander effects would change the slope of the second component. However, to add an extra degree of certainty to our measurements we also plan to use the same fluorescent activated cell sorting techniques indicate above in Item 9. Specifically, the impact of DMSO on the bystander response will be determined by separating the bystander cells and assaying their response specifically. These experiments, detailed in Section D.3e.3, will initially be carried out only for ^{3}HTdR and ^{125}IdU because they have little or no cross irradiation which will interfere with the interpretation of the data.

As indicated at the beginning of this introduction, the critiques of our original submission were highly insightful and thought provoking. The questions raised and suggestions made prompted us to embark on a series of new preliminary studies that have addressed their issues and shaped the Research Design and Methods employed in our revised application in a very positive way. Furthermore, the advances made in our preliminary studies have buoyed our confidence in the model. Accordingly, we look forward to reading the critiques of this revised submission.

A. Specific Aims

It is well recognized that there are several variables that dictate the biological response of tissues that contain radioactivity. Among the many variables are the radiosensitivity of the tissue, distribution of radioactivity at the macroscopic, cellular and subcellular levels, type, number, and relative biological effectiveness (RBE) of radiations emitted (e.g. alpha, beta, Auger electrons), kinetics of uptake and clearance of the radionuclide, dose rate, repair, presence or absence of chemical radioprotectors or sensitizers, and possible bystander effects. Considerable effort has been devoted to correlate biological outcome with repair, presence of radioprotectors and sensitizers, LET, and the kinetics of radiochemicals as it relates to absorbed dose. Consequently, these variables are fairly well understood. However, we have a very limited understanding of the correlation of biological effects with nonuniform dose distributions that result from nonuniform distribution of radioactivity at the cellular and subcellular levels. It is well known that nonuniform dose distributions can have a profound impact on biological response, however, no experimentally verified approach or model has emerged with broad-based acceptance that can be used to predict the impact of nonuniform dose distributions resulting from tissue-incorporated radioactivity. As a result, for lack of a clear and simple alternative, it has become standard practice to simply report the biological response and the mean absorbed dose to the organ or tissue as recommended by the ICRP (*12*) and ICRU (*13*). Furthermore, there is mounting evidence that bystander effects may also play an important role in the biological response to nonuniform dose distributions (*14-16*). These are problems of considerable importance to diagnostic (*17*) and therapeutic nuclear medicine (*18*), as well as to the field of radiation protection (*19, 20*). While microdosimetry (*11, 19, 21-24*) and cellular dosimetry (*25-28*) have been advocated as a means to address this problem, this has largely remained a theoretical exercise without adequate experimental verification that these approaches can consistently predict biological outcome. The exception to this has been in relatively simple geometries such as cell suspensions or monolayers where the cross-dose from one cell to another is minimal (*1, 9, 29, 30*). The reason for this is the complex array of variables indicated above that come into play when the biological response of solid tissue regions is considered. In vivo models and *traditional* in vitro multicellular spheroids do not allow tight control over a sufficient number of variables to isolate and quantitatively assess the biological impact of nonuniform distributions of radioactivity at the cellular level within solid tissues. We hypothesize that the biological response of tissues containing incorporated radionuclides can indeed be correlated with cellular absorbed dose and variables relating to the bystander effect. An in vitro multicellular cluster model, which allows tight control over variables, has been specifically designed to test our hypothesis in a methodical manner. Our Specific Aims are as follows:

1. To experimentally quantify the impact of cellular distribution of radioactivity on cell survival and mutation frequency in a multicellular cluster. This will be achieved by assembling multicellular clusters with two populations of cells: i) cells containing radioactivity, and ii) cells containing no radioactivity. In a given experiment, each cluster will have the same percentage of radiolabeled cells, yet different total cluster activities. After a three day irradiation period, the clusters will be dismantled and the cell survival fraction and mutation frequency determined as a function of cluster activity (kBq/cluster). This process will be repeated for four different percentages of radiolabeled cells: 10%, 50%, and 100%. Four radionuclides with different radiation properties will be considered as representative of the classes of radionuclides encountered in medicine and the environment: i) ^{210}Po: emits 5.3 MeV alpha particles, ii) ^{131}I: emits medium-energy beta particles, iii) ^{3}H: emits very low-energy beta-particles, and iv) ^{125}I: emits numerous low-energy Auger electrons. The radionuclides will be directed to the cytoplasm of the cells using the radiochemicals ^{210}Po-citrate, ^{3}H-methionine, and ($^{125/131}$I)Iodorhodamine.
2. To experimentally quantify the impact of subcellular distribution of the radioactivity on cell survival and mutation frequency in multicellular clusters. This will be achieved by repeating Specific Aim 1 with radiochemicals that target the cell nucleus: dipyrrolidinedithiocarbamato-^{210}Po (^{210}Po-PDC), tritiated thymidine (^{3}HTdR), and $^{125/131}$I-iododeoxyuridine ($^{125/131}$IdU). Subcellular distribution is a known determinant of the biological effects of ^{3}H (*31*) and ^{125}I (*32*), and may also play a role in the case of ^{210}Po (*33*) and ^{131}I (*34*).

3. To experimentally determine the kinetics of radioactivity in the cells and calculate the cellular absorbed dose using a theoretical model which represents the multicellular clusters assembled in Specific Aims 1 and 2. Our established multicellular dosimetry model (*26, 35*) will be adapted to calculate the radiation self-dose and cross-dose to each labeled and unlabeled cell in the cluster. This will carried out with computational efficiency by creating dosimetry tools in the form of tabulations of cross-dose cellular S values to complement existing tables of self-dose cellular S values (*27*). Tabulations will be carried out for the radionuclides to be studied in this proposal and for other radionuclides of interest to nuclear medicine (*36*).

4. To investigate the effect of cell self-irradiation of labeled cells on the survival of unlabeled bystander cells that receive comparatively little or no dose. This will be achieved initially by analyzing the data obtained in Specific Aims 1 and 2. To study the possible mechanisms of observed bystander effects, experiments in Specific Aims 1 and 2 will be repeated in the presence of lindane (inhibitor of gap junctional intercellular communication (GJIC)) 10% DMSO (free radical scavenger). Additional studies will be carried out with GJIC competent and incompetent cells. Finally, experiments will be carried out where labeled cells and unlabeled bystander cells will be separated by fluorescent activated cell sorting (FACS) and separately assayed for survival.

5. In parallel with the studies in Specific Aims 1, 2, and 4, DNA damage will be assessed using the comet assay. Analysis of these data will provide insight into the mechanism of genomic damage produced in bystander cells and help to distinguish between effects due to direct DNA damage and those due indirectly to apoptosis.

6. To develop the elements of a theoretical model to correlate biological response in the experimental multicellular clusters with cellular self-dose and cross-dose (Specific Aim 3), and any variables related to bystander effects observed in Specific Aim 4.

The proposed research will provide new data on the biological effects of nonuniform distributions of radioactivity using a novel approach to specifically control the nonuniformity at the cellular and subcellular levels. These data, along with the dosimetry and the biological response models, are likely to have a major impact on our understanding of radiation effects from incorporated radioactivity. It is therefore anticipated that these data may substantially enhance our capacity to predict biological response of tumor and normal tissue in nuclear medicine and from environmental exposure to radioactivity.

B. Background and Significance

Prediction of radiation risks in diagnostic nuclear medicine and therapeutic outcome in therapeutic nuclear medicine largely relies on calculation of the absorbed dose. Absorbed dose specification is complex due to the wide variety of radiations emitted, heterogeneity in activity distribution, biokinetics, etc. Following the administration of a radiopharmaceutical, the radioactivity is taken up by the various organs within the body and the radioactivity is then eliminated through both biological clearance and physical decay. A general formalism was developed by the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine to calculate absorbed doses from tissue incorporated radioactivity (*13, 37, 38*). This dosimetry formalism was adopted by the International Commission Radiation Units and Measurements (ICRU) (*13*), and is typically used to calculate organ absorbed doses in clinical nuclear medicine. Conventional organ absorbed dose estimates assume that the radioactivity is distributed uniformly throughout the organ and the mean absorbed dose to the organ is calculated (*39*). Risk estimates and prediction of biological response based on the mean absorbed dose to the organ inherently assume that all cells within the organ receive essentially the same absorbed dose. Organs are composed of a variety of cells having very different radiosensitivities (*40*), and sometimes, depending on the radiopharmaceutical, very different degrees of incorporation of radioactivity (*41-43*). Accordingly, the absorbed dose delivered to the various cell populations in the organ may differ markedly. Furthermore, large differences in cellular uptake of radioactivity can occur even within a given cell population,

particularly at very low absorbed doses where the activity concentration is low (*44, 45*). Highly nonuniform uptake of radioactivity is even observed when very large amounts of radioactivity are used such as in cancer therapy where radiopharmaceuticals are targeted to tumors (*46-48*).

The extent to which nonuniform distributions of radioactivity impact the absorbed dose distribution, and ultimately the biological effect, is strongly dependent on the number, type, and energy of the radiations emitted by the radionuclide. Many radionuclides used in nuclear medicine decay by electron capture and/or internal conversion (e.g. ^{51}Cr, ^{67}Ga, ^{99m}Tc, ^{111}In, ^{123}I, ^{201}Tl) and consequently emit a large number of low-energy Auger electrons. The majority of these electrons deposit their energy over subcellular dimensions, which produces highly localized energy density in the immediate vicinity of the decay site. In these instances, the subcellular distribution of the radioactivity can have a dramatic impact on the estimated absorbed dose to the cells in the tissue (*26, 35, 49-51*). Similarly, the short range of alpha particles in biological tissues (40-100 μm) also leads to nonuniform dose distributions when the radioactivity is not uniformly distributed in the tissue (*11, 24, 26, 52, 53*). Energetic beta emitters are somewhat more forgiving because their mean range in tissue is at least several hundred μm. However, distribution of these radionuclides invariably leads to nonuniform dose distributions as well (*46-48, 54-57*). Such nonuniformities are also encountered in brachytherapy (*58*) and external beam radiation therapy (*59*), albeit to a much lesser degree. However, their impact on biological response is carefully considered in these modalities.

As indicated above, prediction of radiation risks in diagnostic nuclear medicine and therapeutic outcome in therapeutic nuclear medicine has largely relied on calculation of the mean absorbed dose to the organ or tissue (*39*). Makrigiorgos et al. (*17*) clearly showed that the dose enhancement observed for Auger emitters when individual cells within an organ accumulated large amounts of activity could lead to significant errors in risk estimates based on mean organ doses in diagnostic nuclear medicine. Similarly, the impact of nonuniform activity distributions on prediction of biological response in therapeutic nuclear medicine has been a topic of considerable importance. Most recently, the discussion has largely centered around nonuniform distributions of radioactivity in radioimmunotherapy and its impact on tumor and normal tissue response (*26, 27, 35, 46, 47, 56, 60-62*). The dependence of absorbed dose distribution on nonuniformities in activity distribution at the macroscopic, multicellular, cellular, and subcellular levels have been explored. However, with few exceptions, this has largely remained a theoretical exercise. This is largely due to the fact that one has total control over the variables that control nonuniform distribution of radioactivity in a theoretical model, whereas investigators have not managed to gain similar control in the laboratory. The distribution of radioactivity in laboratory models generally depends on the radiochemical, details of the experimental model, diffusion, biokinetics, and numerous other variables that do not afford fine control over the degree of nonuniformity. Consequently, it has been difficult to create reproducible nonuniform distributions of radioactivity in models with highly reproducible biological endpoints. Therefore, it has been difficult to correlate absorbed dose and biological response from nonuniform distributions of radioactivity.

There is also mounting interest in the role of "bystander effects" in the biological response of mammalian cells to ionizing radiation (*14-16*). It has long been believed that the principal genetic effects of ionizing radiation in mammalian cells are the direct result of DNA damage in irradiated cells that has not been repaired adequately. Therefore, when cells are exposed to nonuniform distributions of radioactivity, only those cells which receive "hits" from the emitted radiations would be damaged. No effects would be observed in cells that are not "hit". These cells are referred to as bystanders. Studies from a number of investigators suggest that these so-called bystander cells do indeed suffer damage as a consequence of being in the neighborhood of irradiated cells. Nagasawa and Little (*63*) showed that Chinese hamster ovary cells exposed to very low fluences of alpha particles exhibited a much higher incidence of sister chromatid exchanges than expected based on the number of cells that were traversed by alpha particles. They concluded that genetic damage indeed occurred in cells that received no radiation exposure at all. These findings have since been confirmed by others (*64, 65*). Similarly, Mothersill and Seymour (*16*) have demonstrated that bystander effects also influence the survival of cells irradiated with gamma rays. Recent efforts have been made to understand the mechanisms which lead to damage in bystander cells. Gap junctional intercellular communication (GJIC) has been implicated as one of the mechanisms (*14, 16*). Azzam et al. (*14*) have suggested that other factors such as reactive oxygen species, extra-nuclear originating signal pathways, and secreted diffusible factors may also be

involved in the response of bystander cells. All of these findings indicate that the issue of bystander effects is highly relevant to the biological effects of nonuniform distributions of radioactivity. In fact, the data suggest that modeling the biological response of cells to nonuniform distributions of radioactivity solely on the basis of absorbed dose is not likely to adequately predict response even when the dose is calculated at the cellular level. Indeed, our recently published rapid communication in *Radiation Research* supports this premise by demonstrating pronounced bystander effects when ^{3}H is nonuniformly distributed in a multicellular cluster (*66*). This is of major importance to risk estimation in nuclear medicine (diagnostic) and radiation protection (radon, etc.), as well as clinical outcome in therapeutic nuclear medicine.

This project develops an experimental model wherein the variables affecting distribution of radioactivity can be tightly controlled. This will be accomplished by assembling multicellular clusters containing $4x10^{6}$ cells wherein 1%, 10%, 50%, or 100% of the cells are radiolabeled. Additionally, the subcellular distribution of the radionuclide will be controlled through the use of different radiochemicals. This tight control over the nonuniform distribution of radioactivity will allow a thorough investigation of the biological consequences of nonuniform activity distributions at the cellular and subcellular levels for three different classes of radionuclides of relevance to nuclear medicine and radiation protection (alpha, beta, and Auger electron emitters). In addition, the role of bystander effects in the biological response to nonuniform distributions of radioactivity will also be delineated. To enhance our capacity to predict the biological effects of incorporated radionuclides, we will adapt our theoretical multicellular dosimetry model to calculate the absorbed dose to the cluster on a cell-by-cell basis and use this and variables relating to the bystander effect to predict the biological outcome for each cell individually and the cluster as a whole. This combination of tightly controlled experimental and theoretical conditions provide a novel approach to investigate the biological effects of nonuniform distribution of radioactivity.

C. Preliminary Studies

C.1. Radionuclides and Radiochemicals used in Preliminary Studies

C.1a. *Synthesis of ^{131}IdU.* The radionuclide ^{131}I has a physical half-life of 8 d and emits beta particles with mean energy 191 keV and mean range in water of several hundred μm. The radiochemical (^{131}I)Iododeoxyuridine was prepared using procedures described in Narra et al. (*68*). A solution of chloramine T (400 μg in 200 μl of phosphate buffer, 0.1 M, pH 6.95) 2'-deoxyuridine (400 μg in 400 μl of buffer) was stirred at 105°C in an oil bath. One millicurie of $Na^{131}I$ (New England Nuclear), specific activity 770 Ci/mmol, was added and the heating continued for 25 min. Upon cooling to 25°C, $K_2S_2O_5$ (400 μg in 40 μl buffer) was added, the mixture passed through a 0.4 μm filter and purified by HPLC. The HPLC system consisted of an Ultremex C18 column (150×4.6 mm, 5 μm, Phenomenex, Torrence, CA) equilibrated with 8% methanol in water at a flow rate of 1 ml/min. Pure radiolabeled ^{131}IdU was eluted after 12 min. One to two additional passes through the column afforded >98% radiochemical purity, 55% radiochemical yield, and specific activity 300 Ci/mmol. This radiochemical is incorporated into the DNA of dividing cells.

C.1b. *Synthesis of ^{210}Po-citrate.* The radionuclide ^{210}Po emits a single 5.3 MeV alpha particle with a mean range in water of about 40 μm (*69*). The long physical half-life (138 d) of this radionuclide makes it undesirable for nuclear medicine, however, its commercial availability makes it a good radionuclide for laboratory studies on the effects of alpha particle emitters. The radiochemical ^{210}Po-citrate was prepared according to procedures used in Howell et al. (*1*). Stock ^{210}Po in 1 M HCl (Isotope Products, Burbank, CA) was mixed with 1 M sodium citrate (pH 7.0) in the ratio 1:9. This radiochemical sequesters principally in the cytoplasm.

C.1c. *^{3}HTdR.* The radiochemical tritiated thymidine (^{3}HTdR) is incorporated into the DNA of dividing cells. The radionuclide ^{3}H has a physical half-life of 12.3 y and emits beta particles with mean energy 5.67 keV and mean range in water of about 1 μm (*70*). This was obtained from New England Nuclear.

C.2. Experimental Multicellular Cluster Model for Nonuniform Distribution of Radioactivity

C.2a. *Cell Culture.* Chinese hamster V79 lung fibroblasts (kindly provided by AI Kassis, Harvard Medical School) were cultured in minimum essential medium supplemented with 2 mM L-glutamine, 10% fetal

calf serum, 50 U/ml penicillin, and 50 µg/ml streptomycin (MEMA). The pH of the medium was adjusted to 7.0 with $NaHCO_3$. Media and supplements were from Gibco (Grand Island, NY). Cells were maintained in Falcon 175 cm^2 tissue culture flasks at 37°C, 5% CO_2, 95% air, 100% humidity, and subcultured twice weekly.

C.2b. *Preparation of V79 cells used to make Multicellular Clusters.* V79 cells growing as monolayers in 175-cm^2 Falcon flasks were washed with 10 ml of phosphate-buffered saline, trypsinized and suspended at $4x10^6$ cells/ml in calcium-free MEM (MEMB). Aliquots of 1 ml were placed in sterile 12x75-mm Falcon polypropylene round-bottom culture tubes and placed on a rocker-roller for 3-4 h at 37°C in an atmosphere of 95% air and 5% CO_2. After this conditioning period, 1 ml of MEMB containing various activity concentrations of ^{131}IdU, ^{3}HTdR, or ^{210}Po-citrate was added to the culture tubes containing 1 ml of V79 cells. The tubes were then returned to the rocker-roller at 37°C, 95% air and 5% CO_2. Following a 30 min (for ^{210}Po-citrate) or 12 h (for ^{131}IdU and ^{3}HTdR) period of labeling with radioactivity, the tubes were removed and centrifuged at 2000 rpm at 4°C for 10 min. Aliquots of the supernatant were used to check the concentration of radioactivity added. The cells were washed three times with 10 ml of wash MEMA (MEMA prepared with calf serum), resuspended in 400 µl of MEMA. Additional tubes were identically prepared containing cells not labeled with radioactivity.

C.2c. *Assembly of Multicellular Clusters Containing Different Percentages of Radiolabeled Cells.* Multicellular clusters with 0%, 10%, 50%, or 100% labeled cells were assembled using the cells prepared in C.2b as per the following procedures. For 100% labeling, 400 µl of radiolabeled cell suspension ($2.5x10^6$ cells/ml) was transferred directly to a sterile 400 µl polypropylene microcentrifuge tube. The tubes were centrifuged at 1000 rpm for 5 min at 4°C to form a cluster of ~ 1.6 mm diameter. For multicellular clusters containing 10% radiolabeled cells, 360 µl of unlabeled cells were mixed with 40 µl of radiolabeled cells, vortexed, transferred to a 400 µl microcentrifuge tube and centrifuged. For multicellular clusters containing 50% radiolabeled cells, 200 µl of unlabeled cells were mixed with 200 µl of radiolabeled cells, vortexed, transferred to a 400 µl microcentrifuge tube and centrifuged. The resulting clusters contained $4x10^6$ cells.

C.2d. *Cell Survival Fraction in Multicellular Clusters Assembled with Radiolabeled Cells.* The capped microcentrifuge tubes containing the clusters were placed in a perforated microcentrifuge tube rack and moved to a refrigerator at 10.5°C. This temperature was selected because the cells can remain in this configuration at this temperature for long periods of time (up to 72 h) without decrease in plating efficiency (*71*). Therefore, the cells accumulate the preponderance of their radioactive decays while in the cluster configuration as opposed to the radiolabeling and colony forming periods. After 72 h at 10.5°C, the supernatant was carefully removed and the tube was vortexed to disperse the cell cluster. The cells were resuspended in MEMA, transferred to 17x100-mm Falcon polypropylene tubes, washed three times with 10 ml of wash MEMA, resuspended in 2 ml of wash MEMA, serially diluted, seeded in triplicate into 60x15 mm Falcon tissue culture dishes, and placed in an incubator at 37°C with 95% air and 5% CO_2. Aliquots were taken from each tube before serial dilution as above and the mean radioactivity per cell was determined (*72*). The culture dishes were removed from the incubator after 1 week and the resulting colonies were washed 3 times with normal saline, washed 2 times with methanol and finally stained with 0.05% crystal violet. The number of colonies were counted under fluorescent light. A colony count of 25-250 was considered as a valid data point for each dish. The surviving fraction compared to parallel controls was determined for each radioactivity concentration employed.

The preliminary results in the multicellular cluster model are given in Fig. 1 for ^{210}Po-citrate and ^{131}IdU. In each case, the surviving fraction of cells in the V79 multicellular cluster ($4x10^6$ cells) are plotted as a function of the total activity in the cluster. As shown in Fig. 1A, the alpha particles emitted by the radionuclide ^{210}Po impart lethal effects when either 100% or 10% of the cells in the multicellular cluster are labeled. However, 10% labeling is less toxic than 100% labeling for the same activity in the cluster. Least squares fits to the data using a single exponential response function yields mean lethal cluster activities of 0.81 nCi (0.030 kBq) and 1.9 nCi (0.070 kBq) for the 100% and 10% labeling cases, respectively. Since the mean absorbed dose to the cluster is directly proportional to the cluster activity, this confirms that mean absorbed dose to the cluster is not a good predictor of biological effect from nonuniform distributions of alpha particle emitters. Clearly, cellular distribution of radioactivity (e.g. % of cells labeled) is an important variable. Figure 1B shows the survival of cells when 10% of the cells are labeled with ^{131}IdU. A least squares fit of these data to a single component exponential function yields a mean lethal cluster activity of 100 nCi (0.37 kBq).

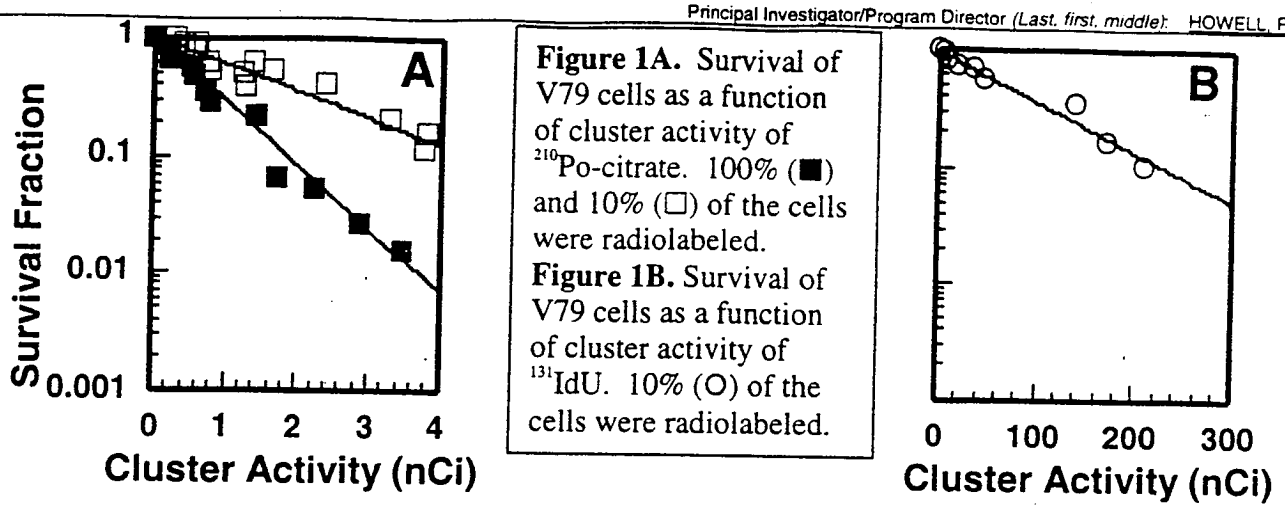


Figure 1A. Survival of V79 cells as a function of cluster activity of ^{210}Po-citrate. 100% (■) and 10% (□) of the cells were radiolabeled.
Figure 1B. Survival of V79 cells as a function of cluster activity of ^{131}IdU. 10% (O) of the cells were radiolabeled.

Studies for ^{3}HTdR are shown in Fig. 2 for 10%, 50% and 100% labeling of the cells. The 100% labeling data in Fig. 2 can be least squares fit to a single exponential response where a mean lethal cluster activity of 2.44 kBq is obtained (*66*). In contrast, the 50% and 10% labeling cases require fits to a two-component exponential function:

$$S = (1-a)\exp(-A/A_1) + a\exp(-A/A_2) \qquad (C.1)$$

These fits result S(50%) = 0.33 exp(-A/0.81) + 0.67 exp(-A/11.8) and S(10%) = 0.13 exp(-A/0.39) + 0.87 exp(-A/19.8), where the cluster activity A is in kBq (*66*). These results are indeed curious because beta particles emitted by ^{3}H have a spectrum of energies from 0-18.6 keV (*73*) with ranges in water from 0-7 μm. The mean energy is only 5.7 keV which has a range of 1 μm in water. The electrons must travel a minimum of 2 μm (range of 10 keV electron) just to get from the perimeter of the nucleus of a labeled cell to the perimeter of a nucleus of an unlabeled cell which presumably contains the primary radiosensitive targets. Since the electrons are emitted by decays occurring randomly throughout the nucleus, nearly all of them will have to travel substantially more than 2 μm to reach the cell nucleus of an unlabelled cell. Given that very few of the beta particles emitted are in excess of the minimum requirement of 10 keV, the cross-dose received by cells in the cluster is negligible. This is supported by the calculations of Goddu et al. (*26*) that show that the cross-dose for electrons in this energy range is negligible when the radioactivity is localized in the cell nucleus. Therefore, in the absence of bystander effects, we should expect to see essentially no killing of unlabeled cells. At high cluster activities, this should translate into a 50% and 10% survival fraction in the case of 50% and 10% labeling, respectively. The first components of the fits indicate that about 50% and 10% of the cells are killed at low cluster activities, however, the second component indicates that cells continue to be killed even though they are not significantly irradiated. This suggests that a bystander effect is responsible for killing of unlabeled cells.

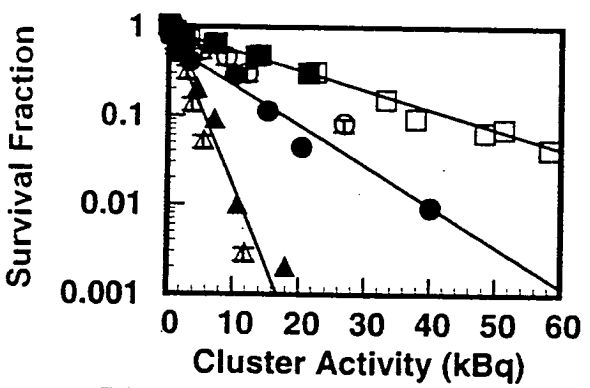


Figure 2. Survival of V79 cells as a function of cluster activity of ^{3}HTdR. Data are shown for experiments where 10% (■,□), 50% (●,O) or 100% (▲,△) cells were radiolabeled in multicellular clusters which were maintained at 10.5°C for 72 h and then the survival fraction was determined compared to unlabeled cells. A clearer view of the two-component nature of the 50% case can be seen in Fig. 3 of Attachment #1 (*66*).

C.2e. *Optimum Concentration and Impact of the Gap-Junction Inhibitor Lindane.* To assess the impact of GJIC on the biological response, it was necessary to determine the optimal concentration of lindane, a known inhibitor of GJIC (*14*). Multicellular clusters were prepared wherein 50% cells were labeled with a fixed activity concentration of ^{3}HTdR (148 MBq/ml). The clusters were maintained at 10.5°C for 72 h in the presence of 20-

200 μM of lindane. To achieve this, lindane was first dissolved in DMSO (5 mg/ml), filtered through a Millex®-HV filter, and diluted with MEMA to a final concentration 20-200 μM lindane, 0.58% DMSO. Parallel controls were maintained where clusters of unlabeled cells were maintained at the same concentrations of lindane with 0.58% DMSO. The solvent 0.58% DMSO had no impact on the response of the V79 cells (*66*). Thus, for each concentration of lindane, two tubes were prepared - one having a cluster of radiolabeled cells (50%) and one having a cluster of unlabeled cells. After 72 h period, the cluster was dismantled, the cells were washed 3X, and the mean activity per cell was determined, and the cell survival compared to its matched control determined as per the procedure outlined above. As shown in Fig. 3, the optimal concentration was 100 μM.

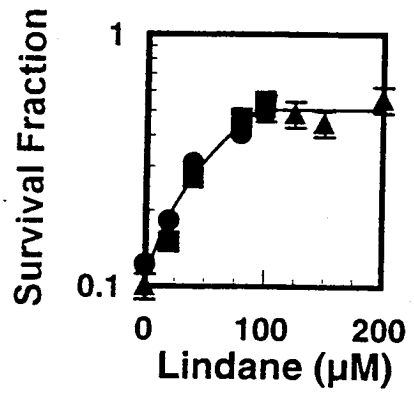


Figure 3. Effect of lindane concentration on survival of V79 cells from multicellular clusters wherein 50% of the cells are labeled with ^{3}HTdR. The survival fraction increased with lindane concentration up to 100 μM, after which no additional beneficial effect was observed. Data from three independent experiments are indicated by three symbols (●,▲,■). Representative standard deviations are indicated by the error bars.

C.2f. *Response of Multicellular Clusters to ^{3}HTdR in the Absence and Presence of Lindane and DMSO.* To elucidate the potential mechanisms responsible for the bystander effect observed in Fig. 2, the gap-junction inhibitor lindane and/or DMSO was added to the culture medium prior to forming the multicellular clusters wherein 50% of the cells were labeled with ^{3}HTdR. Figure 4 shows that 10% DMSO has some impact on the survival of bystander cells because the A_2 value in Eq. C.1 changes from 11.8 kBq to 13.1 kBq. A substantial effect is afforded by 100 μM lindane with an A_2 of 41.6 kBq. Finally, the combination of lindane and DMSO resulted in an A_2 of 59.0 kBq. These data suggest that since lindane is known to be a gap-junction inhibitor (*74, 75*), and it has been demonstrated in the present study that V79 cells form gap-junctions at 10.5°C (see below), it is likely that the bystander effects observed when 50% of the cells in the cluster are labeled with ^{3}HTdR are primarily due to gap junctional intercellular communication (*66, 76*). The change in slope of the second component obtained in the 10% DMSO case suggests that free radicals may also play a role in this process.

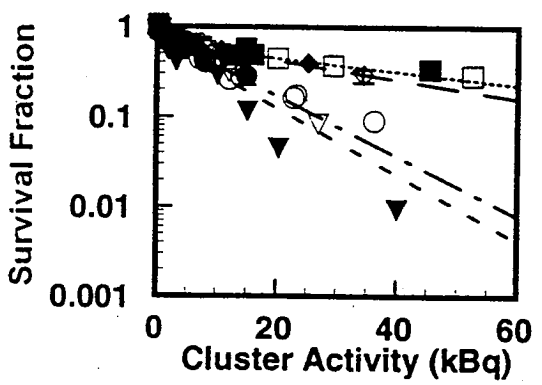


Figure 4. Survival of V79 cells as a function of cluster activity of ^{3}HTdR when 50% of the cells were labeled. Multicellular clusters were maintained at 10.5°C for 72 h in the presence of ^{3}HTdR (▼,▽) (*66*), ^{3}HTdR + 10% DMSO (●,○), ^{3}HTdR + 100 μM lindane (◆,◇) (*66*), and ^{3}HTdR + 10% DMSO + 100 μM lindane (■,□). Data from two independent experiments are plotted for each treatment condition, and differentiated as open and closed symbols, respectively.

C.2g. *Measurement of Functional Gap-Junctional Intercellular Communication in Monolayers and Multicellular Clusters.* The presence of functional GJIC between V79 cells maintained at 10.5°C was measured both in monolayers and multicellular clusters. For monolayers, the scrape-loading and dye transfer technique of El-Fouly *et al.* (*77*) was used with slight modification. Confluent monolayers were maintained at 10.5°C for 72 h and then the transfer of the fluorescent dye, Lucifer yellow (Molecular Probes, Eugene, OR), was examined after scraping the monolayer with a sterile scalpel. Figure 8 in Ref. (*66*) (Attachment #1) shows that V79 cells retain their ability to form membrane channels through gap-junctions even at 10.5°C as evidenced by positive

dye transfer into contiguous cells. While this serves as evidence of GJIC at 10.5°C, the scrape-loading and dye transfer technique in monolayers does not mimic the multicellular cluster system. Accordingly, the presence of functional GJIC between V79 cells in multicellular clusters maintained at 10.5°C was monitored by flow cytometry. The method of Goldberg et al. (*78*) was used with modifications. Cells ($2x10^6$) were loaded with dicarboxy-dichlorofluorescein diacetate (CDCF, Molecular Probes), a fluorescent dye (excitation 488 nm, emission 520 nm) that becomes membrane impermeant upon entering the cell, however, it can pass through functional gap junctions (*79*). The loading was achieved by incubating the cells for 15 min at 37°C in the presence of 2 ml dye solution (10 mM CDCF in DMSO diluted with PBS to 5 μM), washing 1X, resuspending in prewarmed MEMA and incubated for 30 min, centrifuged, and the supernatant decanted. Undyed cells ($2x10^6$) were treated similarly, resuspended in 5 ml MEMA, and transferred to the tube containing the dyed cells. These cells were used to form a multicellular cluster wherein 50% of the cells were dyed. The cluster was maintained at 10.5°C for 72 h. After this period, functional GJIC was interpreted as the ability of CDCF to pass from pre-dyed cells to undyed cells. This was determined by measuring the fluorescence of cells with a Beckton-Dickinson FACSCaliber flow cytometer using the modified technique of Tomasetto et al. (*80*). For this, the cells from clusters were washed with PBS and resuspended in 5 μM EDTA in PBS to about 10^7 cells/ml. The CDCF fluorescence was detected using a 530 nm bandpass filter. Fluorescent signals were processed over a four decade logarithmic range. Clusters containing 100% dyed and undyed cells served as positive and negative controls. Single parameter histograms based on 10,000 events were analyzed using CELLQuest software (BDIS, Mansfield, MA).

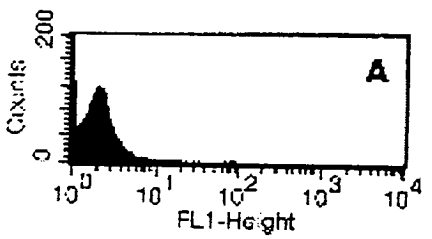


Figure 5A. Single parameter histogram from flow cytometry of V79 cells from a cluster containing 100% undyed cells.

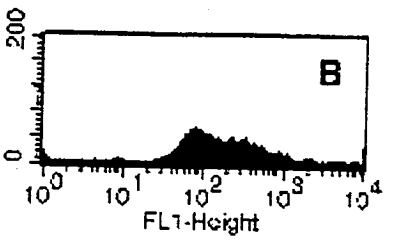


Figure 5B. Single parameter histogram from flow cytometry of V79 cells from a cluster containing 100% dyed cells.

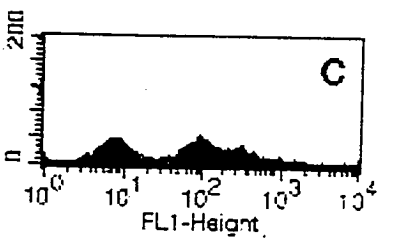


Figure 5C. Single parameter histogram resulting from flow cytometry of V79 cells from a cluster with 50% dyed cells.

As shown in Fig. 5A, the background fluorescence associated with undyed cells results in an peak centered at an FL-1 height of approximately $2x10^0$. When 100% of the cells are dyed, a peak with a maximum FL-1 height of 10^2 is observed (Fig. 5B). Finally, when 50% of the cells are loaded with the dye, some of the dye is transferred to undyed cells as evidenced by the two peaks in Fig 5C. Based on the shape and maximum (FL-1 height = 10^2) of the peak on the right, it corresponds to cells that were initially loaded with the dye. The peak on the left of Fig. 5C is centered at an FL-1 height of 10^1. Therefore, the peak corresponding to initially undyed cells has shifted to the right by one log indicating that dye has transferred from the dyed to the undyed cells. Hence, functional GJIC is present in the V79 multicellular clusters maintained at 10.5°C.

C.2h. *Cell Survival Fraction in Multicellular Clusters Irradiated with External Gamma Rays.* Microcentrifuge tubes containing multicellular clusters prepared with unlabeled cells were moved to a refrigerator at 10.5°C. The tubes were placed at different distances from a 10 mCi ^{137}Cs source housed in a small stainless steel capsule. The cumulated dose to the cells was measured using a Thomson-Nielsen miniature MOSFET dosimeter system. After 72 h of chronic irradiation at 2-20 cGy/h, the cells were processed to determine the cell survival fraction. The response of the multicellular cluster to acute ^{137}Cs gamma rays was also studied by maintaining multicellular clusters prepared with unlabeled cells at 10.5°C for 72 h and then irradiating the clusters acutely (~ 1-1.7 Gy/min) at 10.5°C in a J.L. Shepherd Mark I irradiator. The acutely

irradiated clusters were subsequently disassociated and processed for survival fraction. Figure 6 shows the dose response curves for V79 multicellular clusters exposed to chronic and acute ^{137}Cs gamma irradiation at 10.5°C. The shouldered dose response curves are characteristic of the response of mammalian cells to radiations with low linear energy transfer (LET). It is clear that the response of the multicellular clusters is dependent on the dose rate. The chronic dose rates are similar to the dose rates encountered with incorporated radionuclides, therefore, the α and β coefficients for the chronic irradiation can be taken as representative of the coefficients one would expect for the response to cross-dose from low-LET radiations emitted by the radionuclides.

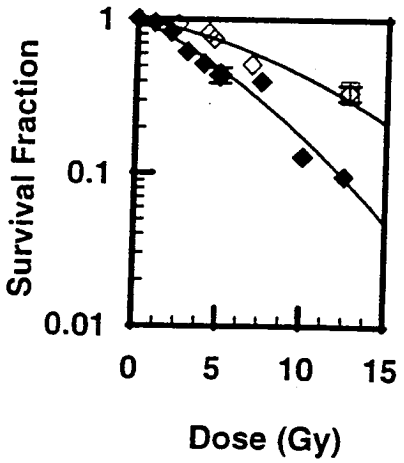


Figure 6. Survival of V79 cells following acute (◆) and chronic (◇) irradiation of multicellular clusters with ^{137}Cs gamma rays. Irradiations were carried out under the same conditions as those maintained in the radionuclide studies. A least squares fit of the data to the linear-quadratic model ($SF = Exp(-\alpha D-\beta D^2)$) yielded the following:

$SF(chronic) = Exp(-4.4x10^{-2} D - 3.9x10^{-3} D^2)$
$SF(acute) = Exp(-1.18x10^{-1} D - 5.6x10^{-3} D^2)$

where the α and β coefficients are in Gy^{-1} and Gy^{-2}, respectively

C.2i. *Mutagenesis and Survival Studies with External Gamma Rays: The Question of Hypoxia in the Clusters.* In this experiment, the protocol used in the above acute gamma ray experiment was followed except that immediately prior to irradiation, cells in half the tubes were resuspended to replace depleted oxygen while the cells in the remaining tubes were continued as pellets. Cells in all tubes were plated to evaluate colony-forming ability. Fig. 7A shows that the cells that remained in clusters were somewhat more resistant to killing by acute gamma irradiation relative to those that had been resuspended. Curve fits to the linear quadratic model resulted in α(susp) = 0.24 Gy^{-1}, β(susp) = 0.0022 Gy^{-2}, α(pellet) = 0.12 Gy^{-1}, and β(pellet) = 0.0070 Gy^{-2}. Fig. 7B shows that the same is true for induction of mutations at the HPGRT locus. Least squares fits to the number of mutants per cell plated *F* yield: *F*(susp) = $3.9x10^{-5}$ per Gy and *F*(pellet) = $2.5x10^{-5}$ per Gy. For this latter arm of the experiment, the Banbury Protocol was followed (*81*). The oxygen enhancement ratio (OER) for survival was about 1.4, and for mutation was approximately 1.6.

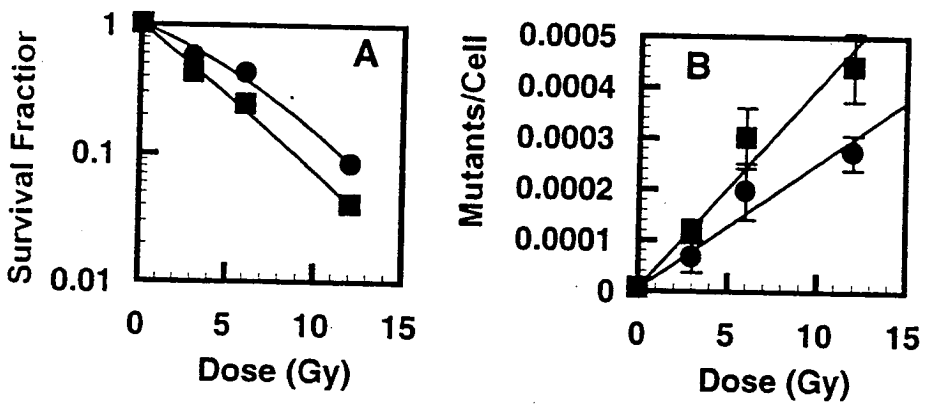


Figure 7. Response of V79 cells following acute irradiation with ^{137}Cs gamma rays at 10.5°C when clusters are maintained at 10.5°C for 72 h and then irradiated intact (■) or after dissociating (□). Two endpoints are examined: A) cell survival, and B) mutations at the HGPRT locus.

This experiment demonstrates that, after the 72 h incubation, hypoxia is present in the clusters. However, it appears to be uniform throughout the pellet since differentially hypoxic populations would result in a two- or more component exponential response to uniform irradiation. This is an important point because differential hypoxia would make data interpretation difficult. The OER is substantially less than 2.5 to 3.0, the maximum range expected for anoxia (Ref. (*82*), pg. 135) so the clusters are not completely hypoxic. In fact,

reference to the relative radiosensitivity versus oxygen tension suggests an oxygen tension of approximately 3 mm Hg or ½% (Ref. (*82*), p. 138, Figure 8-5).

This experiment further shows that the strain of V79 that we have used for our experiments is suitable for mutagenesis studies at the HGPRT locus and that mutagenesis is reduced but not eliminated in the clusters compared to suspensions. Our finding of reduced mutations in the clusters attributed to hypoxia is consistent with the findings of Suzuki and Okada (*83*), Grdina et al. (*84*) and Denault and Liber (*85*). However, because the limited hypoxia appears to be uniform throughout the cluster, we do not anticipate that this will make interpretation of the data difficult. It also should be noted that bystander effects are observed, gap junctions do form, and translocation of effector molecules can take place under these conditions of reduced O_2-tension.

C.2j. *Separation of Labeled and Unlabeled Cells after Disassembly of Cluster.* In the addendum to our original submission, we had proposed to follow the radioactivity in the cells by dyeing unlabeled cells with carboxyfluorescein diacetate succinimidyl ester (Vybrant™ CFDA SE, Molecular Probes) prior to assembling the multicellular cluster with a mixture of radiolabeled and unlabeled cells. This dye is designed specifically as a tightly bound cell tracer. After maintaining the clusters for three days to accumulate decays, the labeled and unlabeled cells were to be separated using fluorescent cell sorting techniques (FACS). We have acquired preliminary data to show that this dye will indeed serve this purpose admirably. In addition, we have found that the dye can also be used to separate the labeled and unlabeled cells for independent determination of cell survival and other biological endpoints including mutagenesis and the comet assay (see Section D.3f).

V79 cells growing as monolayers were removed from flasks and suspended at $2x10^6$ cells/ml in MEMB. Aliquots of 1 ml were placed in sterile 17x100-mm culture tubes and placed on a rocker-roller in standard conditions. After this conditioning period, 1 ml of MEMB containing ^{3}HTdR (0 or 148 kBq) was added to the culture tubes containing 1 ml of V79 cells. All tubes were then returned to the rocker-roller. Following a 12 h period of labeling with radioactivity, the tubes were removed and centrifuged at 2000 rpm at 4°C for 10 min. Aliquots of the supernatant were used to check the concentrations of radioactivity. The remaining supernatant was decanted, the cells were resuspended in 10 ml phosphate-buffered saline (PBS), and transferred into a 15 ml centrifuge tube. The cells were washed three times with 10 ml of PBS and aliquots of washed cells were taken out to perform cell count and to determine the mean radioactivity per cell. The radiolabeled cells were then labeled with the fluorescent dye using a Vybrant™ CFDA SE cell tracer kit (a dye concentration of 0.05 µM CFDA SE was found to be optimal for V79 cells). The radiolabeled and subsequently dyed cells ($2x10^6$) were mixed with equal number of cells that were neither labeled with radioactivity nor dyed but were treated exactly the same manner as the labeled cells. Finally, the pooled cells were formed into a cluster as per Section C.2c. The resulting clusters contained a total of at $4x10^6$ cells of which 50% cells were radiolabeled/dyed. The tubes containing the clusters were transferred to a refrigerator at 10.5°C. After 72 h at 10.5°C, the supernatant was carefully removed and the tube was vortexed to disperse the cell cluster. The cells were resuspended in PBS, transferred to 15 ml centrifuge tubes, washed three times with 10 ml PBS, resuspended in PBS with 5 µm EDTA to a concentration of 10^7 cells/ml, and passed through a 21-G needle five times to produce single cell suspension. The cells were sorted with the FACSCalibur flow cytometer. An air-cooled 488-nm Argon-ion laser was used to excite the dye. The excitation and emission peaks of the fluorescent dye were 492 and 517, respectively. Fluorescence in the FL-1 channel was collected along with forward-angle and 90° light scatter. The cells were sorted for dye-negative cells. After sorting, the dye-negative cells (unlabeled and undyed) were collected, pooled, washed with 10 ml PBS-EDTA and resuspended in 2 ml PBS-EDTA. Aliquots were taken and the cell concentration was determined. An additional 0.5 ml of the suspension was subjected to FACS for the second time to check the purity of sorted cells (i.e., absence of dye-positive cells). Aliquots were taken to determine the mean radioactivity per cell.

Our above preliminary experiments showed that the excitation and emission characteristics of Vybrant™ CFDA SE (492 and 517 respectively) are well suited for cell sorting by FACS. Figure 8 shows the distribution of dye-positive and dye-negative cells. The FACS analysis of cells from a 50% dye-labeled cell population after maintaining the cluster with 50% dyed cells at 10.5°C for 72 h, showed no appreciable dye transfer from dyed to undyed cells.

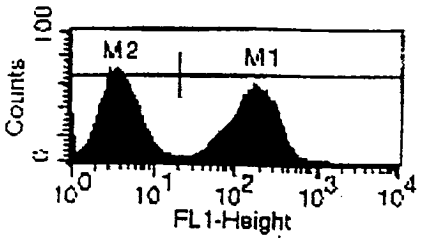


Figure 8. Distribution of dye-positive (M1 gate) and dye-negative (M2 gate) cells after analysis (FL-1 channel) of V79 cells with a FACSCalibur system. These cells were obtained by dispersing multicellular clusters containing 50% radiolabeled / dyed cells maintained at 10.5°C for 72 h. Analysis indicated that 49.58% of the cells were dye-positive.

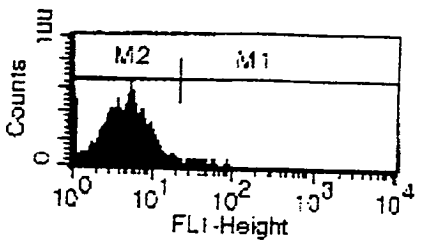


Figure 9. Distribution of dye-positive (M1 gate) and dye-negative (M2 gate) cells after purification of dye-negative V79 cells with a FACSCalibur system. Analysis indicated that 96.70% of the cells were dye-negative indicating that a high degree of purity was obtained.

Figure 9 shows that when multicellular clusters consisting of 50% radiolabeled/dyed cells were maintained at 10.5°C for 72 h, dispersed, and then sorted using the FACSCalibur system, undyed cells (M2 gate) were separated with a high degree of purity (~97%). Aliquots of the M2 gated (undyed) cells in Fig. 8 were counted for radioactivity and it was determined that these cells contained an average of 0.013 mBq/cell. The M1 gated (dyed) cells in Fig. 8 contained 1.5 mBq/cell. The very small amount of radioactivity in the M2 gated cells can be attributed to the lack of 100% purity in the sorted population as indicated in the caption of Fig. 9. These data provide strong evidence that there is no migration of tritiated thymidine from radiolabeled cells to surrounding unlabeled cells under the experimental conditions employed. Furthermore, this procedure can be used in Specific Aim 3 to monitor the kinetics of radioactivity in the labeled and unlabeled cells for all of the radiochemicals proposed. Perhaps even more important, this dye can be used to separate the labeled and unlabeled cells for independent determination of cell survival and other biological endpoints.

C.3. Significance of the Preliminary Studies

Radiopharmaceuticals are widely used in clinical medicine to diagnose and treat a variety of medical conditions. It is well known that when radiopharmaceuticals are administered to the patient, the radioactivity localizes in different tissues in the body and its distribution at the macroscopic and microscopic levels is nonuniform. The degree of nonuniformity can vary widely depending on a variety of factors. The biological consequence of nonuniform distributions of radioactivity in a given tissue can also vary substantially. Despite these well-known facts, current internationally accepted methods for assessing risks from diagnostic nuclear medicine procedures assume that the radioactivity in organs and tissues is uniformly distributed (*86*). The same assumption is frequently made in assessing risks from environmental and accidental exposures to radioactivity. Adelstein et al. (*49*) and Makrigiorgos et al. (*42*) have raised important concerns regarding these assumptions and their impact on risk estimates. Perhaps even more significant is the impact of nonuniform distribution of radioactivity on therapeutic nuclear medicine. Numerous investigators have made important contributions concerning the impact of nonuniform distributions of radioactivity on the resulting absorbed dose profile in the target tissues and the ultimate therapeutic outcome (*46, 47, 87-92*). However, one of the major stumbling blocks in developing models to predict the biological response of tissues with nonuniform distributions of radioactivity has been the absence of models that allow tight control over the distribution of the radioactivity.

The preliminary studies described above confirm that nonuniform distribution of radioactivity in tissues can have a dramatic impact on the biological effect of the radionuclide. It is clear that for the same cluster activity, dividing the radioactivity among 100% of the cells is more lethal than dividing it among 50% or 10% of the cells in the cluster. This emphasizes the fact that the conventionally calculated mean absorbed dose to the cluster, is a poor predictor of biological outcome when the radioactivity is distributed nonuniformly. These

preliminary studies are highly significant in that they demonstrate that multicellular clusters can be *assembled* by mixing radiolabeled and non-radiolabeled cells to achieve a *controlled* degree of nonuniformity of radioactivity in an in vitro multicellular cluster model. This new model provides a solution to this problem by allowing total control over the percentage of radiolabeled cells in the cluster. The use of different radiochemicals provides control over the subcellular distribution of the radiolabeled cells. These degrees of control over the model are a major departure from past in vitro spheroid models. The preliminary studies also show our capacity to synthesize, purify, and work with several different radiochemicals that are an essential part of the project. In addition, these studies establish the response of V79 multicellular clusters to incorporated radioactivity. These data show a strong indication that bystander effects may play a significant role in determining the biological effect of incorporated radioactivity. Our studies with lindane provide strong evidence that gap junctional intercellular communication plays an important role in the bystander response. Evidence of functional gap junctional intercellular communication in the clusters is provided by flow cytometry. Preliminary studies with 10% DMSO indicate that radical species may play some role in the bystander effect. Data are now provided for a new assay that has been added to the Research Design, namely mutations at the HGPRT locus. Perhaps the most significant aspect of the preliminary data is that the labeled cells can be separated from the unlabeled cells using fluorescent activated cell sorting technology. This enables us isolate the bystander cells and specifically assay the biological effects in this population. This is a major advantage of our cluster model over other models that have been used to study bystander effects.

D. Research Design & Methods

D.1 Radionuclides and Radiochemicals

D.1a. *Radionuclides.* The biological response of multicellular clusters will be ascertained for alpha (^{210}Po), beta (^{3}H, ^{131}I), and Auger electron (^{125}I) emitting radiochemicals as these encompass three major categories of radionuclide emissions. The radionuclides ^{125}I and ^{131}I are emitters of Auger electrons and beta particles, respectively. Their corresponding physical half-lives are 60 d and 8 d, respectively. The beta emitter ^{131}I emits particles with mean range of several hundred μm whereas ^{125}I emits numerous Auger electrons with subcellular ranges. ^{131}I is presently being used clinically in radionuclide therapy, and ^{125}I is being investigated to capitalize on its high-LET type radiotoxicity. The radionuclide ^{210}Po emits a single 5.3 MeV alpha particle in its decay to stable ^{206}Pb. The long physical half-life (138 d) of this radionuclide makes it undesirable for radionuclide therapy, however, its long half-life and commercial availability make it a good radionuclide for laboratory studies on the effects of alpha particle emitters. The radionuclide ^{3}H has a physical half-life of 12.3 y and emits beta particles with mean energy 5.67 keV and mean range in water of about 1 μm (*70*). The ^{210}Po and ^{3}H activity will be assayed with a Beckman LS3800 automatic liquid scintillation counter, whereas the photopeaks of ^{131}I and ^{125}I will be assayed using the requested automatic gamma counter.

D.1b. *Radiochemicals.* The radiochemicals ($^{125/131}$I)Iodorhodamine ($^{125/131}$I-Rh 123) localize entirely in the cytoplasm of cells (*93*). It is well-known that the radiotoxicity of cytoplasmically localized ^{125}I is akin to low-LET radiations such as the beta particles emitted by ^{131}I (*68*). The radiochemicals ($^{125/131}$I)Iododeoxyuridine ($^{125/131}$IdU) incorporate into the DNA in the cell nucleus. When labeled with ^{125}I, this radiochemical is known to impart high-LET type biological effects (*1*). Therefore, these two radiochemicals will delineate the response of multicellular clusters as a function of the subcellular distribution of ^{125}I and ^{131}I. The radiochemical dipyrrolidinedithiocarbamato-^{210}Po(II) will localize in the cell nucleus (*9*). ^{210}Po-citrate is known to distribute primarily in the cytoplasm (*1*). For ^{3}H, tritiated thymidine (^{3}HTdR) and ^{3}H-methionine will be used to localize the radionuclide in the nucleus and cytoplasm (*94*), respectively.

D.1c. *Synthesis of ($^{125/131}$I)Iodorhodamine.* The procedures of Harapanhalli et al. (*93*) will be used to prepare $^{125/131}$I-Rh 123. To 20 μl of rhodamine 123 (methyl *o*-(6-amino-3-imino-3*H*-xanthen-9-yl)benzoate monochloride solution (1 mg/ml in 0.04 M acetate buffer, pH 5.0) placed in a screw-cap vial, 50 μl of aqueous peracetic acid (0.032%) will be added followed by 0.5-1 mCi of Na$^{125/131}$I solution in aqueous sodium hydroxide. After vortexing and incubation at ambient temperature for 1 h, C_{18} TLC will be performed to check the extent of

the reaction. Subsequently, the reaction mixture will be extracted with dichloromethane (3 x 1 ml), and the organic phase will be washed with 10% sodium bisulfite, water and brine. The solution will be evaporated and the solid residue will be dissolved in 50 µl of ethanol and the resultant solution will be diluted to 1 ml with water and then it will be passed through a C_{18} Sep-Pak cartridge preconditioned with water, methanol and water. Successive elutions from water to methanol will be performed to get the product in 50% aqueous methanol in 40-45% yield and over 98% chemical as well as radiochemical purity. Alternatively, the reaction mixture following a 1-h incubation period will be directly injected into the HPLC column and the product will be eluted at 18 min. A reversed-phase separation will be accomplished using a mobile phase of 0.02 M phosphate buffer, pH 7 (mobile phase A), and acetonitrile (mobile phase B). A linear gradient of 10-80% B in 15 min on a Spheri-5 RP-8 column (100 X 4.6 mm, 5 µm, Applied Biosystems, Foster City, CA) with a precolumn (30 x 4.6 mm) at a flow rate of 2 ml/min will provide separation. A pair of Waters 510 pumps, 486 Absorbance Detector, and 660 Solvent Programmer will be used. If there is any carryover of unlabeled rhodamine 123, the eluate will be evaporated, redissolved in dichloromethane and washed with water to remove excess of rhodamine 123. Final purity will be checked by silica gel and C_{18} TLC by cospotting with authentic I-rhodamine 123.

D.1d. *Synthesis of ($^{125/131}$I)Iododeoxyuridine, ^{210}Po-citrate.* These radiochemicals will be synthesized using our well established procedures described in the Preliminary Studies section.

D.1e. *Synthesis of dipyrrolidinedithiocarbamato-^{210}Po(II).* This radiochemical will be synthesized using the same procedures described by Azure et al. for dipyrrolidinedithiocarbamato-^{212}Pb(II) (*9*). ^{210}Po in 1 M HCl will be allowed to dry in a fume hood at room temperature, resuspended in a minimum of absolute ethanol (2.5 ml), and transferred to a pear shaped 5 ml flask containing a magnetic stir bar. The ligand solution will be prepared by dissolving the ammonium salt of pyrrolidine dithiocarbamate (MW 164) (Aldrich Chemical Co., Milwaukee, WI) in absolute ethanol. A stoichiometric amount of the ligand will be added to the reaction vessel and the solution maintained at 50°C for 1 h. After cooling to room temperature, the contents of the flask will be transferred to a 12 x 75 mm borosilicate glass tube, allowed to evaporate at room temperature, and resuspended in sterile deionized water. Finally, the $^{210}Po(PDC)_2$ solution will be sterilized by filtration through a Gelman 0.22 µm filter and the activity assayed. The radiochemical purity will be ascertained by thin layer chromatography on aluminum backed silica gel with fluorescent indicator (0.2mm DC-Karten SI F 254 nm obtained from Riedel-de Haën AG, D-3016 Seelze 1) with acetone:toluene:H_2O as solvent system (70:24:6).

D.1f. *Tritium Radiochemicals.* The tritiated radiochemicals, ^{3}HTdR and ^{3}H-methionine will be obtained from New England Nuclear (Billerica, MA).

D.2 Cell Lines

D.2a. *Chinese hamster V79 lung fibroblasts.* V79 cells (kindly provided by AI Kassis, Harvard Medical School) will be used in our studies and survival, mutations at the HGPRT locus, and the comet assay will serve as biological endpoints. As in the preliminary studies, the cells will be cultured in minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 10% fetal calf serum, 50 units/ml penicillin, and 50 µg/ml streptomycin. The pH of the culture medium will be adjusted to 7.0 with $NaHCO_3$. Cells will be maintained in Falcon 175 cm^2 sterile tissue culture flasks under standard conditions (37°C, 5% CO_2, 95% air, 100% humidity) and subcultured twice weekly. Media and supplements will be obtained from Gibco (Grand Island, NY).

D.2b. *Rat Liver Epithelial Cells.* To explore the impact of gap junctional intercellular communication on the biological response of mammalian cells to nonuniform distributions of radioactivity, three new cell lines of rat liver origin (*95*) have been added to our protocols that are GJIC competent ($GJIC^+$) or incompetent ($GJIC^-$). These include i) WBs, wild-type $HGPRT^+$, $GJIC^+$, Fischer 344 rat liver epithelial cell line (WB F-344); ii) WB^r, a WBs mutant that is $HGPRT^-$, $GJIC^+$; and iii) aB1, a WB^r mutant that is $HGPRT^-$, $GJIC^-$. These cell lines have been provided by our consultant JE Trosko (University of Michigan). These cell lines are cultured in a modified Eagle's medium (*96*) with Earle's balanced salt solution supplemented with 50% more vitamins and essential amino acids except glutamine, 100% more nonessential amino acids, 1 mM sodium pyruvate, and 5% fetal bovine serum. Cells are maintained under standard conditions. These cell lines are good models to study the impact of GJIC on bystander effects because the $GJIC^+$ cells have large gap junctions and are highly coupled, and the $GJIC^-$ cells allow one to study the impact of eliminating GJIC without resorting to chemical

agents such as lindane. Survival studies will be carried out with all three cell lines, while mutation studies will be selectively carried out with the wild-type WBs which are HGPRT$^+$.

D.3 Experimental Methods and Design

D.3a. *Survival of Cells in Multicellular Clusters.* As indicated in Specific Aims 1 and 2, the cell survival fraction of multicellular clusters will be ascertained for each radiochemical under conditions where either 1%, 10%, 50% or 100% of the cells are labeled with the radiochemical. The procedures for labeling the cells and assembling the multicellular clusters is described in detail in Section C.2c. Briefly, cells in suspension will be labeled in several different tubes containing different concentrations of the radiochemical to achieve various activities per cell (e.g. mBq/cell). After washing the cells free of extracellular activity, the activity per cell will be determined and the remaining labeled cells will be mixed with unlabeled cells to obtain the desired % labeling and $4x10^6$ cells in 400 µl of culture medium. The cell suspension will be transferred to a sterile 400 µl microcentrifuge tube, capped, and centrifuged gently at 1000 rpm for 5 min to form a close-packed multicellular cluster. The tubes will then be transferred to a 10.5°C environment for three days (72 h) to accumulate radioactive decays. This temperature was selected based on our earlier studies that showed the cells maintain their plating efficiency and do not divide (*71*). This is one additional element of control over the radiobiology of the cluster in that the distribution of activity in that the cluster remains fixed because the cells do not divide. After three days, the cells will be gently removed from the tubes, vortexed, resuspended in 2 ml of culture medium, and gently passed through a 21 g needle several times to break up cell clumps. Aliquots of the cell suspension will be taken to determine the cluster activity and the average activity per cell (e.g. mBq/cell). The cluster activity will simply be the total activity in the tube of suspended cells. The activity per cell will be determined using well established procedures (*1, 72*). Finally, the cells will be washed three times with wash medium, serially diluted, seeded into culture dishes, and placed in an incubator at 37°C, 5% CO_2, 95% air. After one week, the colonies will be washed with 0.9% saline, fixed with methanol, stained with crystal violet, and scored (> 50 cells constitutes a colony). After ensuring the absence of chemical toxicity which is not expected for these high specific activity radiochemicals, the survival fraction compared to untreated controls will initially be plotted as a function of the total activity in the cluster and the cellular uptake in the labeled cells (see Section D.4). This will be repeated for each radiochemicals in Specific Aims 1 and 2. These studies will be carried out for the V79 cells and for the WB^r and aB1 cells (see Section D.3e.2 for rationale) as indicated in Table 1 in the Timeline. These studies will provide information on the lethality of nonuniform distributions of radioactivity which is an important topic in therapeutic nuclear medicine both in terms of eliminating tumor cells as well as dose limiting organ toxicity.

D.3b. *Mutation of Cells in Multicellular Clusters.* Mutagenesis will be followed according to the Banbury Protocol (*81*). After the 72 h incubation at 10.5°C, 10^6 cells will be plated from each experimental condition examined in Specific Aims 1 and 2 and allowed to undergo 10 cell divisions in culture medium to allow for mutant expression. The resulting cells will be challenged with 6-thioguanine (Sigma Chemical Co.) to evaluate mutations at the HGPRT locus. This will be achieved by plating $2x10^5$ cells into five 100 mm culture dishes in culture medium containing reduced fetal calf serum (5%) and 10 µM 6-thioguanine. The resulting mutant colonies will be stained and scored as per methods described above. Plating efficiency will be determined for each data point by plating 200 cells into 60 mm culture dishes containing the same culture medium without 6-thioguanine. Controls will consist of clusters assembled with unlabeled cells or 100% labeled cells which have also been incubated at 10.5°C. The resulting data will be used to calculate the number of mutants per cell plated according to the Banbury Protocol. These studies will be carried out with the V79 cells and the wild type WBs cells. No mutagenesis studies will be carried out with the WB^r and aB1 populations since they are HGPRT$^-$. If the bystander effect involves nuclear interactions, then there should be more mutants in the mixed population than one would predict based on the mutation frequencies observed in the 0% and 100% cases. If, on the other hand, the bystander effect does not involve nuclear interactions, the mutation frequency should be close to the predicted value and one would have to conclude that the lethal bystander effect is the result of cell membrane or cytoplasmic interactions. Perhaps more importantly, these data will provide information on the risk of exposure to nonuniform distributions of radioactivity. This is of considerable importance to radiation protection.

D.3c. *Subcellular Distribution and Kinetics of Radioactivity in the Cells.* To ascertain the absorbed dose received by the cells, it is essential to follow the kinetics of uptake and clearance of the radioactivity from the cells. This will be accomplished by drawing on our previous experience with these procedures (*71*). It is expected that the kinetics in the V79 cells will be essentially the same as those encountered in our studies with V79 cell suspensions which are shown in Fig. 10 (*71*). The area under the curve is proportional to the cumulated decays in the cells. The time period of 0-12 represents the uptake of the radiochemical at 37°C after which the cells were washed free of extracellular activity. The shaded region represents the 72 h period where the cells were maintained at 10.5°C in culture medium. Finally, the curved region corresponds to the colony forming period at 37°C where the cellular activity has an effective half-time of ~ 12 h in V79 cells (*1*).

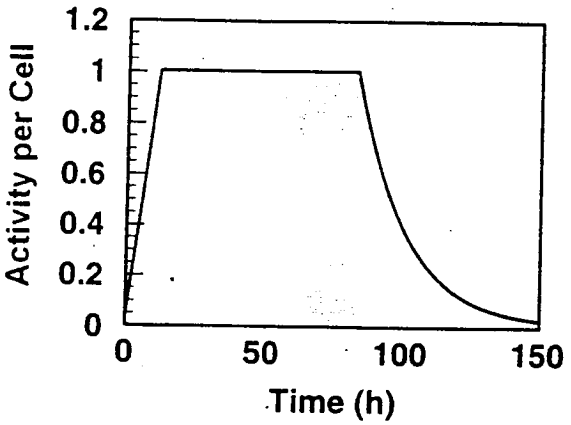


Figure 10. Temporal dependence of intracellular activity of $^{125/131}$IdU in V79 cells (*71*). The units of activity are arbitrary. The area under the curve is proportional to the cumulated decays in the V79 cell nucleus. The time period of 0-12 h represents the uptake of the radiochemical at 37°C after which the cells were washed free of extracellular activity. The shaded region represents the 72 h period where the cells were maintained at 10.5°C in culture medium. Finally, the curved region corresponds to the 1 week colony forming period at 37°C where the cellular activity has an effective half-time of ~ 12 h (*1*). As denoted by the shaded region, 76% of the intracellular decays occur when the cells were maintained at 10.5°C.

We will follow the same procedures to determine the kinetics of activity in the cells during the three phases of the experiment: 1) radiolabeling of the cells (uptake), 2) three days at 10.5°C, and 3) colony formation period. The cellular uptake of radioactivity will be assayed at four time points during the uptake phase, after washing the cells free of extracellular radioactivity, after the three days at 10.5°C, and at four points during the colony forming period. As in our previous studies, measurements will be made using the method of Kassis and Adelstein (*72*). The procedures to be followed for each phase are described below.

1) Radiolabeling Phase. One ml aliquots of cells (suspended in MEMB at 4.0×10^6 per ml) will be transferred into several 12 ml round bottom culture tubes, capped loosely, and placed on a rocker-roller at 37°C, 5% CO_2, 95% air. After 4 h, one additional ml of MEMB containing the radiochemical will be added and the tubes returned to the roller. At 3, 6, 9 , and 12 h after addition of the radiochemical, 0.6 ml of the cell suspension is removed and transferred to a 1.5 ml microcentrifuge tube. One hundred µl of the cell suspension will be layered over 300 µl of calf serum in a 400 µl microcentrifuge tube in duplicate. An additional 1 ml of cells is used to confirm the cell concentration using a Coulter Counter. The remaining 400 µl of cell suspension is centrifuged in a microcentrifuge for 1 min. One hundred µl of the supernatant will be layered over the 300 µl of calf serum in duplicate. The tubes will be centrifuged for 1. min, frozen in liquid nitrogen, the tips cut off and activity determined, and activity/cell will be calculated.

2. Three Days at 10.5°C. The cells in the remaining 12 ml round bottom tubes will be washed three times with wash MEMA, resuspended in 400 µl, pooled, and passed gently through a 21 g needle. The activity per cell will be determined as described above. The remaining cells will be used to form multicellular clusters as described above and the tubes containing the clusters will be transferred to a refrigerator at 10.5°C. After three days, each cluster will be gently transferred to a 12 ml tube, the cluster broken up by gentle tapping and vortexing, resuspended in 2 ml MEMA, passed through a 21 g needle several times to achieve a suspension, and the activity per cell determined again.

3. Colony Forming Period. Cells that have completed Steps 1 and 2 above will be transferred to four 75 cm^2 culture flask containing 10 ml MEMA and placed in an incubator under standard conditions. On each of

the four following days, a flask will be removed from the incubator, washed with 10 ml PBS, the cells removed with trypsin, resuspended in 5 ml MEMA, and the activity per cell determined.

Using these data, the cumulated activity in the labeled cells will be determined and used in the absorbed dose calculations as described in Section D.4. These data will be acquired for each cell line.

It should be noted that Steps 1-3 described above only provide the average activity per cell. Therefore, in multicellular clusters where less than 100% of the cells are labeled, the activity per cell labeled cell can only be inferred from knowledge of the activity per labeled cell prior to assembling the cluster and the percentage of cells labeled. This approach cannot ensure that radioactivity does not migrate from the labeled cells to the unlabeled cells. Such migration will have an impact on the absorbed dose calculations. In view of our preliminary data in Section C.2j, we do not anticipate dosimetrically significant migration. However, additional experiments will be performed for each radiochemical to track the activity per cell in the labeled and unlabeled cells. This will be accomplished according to the procedures detailed in the preliminary studies in Section C.2j.

Determination of the subcellular distribution of the radiochemicals is an essential element required for cellular dosimetry. Therefore, the subcellular distribution of each radiochemical will be determined in each cell line according to standard procedures (*1, 97*). These data and the kinetics data described above are necessary to accomplish Specific Aim 3.

D.3d. *Assessment of Survival and Mutations after Separation of Unlabeled Cells from Labeled Cells using FACS.* Our preliminary experiments showed that FACS using Vybrant™ CFDA SE fluorescent dye is well suited for separating radiolabeled cells from unlabeled cells after disassembly of the cluster. This is because, in the cluster environment, there is no appreciable transfer of this particular dye from dyed to undyed cells. Thus, selected experiments from Specific Aims 1, 2, and 4 will be repeated by first dyeing the radiolabeled cells with CFDA SE prior to assembling the cluster. Once assembled, the clusters will be maintained at 10.5°C for 72 h, dissociated, and the labeled and unlabeled cells separated by FACS. The survival and mutation frequency will then be determined separately for the labeled and unlabeled cells. Ideally, all experiments would be carried out with this procedure, however, given the time consuming nature of the cell sorting process, only selected experiments can be performed. Thus, sorted experiments will initially be carried out for ^{3}HTdR and ^{125}IdU. These two radiochemicals have been selected because the bystander cells are not irradiated in the case of ^{3}HTdR and only minimally irradiated in the case of ^{125}IdU. Therefore, the biological effects observed in the unlabeled cells will principally be due to bystander effects and not due to cross-irradiation received by the unlabeled cells. Additional radionuclides will be studied as sorting time allows.

D.3e. *Bystander Effects and Potential Mechanisms.* Our preliminary studies with ^{3}HTdR, described in Section C and Attachment #1 (*66*), indicate that bystander effects play an important role in the biological effects of nonuniform distribution of incorporated radionuclides. Accordingly, this will be investigated in detail as per Specific Aim 4. Initially, experimental results in Specific Aims 1 and 2 will be analyzed with the help of the theoretical dosimetry model described below in Section D.4 to ascertain the extent of the role of bystander effects in reducing the surviving fraction of cells in the multicellular cluster. It is anticipated that this phenomenon will be observed to the greatest extent in those cases where the cross-dose to unlabeled cells is very small compared to the self-dose to the labeled cells. Section C.2 and Attachment #1 (*66*) indicate that this is indeed the case for ^{3}HTdR when 10% or 50% of the cells are labeled. No bystander effects were observed for 100% labeling with ^{3}HTdR. Based on our earlier theoretical calculations, this will also be the case for ^{125}IdU when 50% or less of the cells are labeled and the remaining radionuclides when less than 10% of the cells are labeled (*26*). The theoretical dosimetry calculations in Specific Aim 3 will provide more detailed information than our earlier model (*26, 98*) on the self- and cross-dose received by the labeled cells and the cross-dose received by the unlabeled cells. This information will be helpful in terms of delineating radiation effects as opposed to bystander effects (i.e. cell kill in the absence of significant radiation dose). In those cases where bystander effects appear to play a role in the biological response of the cells, additional experiments will be carried out in an attempt to elucidate the mechanism of the effect. These approaches are described below.

D.3e.1. Chemical Inhibition of Gap Junctional Intercellular Communication. Selected experiments with multicellular clusters will be repeated in the presence of an inhibitor of gap junctions that mediate intercellular communication. V79 cells have been shown to have substantial GJIC (*77*), and more in the WBs cell line (*95*).

As in our preliminary studies (*66*), and those of Azzam et al. (*14*), lindane (γ-isomer of hexachlorocyclohexane) will be used as an inhibitor of this communication (*74, 99*). We have shown that 100 μm lindane is nontoxic and provides maximum effect for survival studies with V79 cells (*66*). Similar studies, as detailed in Section C.2e, will be repeated to find the optimal concentrations for the mutation assays for both the V79 cells and the wild type WBs cells. The WBs cell line of the WB series was chosen for mutation studies because of its outstanding GJIC and it is HGPRT$^-$. Once the appropriate non-toxic lindane concentration is established for each endpoint and cell line indicated above, selected experiments in Section D.3a and D.3b will be repeated in the presence of lindane. Specifically, we will begin by examining ^{3}HTdR (1%, 10%, 50%), ^{125}IdU (1%, 10%, 50% of cells labeled), ^{210}Po-PDC (1%, 10%), and ^{131}IdU (1%, 10%). Experiments for 100% labeling are not included because we have shown that bystander effects are not observable under these conditions (see Attachment #1), thereby suggesting that GJIC does not play a significant role in the survival of cells in multicellular clusters when the cells are more uniformly irradiated. Following the lead of Specific Aim 2, these experiments will also be repeated with the cytoplasmically localized radiochemicals to explore whether cell killing due to GJIC depends on the subcellular distribution of the radiochemical.

D.3e.2. Elimination of Gap Junctional Intercellular Communication with GJIC$^-$ Cell Lines. While lindane can be used to inhibit GJIC, one can entirely eliminate the presence of GJIC by using aB1 cells from the WB series. Accordingly, as already indicated in Section D.3a, WB$^+$ (GJIC$^+$) and aB1 (GJIC$^-$) cells will be used to carry out cell survival studies that compare the lethality of incorporated radionuclides in the presence and absence of GJIC. This pair of cell lines has already been used extensively to compare the effects of toxic chemicals in the presence and absence of GJIC (*100*). Thus, this system has the capability of isolating the radiation effects from the bystander effect.

D.3e.3. Protection of Bystander Cells with a Radical Scavenger. Azzam et al. (*14*) have suggested that reactive oxygen species may play some role in the bystander effect. Our preliminary studies with ^{3}HTdR and 10% DMSO suggest that the bystander effect in V79 cells may be mediated in part by free radicals (Section C.2f). The extent of free radical participation may be related to percentage of cells labeled, the cell line, and the type of radiochemical involved. Therefore, we will examine this carefully in both V79 and the WB cell lines.

Careful consideration must be given to the fact that DMSO can, in principle, afford protection against both radiation effects and bystander effects. We have considerable experience using DMSO as a radioprotector against the effects of incorporated radionuclides both in vitro and in vivo (*71, 101*). Our published in vitro studies with suspensions of V79 cells indicate that 5% DMSO in the culture medium does not afford protection against ^{3}HTdR, ^{131}IdU, or ^{125}IdU when the radiolabeled cells are maintained in suspension for three days at 10.5°C (*71*). However, our recent studies using the same experimental conditions show that 10% DMSO provides very good protection against ^{131}IdU and ^{125}IdU with only minimal chemotoxicity associated with the DMSO. The ^{125}IdU data are shown below to illustrate the protection provided by 10% DMSO. Therefore, changes in survival fraction or mutation frequency in a cluster containing a mixture of labeled and unlabeled cells could be due to mitigation of radiation effects and/or bystander effects. However, for ^{3}HTdR, if DMSO only mitigated radiation effects, one would expect an upward shift of the entire second component with no change in the slope of the two-component exponential survival curve (Eq. C.1). In contrast, mitigation of the bystander effects would change the slope of the second component. In any case, because of the concerns over mitigation of radiation effects, DMSO experiments will benefit greatly from cell sorting since the effects on bystander cells can be specifically measured. Therefore, labeled and unlabeled cells will be sorted prior to assaying biological response and initially only ^{3}HTdR and ^{125}IdU will be studied as per the arguments in Section D.3c. As indicated in Specific Aim 4, the multicellular cluster studies described in Sections D.3e.1 will thus be repeated by replacing lindane with 10% DMSO. In addition the studies described in Section D.3e.2 will be repeated in the presence of 10% DMSO. Finally the combination of lindane and DMSO will also be examined for the cases described in Section D.3e.1. These experiments will delineate the extent to which the bystander effect is radical mediated.

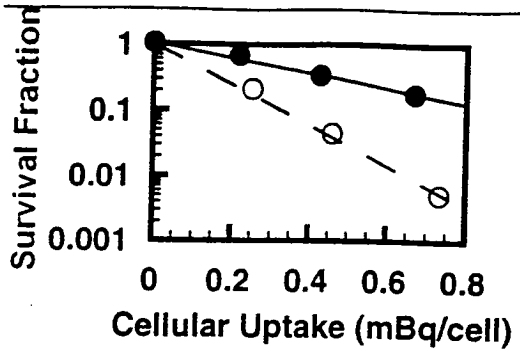


Figure 11. Survival of V79 cells as a function of intracellular activity of ^{125}IdU in the absence (O) and presence (●) of 10% DMSO in the culture medium. The cells were maintained in suspension at 10.5°C for 72 h.

D.3f. *Comet Assay to Determine the Mechanism of Cell Death in the Bystander Cells.* This method of measuring DNA damage has gained continuously in popularity since it was originally described in 1984 (*102*). Recent reviews have summarized the advantages of this assay over other methods (*103, 104*). DNA damage in virtually any eukaryotic cell can be analyzed. Cells do not require radiolabeling nor do they need to be cycling. In fact, DNA synthesis can confound results (*104*). Small numbers of cells are adequate and the slides can be preserved. The greatest advantage of this assay for our purposes is that DNA damage in individual cells can be quantitated. As Olive states in her review, 'The comet assay can identify cells that are hit from those that escape damage'. Since we are dealing with heterogeneous distributions of radioactivity it will be a decided benefit to be able to observe the damage in individual cells. The 10.5°C incubation should suspend the cells in time and reduce or eliminate confusion that could result from DNA synthesis. Furthermore, different mechanisms of death can be distinguished. For example, cells that die from apoptosis can be distinguished from those that die of nuclear damage (*104*). By varying the pH of the lysis mixture and the electrophoresis buffer, single strand breaks (alkaline assay) and double strand breaks (neutral assay) can be differentiated.

The comet assay will be used to study the effects of nonuniform distributions of radioactivity only in those cases where the unlabeled bystander cells are separated from the labeled cells using FACS. Given that the FACS dye resides in the labeled cells, a pure population of bystander cells can be isolated for comet analysis. Thus, in the experiments outlined Section D3.d, 10^5 sorted bystander cells will be taken from each tube to be analyzed for comet assay. In all experiments, controls will consist of 100% labeled and 0% labeled cells. We will follow Dr. Olive's protocol (personal communication) for the alkaline assay and, when appropriate, for the neutral assay. Initially, comets will be analyzed using our fluorescence microscope, in order to get a feeling for the nature of the comets in any given case. Final analysis will be accomplished using a CCD camera available in the Department of Medicine in conjunction with the Image Analysis software (NIH Image) available at http://rsb.info.nih.gov/nih-image/. Response will be scored in two ways: 1) percentage of cells with comets, and 2) tail moment which is the tail length times tail intensity, or percent migrated DNA.

D.3g. *Measurement of Cell Dimensions.* The dimensions of the cell and cell nucleus of V79 cells have already been carefully determined when these cells are in suspension (*1*). A mean nuclear diameter of 8.0 μm and cell diameter of 10.0 μm was obtained. However, to ensure that these dimensions are valid for V79 cells within the assembled clusters, we will employ confocal microscopy to measure the diameters *in situ*. Measurements will also be made for the WBs, WB', and aB1 cell lines. This will be done with the assistance of our faculty expert in confocal microscopy, Jeffrey Gardner, Ph.D., NJMS Department of Pediatrics. Cells will be labeled with a Vybrant™ CFDA SE cell tracer kit as described in Section C.2j and clusters assembled with the dyed cells. The clusters will be maintained at 10.5°C for 72 h and then delicately teased from the microcentrifuge tube to minimize disruption of the cluster. The cluster will be placed on a slide and immediately placed into the Zeiss Laser Scan Microscope LSM 410 invert with ArKr laser (488/568/647 nm). Slices will be acquired by exciting with a wavelength of 488 nm and passage of the resulting CFDA SE fluorescence through a 515-545 bandpass filter. A three dimensional rendering of the cluster geometry will be obtained. The Zeiss software package will be used to obtain the mean diameters and standard deviations of the cell and cell nucleus, as well as the packing density of the cells. These results will be used in the theoretical modeling below.

D.4 Theoretical Cellular Dosimetry and Prediction of Cell Survival

D.4a. *Overview.* The biological response of a given cell in the multicellular cluster is expected to depend principally on three variables: i) absorbed-dose, ii) RBE, and iii) bystander effects. The dependence on i) requires detailed dosimetry calculations that take into account the self-dose from radioactivity within the cell itself and the cross-dose from the other cells in the cluster. These are addressed Specific Aim 3. The impact of a given component of the absorbed dose depends on its RBE. This level of detail is particularly important for ^{125}I because the RBE of Auger electron emitters is strongly dependent on subcellular distribution of the radionuclide (Attachment #4, Refs. (*1, 2, 51, 105, 106*)). We have considerable experience with correlating the biological effects of radionuclides incorporated into cultured cells with absorbed dose. This includes experience with alpha, beta, and Auger-electron emitters (*1, 3, 9, 10, 71, 107*). Cellular dosimetry has been the basis for all of these studies and we have found that this approach is very effective for predicting response. While our earlier studies used a single-cell model, we anticipate that the cellular dosimetry approach will be equally effective for the multicellular model. Both the single-cell and multicellular models involve mixed radiation fields, a problem we dealt with in detail in our article on the effects of ^{212}Pb-PDC which involves the emission of both alpha and beta particles (Attachment #5, Ref. (*9*)). Our approach to relating dose and effect, outlined in Section D.4c and D.4d, takes into account the high RBE expected from the self- and cross-dose for ^{210}Po alpha particles and the self-dose from DNA-incorporated ^{125}IdU and ^{3}HTdR (*1*).

D.4b. *Theoretical Multicellular Cluster Model.* The theoretical multicellular cluster model will be constructed using the same approach described earlier (*26, 27*). The multicellular cluster will be represented as a spherical collection of cells in hexagonal close-packed geometry provided that the measured packing density is close to 74%. Otherwise other packing geometries will be considered to more closely match the measured value. The cell and cell nucleus will be represented as concentric spheres of unit density matter (*108*) with dimensions corresponding to those obtained in Section D.3g for the V79 and WB cell lines. Our preliminary look at the clusters using simple microscopy techniques indicates that the cells are spherical. Although it should be noted that even large deviations from sphericity will not impact the dose calculations by more than about 15% (*27*). To simulate the experimental conditions, a cluster of 4×10^6 cells will be modeled (for cells with a 10 μm diameter, this corresponds to a cluster diameter of 1.754 mm). The radioactivity will be distributed between the cytoplasm (Cy) and the nucleus (N) according to the measured subcellular distribution of the radiochemical.

D.4c. *Cellular Dosimetry.* To achieve Specific Aim 3, the method of dose calculation used in our theoretical cellular and multicellular dosimetry models described in our earlier publications will be revised to calculate the absorbed dose on a cell-by-cell basis (*26, 27*). The mean absorbed dose $D_{k,N}$ to the cell nucleus N of target cell k in the cluster is given by the sum of the self-dose to the target cell and the cross-dose from all other cells in the cluster:

$$D_{k,N} = D_{k,N}^{self} + D_{k,N}^{cross} \tag{D.1}$$

$$D_{k,N}^{self} = \frac{\tilde{a}_k}{m_N} \sum_i \Delta_i \left(f_{Cy} (\phi^{self}_{k,N \leftarrow k,Cy})_i + f_N (\phi^{self}_{k,N \leftarrow k,N})_i \right), \tag{D.2}$$

$$D_{k,N}^{cross} = \frac{1}{m_N} \sum_j \tilde{a}_j \left\{ \sum_i \Delta_i \left(f_{Cy} (\phi^{cross}_{k,N \leftarrow j,Cy})_i + f_N (\phi^{cross}_{k,N \leftarrow j,N})_i \right) \right\}, \tag{D.3}$$

where m_N is the mass of the cell nucleus, Δ_i is the mean energy emitted per nuclear transition for the ith radiation component, and $\tilde{a}_k$ and $\tilde{a}_j$ are the cumulated activities in the kth target cell and the jth non-target cell, respectively. The absorbed fractions $(\phi^{self}_{k,N \leftarrow k,Cy})_i$ and $(\phi^{self}_{k,N \leftarrow k,N})_i$ are the fraction of the ith energy component emitted by radioactivity in the cytoplasm and nucleus within the kth target cell that is deposited in cell nucleus of target cell k, respectively. The absorbed fractions $(\phi^{cross}_{k,N \leftarrow j,Cy})_i$ and $(\phi^{cross}_{k,N \leftarrow j,N})_i$ are the fraction of the ith energy component emitted by radioactivity in the cytoplasm and nucleus within the jth non-target cell that is deposited in cell

nucleus of target cell k, respectively. The quantities f_{Cy} and f_N are the fraction of cellular radioactivity localized in the cytoplasm and nucleus, respectively. Equations 2 and 3 can be written more simply in terms of S (where $S = \Sigma \Delta_i \phi_i / m$), the mean absorbed dose to the target region per unit cumulated activity in the source region (*38*).

$$D_{k,N}^{self} = \tilde{a}_k \left(f_{Cy} S_{k,N \leftarrow k,Cy}^{self} + f_N S_{k,N \leftarrow k,N}^{self} \right), \quad (D.4)$$

$$D_{k,N}^{cross} = \sum_j \tilde{a}_j \left(f_{Cy} S_{k,N \leftarrow j,Cy}^{cross} + f_N S_{k,N \leftarrow j,N}^{cross} \right), \quad (D.5)$$

The cellular S values for calculating the self absorbed dose $S_{k,N \leftarrow k,Cy}^{self}$ and $S_{k,N \leftarrow k,N}^{self}$ have been tabulated for over 200 radionuclides in a MIRD monograph (*27*). Since the radionuclides to be used in the present study are tabulated in the monograph, no new self-dose cellular S values will need to be calculated. The cross-dose S values depend on the separation distance between the source and target cell. These cellular cross-dose S values will be tabulated for separation distances of 1-2000 μm at 1 μm intervals for ^{3}H, ^{125}I, ^{131}I, and ^{210}Po. The absorbed fractions needed for these tabulations will be calculated using analytical techniques as described in our earlier paper (*26*). Tabulation of the S values in this way is essential for minimizing the computation time needed to calculate doses in clusters containing large numbers of cells. As indicated in Specific Aim 5, similar tabulations will be carried out for numerous other radionuclides of interest to nuclear medicine (*27*). Radiation spectra for all radionuclides will be taken from Eckerman et al. (*109*). This compilation of radiation spectra contains the complete beta spectrum for each radionuclide, a feature necessary for cellular and multicellular dosimetry.

When calculating self-dose, the cumulated activity in the target cell $\tilde{a}_k$ is the sum of three components:

$$\tilde{a}_k = \tilde{a}_I + \tilde{a}_M + \tilde{a}_{CF} \quad (D.6)$$

where $\tilde{a}_I$, $\tilde{a}_M$, and $\tilde{a}_{CF}$ are the cellular cumulated activities during the periods of incubation for cellular uptake of radioactivity, maintenance as a cluster for 72 h, and colony formation, respectively. When calculating the cross-dose, only the cumulated activity in the non-target source cell during the multicellular cluster period should be considered. Therefore, the cumulated activity in non-target source cell $\tilde{a}_j$ is simply,

$$\tilde{a}_j = \tilde{a}_M \quad (D.7)$$

Of course, if the target cell is an unlabeled cell, then $\tilde{a}_k = 0$. Similarly, if the non-target cell is unlabeled, $\tilde{a}_j = 0$.

Since the radiopharmaceuticals are taken up by the cells linearly in time (*1*), the cellular cumulated activity during the uptake period $\tilde{a}_I$ is given by

$$\tilde{a}_I = 0.5\, t_I\, a_I \quad (D.8)$$

where a_I is the average cellular activity (mBq/cell) at the end of the uptake incubation period and t_I is the incubation time during which the radioactivity is taken up by the cells ($t_I(^{210}Po) = 0.5$ h, $t_I(^{125/131}IdU$, $^{3}HTdR$, ^{3}H-methionine, $^{125/131}I$-Rh123) = 12 h). During the maintenance period t_M = 72 h, the radioactivity undergoes physical decay only. Therefore, the cumulated activity during this time is given by

$$\tilde{a}_M = a_I \int_0^{t_M} \exp(-0.693t / T_p)\, dt \quad (D.9)$$

The physical half-lives T_p of ^{210}Po, ^{131}I, and ^{125}I are 138 d, 8 d, and 60 d, respectively (*36*). The physical half-life of ^{3}H is so long that no appreciable decay occurs in this time scale. Finally, the cumulated activity during the colony forming period t_{CF} is given by,

$$\tilde{a}_{CF} = \exp(-0.693\, t_M/T_p)\, a_I \int_0^{t_{CF}} \exp(-0.693t / T_{e,CF})\, dt \quad (D.10)$$

where $T_{e,CF}$ is the effective half-time of the radioactivity in the cells during this period.

D.4d. *Modeling the Survival of Cells in the Multicellular Cluster.* The survival probability P_k for a given target cell k will be taken as the product of the survival probabilities resulting from radiation effects R_k and bystander effects B_k.

$$P_k = R_k \times B_k \tag{D.11}$$

The survival probability for radiation effects R_k for the k^{th} target cell will be taken as the product of the survival probabilities resulting from the radiation insults from the self-dose and the cross-dose to the cell nucleus. We have been very successful in using the linear quadratic (LQ) model to predict cell survival when only self-dose from incorporated radioactivity is present (cells not maintained as a cluster). This has been true for alpha, beta, and Auger-electron emitters alike, such as ^{212}Pb, ^{125}I, ^{193m}Pt, ^{3}H, and ^{210}Po (*1, 3, 9*). The former three radionuclides involve the emission of mixed radiation fields so this should not pose undue complications in the proposed studies. The LQ model was also used when the cells were irradiated with external gamma-rays (i.e. only cross-dose) as well (*1*). Therefore, the survival probability for radiation effects R_k in target cell k will be modeled as the product of the survival probabilities resulting from the self-dose and the cross-dose components.

$$R_k = \exp(-\alpha_{self} D_{k,N}^{self} - \beta_{self} (D_{k,N}^{self})^2) \times \exp(-\alpha_{cross} D_{k,N}^{cross} - \beta_{cross} (D_{k,N}^{cross})^2), \tag{D.12}$$

where α_{self}, β_{self}, α_{cross}, β_{cross} are the linear and quadratic coefficients of the LQ model for the self- and cross-irradiation components, respectively. These quantities will depend on the type of cell, the radionuclide, and the subcellular distribution of the radionuclide which depends on the radiochemical. The methods to be used to obtain α_{self}, β_{self}, α_{cross}, and β_{cross} will be as follows:

^{210}Po-citrate and ^{210}Po-PDC:, A high-LET dose response curve with no shoulder is expected to emerge for both self- and cross-dose, therefore, $\beta_{cross} = \beta_{self} = 0$. Despite the differences in subcellular distribution of the two ^{210}Po radiochemicals, our previous cell culture experience with alpha emitters suggests that the slope of the dose-response curves will be the same ($\alpha_{self} = \alpha_{cross}$) (*1, 9, 107*). Thus, if we plot the survival as a function of mean absorbed dose to the cluster for the case of 100% labeling and fit the data to a monoexponential function, we obtain $\alpha_{self} = \alpha_{cross}$ = 1/(experimental mean lethal dose D_{37} to the cluster).

^{125}I-Rh 123 and ^{131}I-Rh 123: These radiochemicals localize entirely in the cytoplasm of cells (*93*) so both the self- and cross-dose will produce low-LET type effects in cultured cells (*51, 93, 110*). Thus, a similar approach will be used for these radiochemicals as was used for ^{210}Po. The survival fraction will be plotted as a function of mean absorbed dose to the cluster for the case of 100% labeling and the dose-response data will be fitted using the linear quadratic model. The mean absorbed dose to the cluster will be calculated using our well-established computer code which accounts for energy losses outside the cluster volume (*87*). The fitted values of α and β will correspond to the desired values where $\alpha_{self} = \alpha_{cross}$ and $\beta_{cross} = \beta_{self}$. It is anticipated that these values will be close to the values obtained for *chronic* ^{137}Cs gamma irradiation in the Preliminary Studies ($\beta = 3.9\text{x}10^{-3}$ Gy^{-2}, $\alpha = 4.4\text{x}10^{-2}$ Gy^{-1}).

^{125}IdU: This radiochemical localizes entirely in the cell nucleus (*1*). For the cross-dose, the values α_{cross} and β_{cross} from ^{125}I-Rh 123 will be used. In the case of the self-dose, there is overwhelming evidence both from our laboratory and others that a shoulderless high-LET type response will be operative (*1, 29, 111*), therefore, $\beta_{self} = 0$. Our earlier multicellular dosimetry calculation show that when ^{125}I is in the cell nucleus and 100% of the cells are labeled, over 90% of the cellular absorbed dose comes from the self-dose (*26*). Furthermore, the RBE of this self-dose is approximately 9 (*1*) so the cross-dose will have a minimal impact on the biological outcome when 100% of the cells are labeled. Therefore, α_{self} = 1/(mean lethal self-dose to the cell nucleus for 100% labeling).

^{131}IdU: For the cross-dose, the values α_{cross} and β_{cross} from ^{131}I-Rh 123 will be used. Since ^{131}I is a medium-energy beta emitter, the same values will also be used for α_{self} and β_{self}.

^{3}HTdR: Figure 2 of Attachment #2 shows that the cross-dose is negligible for this radiochemical which localizes in the nucleus (*71*). Therefore, we can safely set $\alpha_{cross} = \beta_{cross} = 0$. In the case of the self-dose, our preliminary data for 100% labeling shows that a shoulderless high-LET type response is obtained, therefore,

$\beta_{self} = 0$. In view of the extremely short range of the ^{3}H beta particles, α_{self} = 1/(mean lethal self-dose to the cell nucleus for radioactivity in the nucleus for 100% labeling).

^{3}H-methionine: Figure 2 of Attachment #2 shows that the cross-dose is also negligible for this radiochemical which localizes largely in the cytoplasm (*71*). Therefore, we can safely set $\alpha_{cross} = \beta_{cross} = 0$. The fitted values of α_{self} and β_{self} will be used upon plotting the self-dose to the cell nucleus from radioactivity in the cytoplasm for 100% labeling.

Our preliminary data for ^{3}HTdR indicate quite clearly that a *bystander effect in unlabeled cells* is indeed present and it is *dependent on the absorbed dose delivered to the labeled cells*. The monoexponential dose-response for 100% labeling and the two-component exponential response observed for 50% and 10% labeling shown in Figs. 2 and 4 provide a strong argument for an exponential response for the bystander effect. As pointed out in Attachment #1 (*66*), the first component is due to radiation effects in the labeled cell population and the second component is due to bystander effects in the unlabeled cell population. If the data in Fig. 2 are replotted as a function of the cellular uptake of radioactivity in the *labeled* cells, one obtains Fig. 12. As indicated in the caption, the solid lines correspond to a least squares fit to a three-parameter, two-component, exponential response function. The dotted lines are a one-parameter fit to SF = (1-c) exp(-a_I/a_2(100%)) + c exp(-$f\,a_I$/b) where c is only fitted parameter. Reasonably good fits ($r^2 > 0.95$) are obtained indicating that, in the case of ^{3}HTdR, the bystander response is exponentially dependent on the fraction of cells in the cluster that are labeled f and the uptake of radioactivity in the labeled cells a_I (i.e. absorbed dose to labeled cells).

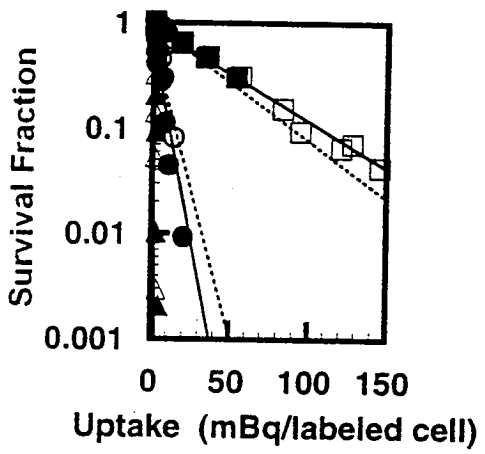


Figure 12. Survival of V79 cells as a function of cellular uptake of ^{3}HTdR in the *radiolabeled* cells. Data are shown for experiments where 10% (■,□), 50% (●,○) or 100% (▲,△) cells were radiolabeled in multicellular clusters which were maintained at 10.5°C for 72 h and then the survival fraction was determined compared to unlabeled cells. The solid lines are a least squares fit of the data to a two-component exponential function: SF = (1-c) exp(-a_I/a_1) + c exp(-a_I/a_2), where c=0.87, a_1=0.96 mBq, a_2=49.4 mBq for 10% labeling; c=0.67, a_1=0.41 mBq, a_2=5.9 mBq for 50% labeling; and c=1.0, a_2=0.51 mBq for 100% labeling. The dotted lines are a least squares fit to SF = (1-c) exp(-a_I/a_2(100%)) + c exp(-$f\,a_I$/b) where a_2(100%) = 0.51 mBq, b = 4 mBq, f is the fraction of cells labeled, and c is the fitted parameter: c(10%) = 0.17, c(50%) = 0.42, and c(100%) = 1.0.

More generally, this implies that the bystander response of the target cell k depends on the magnitude of the individual absorbed dose received by each of the neighboring cells j. In view of this, we will initially assume the survival probability for the bystander response is of the form,

$$B_k = \prod_j \exp(-(D_{j,N})/D_B), \qquad (D.13)$$

where D_B is a radiochemical specific fitted parameter and the product over j will initially include only the 13 cells immediately surrounding the target cell of interest. Additional layers of surrounding cells may have to be included for the WB cell lines which form a large network of gap junctions capable of communicating across numerous cells. Interestingly, the data of Mothersill and Seymour (*16*) suggest that absorbed dose may not be an important variable. However, our preliminary data for ^{3}HTdR argue that the dose to neighboring cells is important in that the surviving fraction of V79 cells continues to drop with increasing activity per labeled cell while maintaining the percentage of labeled cells constant.

A Monte Carlo approach will be adopted to calculate the survival fraction SF for the multicellular cluster. This method will entail consideration of the survival probability for each individual cell in the cluster and making a determination of the fate of the cell. Briefly, a random number (RAND) will be generated

between zero and unity. If $R_k \times B_k > RAND$, the cell will be scored as dead; otherwise, the cell will be scored as a survivor. This process will be carried out for each cell in the cluster and the survival fraction will be calculated according to the equation below.

$$SF = (\text{number of survivors}) \div 4x10^6, \tag{D.14}$$

The SF will be plotted against the cluster activity and the resulting curve will be compared with the corresponding experimental survival curves obtained in Specific Aims 1-2. We have chosen to initially plot the SF against cluster activity since the *raw experimental data* is in this form. Therefore, this is the data that we have to ultimately match with our theoretical model. In addition, the data will be plotted as a function of cellular uptake of radioactivity in the labeled cells. However, plots as a function of dose-related variables will likely prove to be more informative but will have to await further data collection to ascertain the best mode of presentation.

Essentially the same modeling approach will be adopted for the mutation assay. The mutation probability P_k for a given target cell k will be taken as the sum of the mutation probabilities resulting from radiation effects R_k and bystander effects B_k.

$$P_k = R_k + B_k \tag{D.15}$$

$$R_k = \alpha_{self} D_{k,N}^{self} + \beta_{self} (D_{k,N}^{self})^2 + \alpha_{cross} D_{k,N}^{cross} + \beta_{cross} (D_{k,N}^{cross})^2, \tag{D.16}$$

where the parameters α_{self}, β_{self}, α_{cross}, β_{cross} are assay specific and radiochemical specific. For bystander effects, a probability function of the form

$$B_k = \sum_j D_{j,N} / D_B, \tag{D.17}$$

is expected to emerge. Again, the quantity D_B is assay specific and radiochemical specific.

It is expected that perfect agreement between theory and experiment will not emerge immediately. There are several aspects of the theoretical model that can be improved if necessary. First, but perhaps not foremost, the theoretical cluster model assumes spherical geometry. The cluster is actually packed in a 400 µl microcentrifuge tube. The cell number of $4x10^6$ was chosen because this number of cells most closely takes the shape of a sphere (too few cells makes a pancake and too many cells makes a tall cylinder). The actual shape is closer to a cone with a broad round bottom and a slightly concave flat top. This shape can be theoretically modeled if necessary to help bring the theory and experiment into accord. Backscattering of the particulate radiation from the polypropylene tube could contribute somewhat to the dose to the outermost cells in the cluster. Based on the work of Nunes and Prestwich (*112*), this is not anticipated to significantly impact our calculations. Another potential area that may need to be addressed is mixed radiations. Equation D.12 assumes that the biological impact of two different types of radiation can be treated independently. Lam (*113*) suggests that mixtures of high- and low-LET radiation are synergistic with respect to their radiobiological effects. However, our experience with incorporated alpha and beta emitters suggests that different radiations can be treated independently (*9, 114*). Therefore, we do not anticipate having to make any modifications in this regard. Another potential area that will need exploration is the absorbed dose to the cytoplasm. Wu et al. (*115*) and others have used alpha particle microbeams to show that selective irradiation of the cytoplasm leads to induction of mutations in mammalian cells and that this effect is radical mediated. This suggests that the absorbed dose to the cytoplasm of a given target cell may have an impact on both the response of the target cell and bystander cells. This can be readily explored using the same cellular dosimetry techniques by simply considering the cytoplasm as a target region.

Finally, our theoretical modeling utilizes radiochemical specific α and β values for both the self- and cross-dose. Therefore, for any given radiochemical, we anticipate our theoretical model will do a very good job of predicting the biological response of cells in the cluster for any percentage of cells labeled with a known activity. Furthermore, the model will provide good estimates of the absorbed doses received by each of the cells in the cluster so that one can also predict the biological response of the cluster after calculation of the self- and

cross-doses delivered to the cells in the cluster. These are critical elements in the understanding of the biological effects of nonuniform distributions of radioactivity. This is the essence of Specific Aim 6. Specific Aim 6 is not necessarily intended to develop a universal dosimetry method that arrives at a single set of universal parameters (e.g. α and β) that would applicable for all radiochemicals/radiation types. That is not to say, however, that these data will not lead to progress in this area. This proposal will generate a very large database on the biological response of mammalian cells to nonuniform distributions of radioactivity. *These data will be made available as they are collected to all scientists by establishing a web site at the New Jersey Medical School.* The web site will house protocols, raw data, graphs, and curve fits. We will seek to advertise the web site in the Radiation Research Society Newsletter, Newsline in the Journal of Nuclear Medicine, Health Physics Society Newsletter, and Medical Physics newsletter. In this way, data can be freely downloaded by interested theorists who may not otherwise have access to experimental data, and of course by experimentalists who may wish to compare their own data with our results. Furthermore, this creates an opportunity to establish an international dialogue that may lead to protocol alterations that maximize the information provided by the data. Thus, rather than restricting the theoretical interpretation of the data to a single group, this opens the doors for analysis of the data using microdosimetry (*11, 19, 24, 61, 62, 116-119*) and other theories (*113, 120-122*). Thus numerous theories can be explored in parallel. Of course, we do not intend to leave this endeavor only to others. We will seek to adapt our cellular dosimetry approach with the same goal in mind. One avenue we will explore will be based on recommendations in a report by the American Association of Physicists in Medicine (AAPM) (*8*). For example, for mutagenic radiation effects, the following form will serve as a starting point:

$$R_k = \alpha_{self} H_{k,N}^{self} + \beta_{self} (H_{k,N}^{self})^2 + \alpha_{cross} H_{k,N}^{cross} + \beta_{cross} (H_{k,N}^{cross})^2, \quad (D.18)$$

where H, the equivalent dose, is equal to the product of the absorbed dose D and the radiation weighting factor w_R (*8, 12*). The radiation weighting factor will be based on experimental RBE values (*12*). Thus, H contains the radiochemical specific information. Therefore, the parameters α_{self}, β_{self}, α_{cross}, β_{cross} should only be cell line specific. They should not depend on the radionuclide/radiation, and the subcellular distribution of the radionuclide. This approach should work well for mutation dose response relationships that are essentially linear (i.e. $\beta = 0$) such as those we obtained in Fig. 7B for external gamma rays. In these instances, the RBE values (and therefore w_R) will not depend on the absorbed dose. The will depend on the subcellular distribution in the case of Auger emitters and mechanisms to tackle this problem have already been addressed in the AAPM report (*8*). In the case of survival, modifications to accommodate dose dependence of the RBE will have to be made (*10*). The specifics of these alterations will have to wait until adequate experimental data is collected.

D.5 Timeline

The following timeline will be adopted to complete the proposed work in a matter that will maximize the overlap of experimental data acquisition and theoretical modeling. If four radionuclides are studied, this entails carrying out experiments at four different levels of cell labeling (1%, 10%, 50%, 100%), two subcellular distributions (nucleus, cytoplasm), three biological endpoints (survival, mutations, DNA damage via comet assay), different environments (MEMA, MEMA + lindane, MEMA + DMSO, MEMA + lindane + DMSO), and multiple cell lines. In addition, kinetics studies, confocal microscopy measurements, and cell sorting studies, will also be performed. All experiments will have to be repeated at least once to ensure reproducibility and adequate statistical certainty of the data. Theoretical modeling and web site maintainence are also involved. Therefore, a five year program is required to complete the proposed work.

Year 1.	Complete specific aims 1-6 for ^{3}H radiochemicals. Initiate web site.
Year 2.	Begin specific aims 1-6 for ^{210}Po radiochemicals.
Year 3.	Complete ^{210}Po radiochemicals and begin specific aims 1-6 for ^{125}I radiochemicals
Year 4.	Complete ^{125}I radiochemicals and begin specific aims 1-6 for ^{131}I radiochemicals
Year 5.	Complete specific aims 1-6 for ^{131}I radiochemicals and correlate all data.

E. Human Subjects: none

F. Vertebrate Animals: none

G. Literature Cited

1. R. W. Howell, D. V. Rao, D.-Y. Hou, V. R. Narra, and K. S. R. Sastry, The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. *Radiat. Res.* **128,** 282-292 (1991).
2. R. W. Howell, V. R. Narra, K. S. R. Sastry, and D. V. Rao, On the equivalent dose for Auger electron emitters. *Radiat. Res.* **134,** 71-78 (1993).
3. R. W. Howell, A. I. Kassis, S. J. Adelstein, D. V. Rao, H. A. Wright, R. N. Hamm, J. E. Turner, and K. S. R. Sastry, Radiotoxicity of ^{195m}Pt labeled *trans*-platinum(II) in mammalian cells. *Radiat. Res.* **140,** 55-62 (1994).
4. K. S. R. Sastry, and D. V. Rao, Dosimetry of low energy electrons. In *Physics of Nuclear Medicine: Recent Advances* (D. V. Rao, R. Chandra, and M. Graham, Eds.), pp. 169-208. American Institute of Physics, New York, 1984.
5. K. S. R. Sastry, R. W. Howell, D. V. Rao, V. B. Mylavarapu, A. I. Kassis, S. J. Adelstein, H. A. Wright, R. N. Hamm, and J. E. Turner, Dosimetry of Auger-emitters: Physical and phenomenological approaches. In *DNA Damage by Auger Emitters* (K. F. Baverstock, and D. E. Charlton, Eds.), pp. 27-38. Taylor & Francis, London, 1988.
6. H. A. Wright, R. N. Hamm, J. E. Turner, R. W. Howell, D. V. Rao, and K. S. R. Sastry, Calculations of physical and chemical reactions with DNA in aqueous solution from Auger cascades. *Radiat. Prot. Dosim.* **31,** 59-62 (1990).
7. R. W. Howell, Radiation spectra for Auger-electron emitting radionuclides: Report No. 2 of AAPM Nuclear Medicine Task Group No. 6. *Med. Phys.* **19,** 1371-1383 (1992).
8. J. L. Humm, R. W. Howell, and D. V. Rao, Dosimetry of Auger electron emitting radionuclides: Report No. 3 of the AAPM Nuclear Medicine Task Group No. 6. *Med. Phys.* **21,** 1901-1915 (1994).
9. M. T. Azure, R. D. Archer, K. S. R. Sastry, D. V. Rao, and R. W. Howell, Biologic effect of ^{212}Pb localized in the nucleus of mammalian cells: Role of recoil energy in the radiotoxicity of internal alpha emitters. *Radiat. Res.* **140,** 276-283 (1994).
10. R. W. Howell, V. R. Narra, D. Y. Hou, D. A. Terrone, R. S. Harapanhalli, K. S. R. Sastry, and D. V. Rao, Relative biological effectiveness of Auger emitters for cell inactivation: *In vitro* versus *in vivo*. In *Biophysical Aspects of Auger Processes* (R. W. Howell, V. R. Narra, K. S. R. Sastry, and D. V. Rao, Eds.), pp. 290-318. American Institute of Physics, Woodbury, NY, 1992.
11. J. C. Roeske, and T. G. Stinchcomb, Dosimetric framework for therapeutic alpha-particle emitters. *J. Nucl. Med.* **38,** 1923-1928 (1997).
12. ICRP, *1990 Recommendations*, Publication 60. International Commission on Radiological Protection, Pergamon, Oxford, 1991.
13. ICRU, *Methods of Assessment of Absorbed Dose in Clinical use of Radionuclides*, Report 32. International Commission on Radiation Units and Measurements, Bethesda, MD, 1979.
14. E. I. Azzam, S. M. de Toledo, T. Gooding, and J. B. Little, Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiat. Res.* **150,** 497-504 (1998).
15. M. Sigg, N. E. A. Crompton, and W. Burkhart, Enhanced neoplastic transformation in an inhomogeneous radiation field: An effect of the presence of heavily damaged cells. *Radiat. Res.* **148,** 543-547 (1997).
16. C. Mothersill, and C. B. Seymour, Cell-cell contact during gamma irradiation is not required to induce a bystander effect in normal kidney keratinocytes: Evidence for release during irradiation of a signal controlling survival into the medium. *Radiat. Res.* **149,** 252-262 (1998).

17. G. M. Makrigiorgos, S. J. Adelstein, and A. I. Kassis, Limitations of conventional internal dosimetry at the cellular level. *J. Nucl. Med.* **30,** 1856-1864 (1989).
18. J. L. Humm, K. D. Bagshawe, S. K. Sharma, and G. Boxer, Tissue dose estimates following the selective uptake of ^{125}IUdR and other radiolabelled thymidine precursors in resistant tumours. *Br. J. Radiol.* **64,** 45-49 (1991).
19. W. C. Roesch, Microdosimetry of internal sources. *Radiat. Res.* **70,** 494-510 (1977).
20. A. I. Kassis, and S. J. Adelstein, Does nonuniformity of dose have implications for radiation protection? *J. Nucl. Med.* **33,** 384-387 (1992).
21. E. Polig, The localized dosimetry of internally deposited alpha-emitters. *Curr. Top. Radiat. Res.* **13,** 189-327 (1978).
22. J. L. Humm, J. C. Roeske, D. R. Fisher, and G. T. Y. Chen, Microdosimetric concepts in radioimmunotherapy. *Med. Phys.* **20, Pt. 2,** 535-541 (1993).
23. D. R. Fisher, From "micro" to "macro" internal dosimetry. In *Internal Radiation Dosimetry: Health Physics Society 1994 Summer School* (O. Raabe, Eds.), pp. 61-80. Medical Physics Publishing, Madison, WI, 1994.
24. G. Akabani, and M. R. Zalutsky, Microdosimetry of astatine-211 using histological images: Application to bone marrow. *Radiat. Res.* **148,** 599-607 (1997).
25. R. W. Howell, The MIRD schema: From organ to cellular dimensions. *J. Nucl. Med.* **35,** 531-533 (1994).
26. S. M. Goddu, D. V. Rao, and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation and subcellular distribution of radionuclides. *J. Nucl. Med.* **35,** 521-530 (1994).
27. S. M. Goddu, R. W. Howell, L. G. Bouchet, W. E. Bolch, and D. V. Rao, *MIRD Cellular S values: Self-absorbed dose per unit cumulated activity for selected radionuclides and monoenergetic electron and alpha particle emitters incorporated into different cell compartments*. Society of Nuclear Medicine, Reston, VA, 1997.
28. S. J. Adelstein, R. W. Howell, J. L. Humm, G. M. Makrigiorgos, and B. W. Wessels, On the conceptual basis for dose quantities in nuclear medicine. *ICRU News* **1,** 4-10 (1998).
29. A. I. Kassis, K. S. R. Sastry, and S. J. Adelstein, Kinetics of uptake, retention, and radiotoxicity of ^{125}IUdR in mammalian cells: Implications of localized energy deposition by Auger processes. *Radiat. Res.* **109,** 78-89 (1987).
30. D. R. Fisher, M. E. Frazier, and T. K. Andrews, Jr., Energy distribution and the relative biological effects of internal alpha emitters. *Radiat. Prot. Dosim.* **13,** 223-227 (1985).
31. T. Straume, and A. L. Carsten, Tritium radiobiology and relative biological effectiveness. *Health Phys.* **65,** 657-672 (1993).
32. K. S. R. Sastry, Biological effects of the Auger emitter ^{125}I: A review. Report No. 1 of AAPM Nuclear Medicine Task Group No. 6. *Med. Phys.* **19,** 1361-1370 (1992).
33. R. H. Larsen, G. Vaidyanathan, and M. R. Zalutsky, Cytotoxicity of a-particle-emitting 5-[211At]astato-2'-deoxyuridine in human cancer cells. *Int. J. Radiat. Biol.* **72,** 79-90 (1997).
34. J. M. Whaley, and J. B. Little, Efficient mutation induction by I-125 and I-131 decays in DNA of human cells. *Radiat. Res.* **123,** 68-74 (1990).
35. S. M. Goddu, R. W. Howell, and D. V. Rao, Cellular dosimetry: Absorbed fractions for monoenergetic electron and alpha particle sources and S-values for radionuclides uniformly distributed in different cell compartments. *J. Nucl. Med.* **35,** 303-316 (1994).
36. D. A. Weber, K. F. Eckerman, L. T. Dillman, and J. C. Ryman, *MIRD: Radionuclide Data and Decay Schemes*. Society of Nuclear Medicine, New York, 1989.
37. R. Loevinger, and M. Berman, *A revised schema for calculating the absorbed dose from biologically distributed radionuclides*. Society of Nuclear Medicine, New York, 1976.
38. R. Loevinger, T. F. Budinger, and E. E. Watson, *MIRD Primer for Absorbed Dose Calculations*. The Society of Nuclear Medicine, New York, 1991.

39. W. S. Snyder, M. R. Ford, G. G. Warner, and S. B. Watson, *"S," absorbed dose per unit cumulated activity for selected radionuclides and organs*. Society of Nuclear Medicine, New York, 1975.
40. E. F. Oakberg, Spermatogonial stem-cell renewal in the mouse. *Anat. Rec.* **169,** 515-532 (1971).
41. B.-A. Jönsson, S.-E. Strand, and B. S. Larsson, A quantitative autoradiographic study of the heterogeneous activity distribution of different indium-111-labeled radiopharmaceuticals in rat tissues. *J. Nucl. Med.* **33,** 1825-1832 (1992).
42. G. M. Makrigiorgos, S. J. Adelstein, and A. I. Kassis, Cellular radiation dosimetry and its implications for estimation of radiation risks. Illustrative results with technetium-99m-labeled microspheres and macroaggregates. *JAMA* **264,** 592-595 (1990).
43. G. M. Makrigiorgos, S. Ito, J. Baranowska-Kortylewicz, D. W. Vinter, A. Iqbal, A. D. Van den Abbeele, S. J. Adelstein, and A. I. Kassis, Inhomogeneous deposition of radiopharmaceuticals at the cellular level: Experimental evidence and dosimetric implications. *J. Nucl. Med.* **31,** 1358-1363 (1990).
44. L. E. Feinendegen, V. P. Bond, and J. Booz, The quantification of physical events within tissue at low levels of exposure to ionizing radiation. In eds. ICRU NEWS. International Commission on Radiation Units and Measurements, Bethesda, MD, 1994:9-12.
45. NCRP, *Tritium and other radionuclide labelled organic compounds incorporated in genetic material*, Report No. 63. National Council on Radiation Protection and Measurements, 1979.
46. B. W. Wessels, and M. H. Griffith, Miniature thermoluminescent dosimeter absorbed dose measurements in tumor phantom models. *J. Nucl. Med.* **27,** 1308-1314 (1986).
47. J. L. Humm, and L. M. Cobb, Nonuniformity of tumor dose in radioimmunotherapy. *J. Nucl. Med.* **31,** 75-83 (1990).
48. J. L. Humm, R. M. Macklis, K. Bump, L. M. Cobb, and L. M. Chin, Internal dosimetry using data derived from autoradiographs. *J. Nucl. Med.* **34,** 1811-1817 (1993).
49. S. J. Adelstein, A. I. Kassis, and K. S. R. Sastry, Cellular vs. organ approaches to dose estimates. In *Proceedings of Fourth International Radiopharmaceutical Dosimetry Symposium* (A. T. Schlafke-Stelson, and E. E. Watson, Eds.), pp. 13-25. National Technical Information Service, Springfield, VA, 1986.
50. J. L. Humm, and L. M. Chin, Cellular dosimetry. In *Dosimetry of Administered Radionuclides* (S. J. Adelstein, A. I. Kassis, and R. W. Burt, Eds.), pp. 306-330. American College of Nuclear Physicians, Washington, D.C., 1990.
51. K. G. Hofer, C. R. Harris, and J. M. Smith, Radiotoxicity of intracellular Ga-67, I-125, H-3. Nuclear versus cytoplasmic radiation effects in murine L1210 leukaemia. *Int. J. Radiat. Biol.* **28,** 225-241 (1975).
52. J. L. Humm, and L. M. Chin, A model of cell inactivation by alpha-particle internal emitters. *Radiat. Res.* **134,** 143-150 (1993).
53. D. R. Fisher, Alpha-particle emitters in medicine. In *Dosimetry of Administered Radionuclides* (S. J. Adelstein, A. I. Kassis, and R. W. Burt, Eds.), pp. 194-214. American College of Nuclear Physicians, Washington, D.C., 1990.
54. C. S. Kwok, W. V. Prestwich, and B. C. Wilson, Calculation of radiation doses for nonuniformly distributed beta and gamma radionuclides in soft tissue. *Med. Phys.* **12,** 405-412 (1985).
55. W. E. Bolch, L. G. Bouchet, J. S. Robertson, B. W. Wessels, J. A. Siegel, R. W. Howell, A. K. Erdi, B. Aydogan, S. Costes, and E. E. Watson, MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions - radionuclide S values at the voxel level. *J. Nucl. Med.* **40,** 11S-36S (1999).
56. G. Sgouros, S. Chiu, K. S. Pentlow, L. J. Brewster, H. Kalaigian, B. Baldwin, F. Daghighian, M. C. Graham, S. M. Larson, and R. Mohan, Three-dimensional dosimetry for radiotherapy treatment planning. *J. Nucl. Med.* **34,** 1595-1601 (1993).
57. A. K. Erdi, B. W. Wessels, and R. DeJager, Tumor activity confirmation and isodose curve display for patients receiving iodine-131-labelled 16.88 human monoclonal antibody. *Cancer* **73,** 932-944 (1994).
58. C. Thomason, T. R. Mackie, M. J. Lindstrom, and P. D. Higgins, The dose distribution surrounding ^{192}Ir and ^{137}Cs seed sources. *Phys. Med. Biol.* **36,** 475-493 (1991).

59. A. P. Brown, M. M. Urie, G. Barest, E. Cheng, L. Coia, B. N. Emami, J. Galvin, J. Kutcher, J. Manolis, and J. W. Wong, Three-dimensional photon treatment planning for Hodgkin's disease. *Int. J. Radiat. Oncol. Biol. Phys.* **21,** 205-215 (1991).
60. J. L. Humm, Dosimetric aspects of radiolabeled antibodies for tumor therapy. *J. Nucl. Med.* **27,** 1490-1497 (1986).
61. D. R. Fisher, The microdosimetry of monoclonal antibodies labeled with alpha emitters. In *Proceedings of Fourth International Radiopharmaceutical Dosimetry Symposium* (A. T. Schlafke-Stelson, and E. E. Watson, Eds.), pp. 26-36. National Technical Information Service, Springfield, VA, 1986.
62. T. G. Stinchcomb, and J. C. Roeske, Analytic microdosimetry for radioimmunotherapeutic alpha emitters. *Med. Phys.* **19,** 1385-1393 (1992).
63. H. Nagasawa, and J. B. Little, Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* **52,** 6394-6396 (1992).
64. A. Deshpande, E. H. Goodwin, S. M. Bailey, B. L. Marrone, and B. E. Lehnert, Alpha-particle-induced sister chromatid exchange in normal human lung fibroblasts - Evidence for an extranuclear target. *Radiat. Res.* **145,** 260-267 (1996).
65. S. A. Lorimore, M. A. Kadhim, D. A. Pocock, D. Papworth, D. L. Stevens, D. T. Goodhead, and E. G. Wright, Chromosomal instability in the descendants of unirradiated surviving cells after alpha-particle irradiation. *Proc. Natl. Acad. Sci. USA* **95,** 5730-5733 (1998).
66. A. Bishayee, D. V. Rao, and R. W. Howell, Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiat. Res.* **152,** 88-97 (1999).
67. H. L. Liber, P. K. Lemotte, and J. B. Little, Toxicity and mutagenicity of x-rays and [I-125]dUrd or [H-3]TdR incorporated in the DNA of human lymphoblast cells. *Mutat. Res.* **111,** 387-404 (1983).
68. V. R. Narra, R. W. Howell, R. S. Harapanhalli, K. S. R. Sastry, and D. V. Rao, Radiotoxicity of some I-123, I-125, and I-131 labeled compounds in mouse testes: Implications for radiopharmaceutical design. *J. Nucl. Med.* **33,** 2196-2201 (1992).
69. ICRU, *Stopping Powers and Ranges for Protons and Alpha Particles*, Report 49. International Commission on Radiation Units and Measurements, Bethesda, MD, 1993.
70. ICRU, *Photon, Electron, Proton and Neutron Interaction Data for Body Tissues*, Report 46. International Commission on Radiation Units and Measurements, Bethesda, MD, 1992.
71. R. W. Howell, S. M. Goddu, A. Bishayee, and D. V. Rao, Radioprotection against lethal damage caused by chronic irradiation with radionuclides *in vitro*. *Radiat. Res.* **150,** 391-399 (1998).
72. A. I. Kassis, and S. J. Adelstein, A rapid and reproducible method for the separation of cells from radioactive media. *J. Nucl. Med.* **21,** 88-90 (1980).
73. E. Browne, and R. B. Firestone, *Table of Radioactive Isotopes*. Wiley, New York, 1986.
74. X. Guan, and R. J. Ruch, Gap junction endocytosis and lysosomal degredation of connexin43-P2 in WB-F344 rat liver epithelial cells treated with DDT and lindane. *Carcinogenesis* **17,** 1791-1798 (1996).
75. K. A. Criswell, and R. Loch-Caruso, Lindane-induced elimination of gap junctional communication in rat uterine monocytes is mediated by an arachdonic acid-sensitive cAMP-independent mechanism. *Toxicol. Appl. Pharmacol.* **135,** 127-138 (1995).
76. R. Bruzzone, T. W. White, and D. A. Goodenough, The cellular internet: on-line with connexins. *Bioessays* **18,** 709-718 (1996).
77. M. H. El-Fouly, J. E. Trosko, and C. C. Chang, Scrape-loading and dye transfer: A rapid and simple technique to study gap junctional intercellular communication. *Exp. Cell. Res.* **168,** 422-430 (1987).
78. G. S. Goldberg, J. F. Beckberger, and C. C. G. Naus, A pre-loading method of evaluating gap junctional communication by fluorescent dye transfer. *Biotechniques* **18,** 490-497 (1995).
79. W. Zhang, W. T. Couldwell, M. F. Simard, H. Song, J. H. C. Lin, and M. Nedergaard, Direct gap junction communication between malignant glioma cells and astrocytes. *Cancer Res.* **59,** 1994-2003 (1999).

80. C. Tomasetto, M. J. Nepeu, J. Daley, P. K. Horan, and R. Sager, Specificity of gap junction communication among human mammary cells and connexin transfectants in culture. *J. Cell Biol.* **122,** 157-167 (1993).
81. J. P. O'Neill, and A. W. Hsie, The CHO/HGPRT mutation assay: Experimental procedure. In *Banbury Report 2 Mammalian Cell Mutagenesis: The Maturation of Test Systems* (A. W. Hsie, J. P. O'Neill, and McElheny, Eds.), pp. 55-63. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1979.
82. E. J. Hall, *Radiobiology for the Radiologist.* J. B. Lippincott Co., Philadelphia, 1994.
83. N. Suzuki, and S. Okada, Gamma-ray mutagenesis of cultured mammalian cells in vitro and in vivo. *Mutat. Res.* **43,** 81-90 (1977).
84. D. J. Grdina, B. Nagy, C. K. Hill, and C. P. Sigdestad, Protection against radiation-induced mutagenesis in V79 cels by 2-[aminopropyl)amino]ethanethiol under conditions of acute hypoxia. *Radiat. Res.* **117,** 251-258 (1989).
85. C. M. Denault, and H. L. Liber, The effects of hypoxia and cysteamine on X-ray mutagenesis in human cells. I. Dose response and Southern blot analysis of induced mutants. *Radiat. Res.* **135,** 98-107 (1993).
86. ICRP, *Radiation Dose to Patients from Radiopharmaceuticals*, Publication 53. International Commission on Radiological Protection, Pergamon, Oxford, 1987.
87. R. W. Howell, D. V. Rao, and K. S. R. Sastry, Macroscopic dosimetry for radioimmunotherapy: Nonuniform activity distributions in solid tumors. *Med. Phys.* **16,** 66-74 (1989).
88. G. Sgouros, G. Barest, J. Thekkumthala, C. Chui, R. Mohan, R. E. Bigler, and P. Zanzonico, Treatment planning for internal radionuclide therapy: Three-dimensional dosimetry for nonuniformly distributed radionuclides. *J. Nucl. Med.* **31,** 1884-1891 (1990).
89. E. D. Yorke, L. E. Williams, A. J. Demidecki, D. B. Heidorn, P. L. Roberson, and B. W. Wessels, Multicellular dosimetry for beta-emitting radionuclides: Autoradiography, thermoluminescent dosimetry and three-dimensional dose calculations. *Med. Phys.* **20, Pt. 2,** 543-550 (1993).
90. V. K. Langmuir, B. W. Wessels, H. Mendonca, E. Yorke, and L. Montilla, Comparisons of sectioned micro-TLD dose measurements with predicted dose from ^{131}I-labeled antibody. *Med. Phys.* **19,** 1213-1218 (1992).
91. V. K. Langmuir, R. W. Atcher, J. J. Hines, and M. W. Brechbiel, Iodine-125-NRLU-10 kinetic studies and bismuth-212-NRLU-10 toxicity in LS174T multicell spheroids. *J. Nucl. Med.* **31,** 1527-1533 (1990).
92. D. J. Buchsbaum, and B. W. Wessels, Radiolabeled antibody tumor dosimetry. *Med. Phys.* **20, Pt. 2,** 499-502 (1993).
93. R. S. Harapanhalli, A. M. Roy, S. J. Adelstein, and A. I. Kassis, [^{125}I/^{127}I/^{131}I]Iodorhodamine: Synthesis, cellular localization, and biodistribution in athymic mice bearing human tumor xenografts and comparison with [^{99m}Tc]Hexakis(2-methoxyisobutylisonitrile). *J. Med. Chem.* **41,** 2111-2117 (1998).
94. A. I. Kassis, S. J. Adelstein, C. Haydock, and K. S. R. Sastry, Radiotoxicity of Se-75 and S-35: Theory and application to a cellular model. *Radiat. Res.* **84,** 407-425 (1980).
95. S. Y. Oh, E. Dupont, B. V. Madhukar, J. P. Briand, C. C. Chang, E. Beyer, and J. Trosko, Characterization of gap junctional communication-deficient mutants of a rat liver epithelial cell line. *Eur. J. Cell Biol.* **60,** 250-255 (1993).
96. C. C. Chang, J. A. Boezi, and S. T. Warren, Isolation and characterization of a UV-sensitive hypermutable aphidicolin-resistant Chinese hamster cell line. *Somat. Cell Genet.* **7,** 235-253 (1981).
97. A. I. Kassis, K. S. R. Sastry, and S. J. Adelstein, Intracellular distribution and radiotoxicity of chromium-51 in mammalian cells: Auger-electron dosimetry. *J. Nucl. Med.* **26,** 59-67 (1985).
98. R. W. Howell, D. V. Rao, and C. Haydock, Dosimetry techniques for therapeutic applications of incorporated radionuclides. In *Dosimetry of Administered Radionuclides* (S. J. Adelstein, A. I. Kassis, and R. W. Burt, Eds.), pp. 215-256. American College of Nuclear Physicians, Washington, D.C., 1990.
99. X. Guan, W. J. Bonney, and R. J. Ruch, Changes in gap junction permeability, gap junction number, and connexin43 expression in lindane-treated rat liver epithelial cells. *Toxicol. Appl. Pharm.* **130,** 79-86 (1995).

100. J. E. Trosko, B. V. Madhukar, and C. C. Chang, Endogenous and exogenous modulation of gap junctional intercellular communication: toxicological and pharmacological implications. *Life Sci.* **53,** 1-19 (1993).
101. S. M. Goddu, V. R. Narra, R. S. Harapanhalli, R. W. Howell, and D. V. Rao, Radioprotection by DMSO against the biological effects of incorporated radionuclides *in vivo*. *Acta Oncologica* **35,** 901-907 (1996).
102. O. Ostling, and K. J. Johanson, Microelectrophoretic study of radiation-induced DNA damage in individual mammalian cells. *Biochem. Biophys. Research Comm.* **123,** 291-298 (1984).
103. E. Rojas, M. C. Lopez, and M. Valverde, Single cell gel electrophoresis assay methodology and applications. *J. Chromatog.* **B 722,** 225-254 (1999).
104. P. L. Olive, DNA damage and repair in individual cells: Applications of the comet assay in radiobiology. *Int. J. Radiat. Biol.* **75,** 395-405 (1999).
105. D. V. Rao, K. S. R. Sastry, H. E. Grimmond, R. W. Howell, G. F. Govelitz, V. K. Lanka, and V. B. Mylavarapu, Cytotoxicity of some indium radiopharmaceuticals in mouse testes. *J. Nucl. Med.* **29,** 375-384 (1988).
106. A. I. Kassis, R. W. Howell, K. S. R. Sastry, and S. J. Adelstein, Positional effects of Auger decays in mammalian cells in culture. In *DNA Damage by Auger Emitters* (K. F. Baverstock, and D. E. Charlton, Eds.), pp. 1-14. Taylor & Francis, London, 1988.
107. R. W. Howell, V. R. Narra, D. V. Rao, and K. S. R. Sastry, Radiobiological effects of intracellular polonium-210 alpha emissions: A comparison with Auger-emitters. *Radiat. Prot. Dosim.* **31,** 325-328 (1990).
108. A. Cole, Absorption of 20 eV to 50,000 eV electron beams in air and plastic. *Radiat. Res.* **38,** 7-33 (1969).
109. K. F. Eckerman, R. J. Westfall, J. C. Ryman, and M. Cristy, *Nuclear decay data files of the dosimetry research group*, ORNL/TM-12350. Oak Ridge National Laboratory, 1993.
110. A. I. Kassis, F. Fayad, B. M. Kinsey, K. S. R. Sastry, R. A. Taube, and S. J. Adelstein, Radiotoxicity of I-125 in mammalian cells. *Radiat. Res.* **111,** 305-318 (1987).
111. K. G. Hofer, and W. L. Hughes, Radiotoxicity of intranuclear tritium, iodine-125 and iodine-131. *Radiat. Res.* **47,** 94-109 (1971).
112. J. Nunes, and W. V. Prestwich, Experimental determination of ^{32}P dose backscatter factors at and near soft-tissue boundaries. *Med. Phys.* **20,** 223-231 (1993).
113. G. K. Y. Lam, An isoeffect approach to the study of combined effects of mixed radiations - The nonparametric analysis of *in Vivo* data. *Radiat. Res.* **119,** 424-431 (1989).
114. R. W. Howell, S. M. Goddu, V. R. Narra, D. R. Fisher, R. E. Schenter, and D. V. Rao, Radiotoxicity of gadolinium-148 and radium-223 in mouse testes: Relative biological effectiveness of alpha particle emitters *in vivo*. *Radiat. Res.* **147,** 342-348 (1997).
115. L. J. Wu, G. R. Randers-Pehrson, A. Xu, C. A. Waldren, C. R. Geard, Z. L. Yu, and T. K. Hei, Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. *Proc. Natl. Acad. Sci. USA* **96,** 4959-4964 (1999).
116. J. L. Humm, A microdosimetric model of astatine-211 labeled antibodies for radioimmunotherapy. *Int. J. Radiation Oncology Biol. Phys.* **13,** 1767-1773 (1987).
117. D. E. Charlton, J. Booz, J. Fidorra, T. Smit, and L. E. Feinendegen, Microdosimetry of radioactive nuclei incorporated into the DNA of mammalian cells. In *Sixth Symposium Microdosimetry* (J. Booz, and H. G. Ebert, Eds.), pp. 91-110. Harwood Academic, London, 1978.
118. J. Booz, H. G. Paretzke, E. Pomplun, and P. Olko, Auger-electron cascades, charge potential and microdosimetry of iodine-125. *Radiat. Environ. Biophys.* **26,** 151-162 (1987).
119. J. L. Humm, and D. E. Charlton, A new calculational method to assess the therapeutic potential of Auger electron emission. *Int. J. Radiat. Oncol. Biol. Phys.* **17,** 351-360 (1989).
120. D. E. Watt, An approach towards a unified theory of damage to mammalian cells by ionising radiation for absolute dosimetry. *Radiat. Prot. Dos.* **27,** 73-84 (1989).
121. H. G. Paretzke, Radiation track structure theory. In *Kinetics of inhomogeneous processes* (G. R. Freeman, Eds.), pp. 89-170. Wiley, New York, 1987.

122. B. R. Scott, Methodologies for predicting expected combined stochastic radiobiological effects of different ionizing radiations and some applications. *Radiat. Res.* **98,** 182-197 (1984).

H. Consortium/Contractual Arrangements

I. Consultants

Confocal Microscopy:
Jeffrey Gardner, Ph.D. UMDNJ - New Jersey Medical School, Dept. Pediatrics

Cell Sorting.
Thomas Denny UMDNJ - New Jersey Medical School, Dept. Pediatrics

Rat Liver Epithelial Cells and Gap-Junctional Intercellular Communication
James E. Trosko, Ph.D. University of Michigan, Department of Pediatrics and Human Development

Letters indicating their willingness to serve on this project are on the following three pages.

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

Department of Pediatrics
Fax: (973) 972-7597

185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

October 20, 1999

Dr. Roger Howell, Ph.D.
MSB F-451
Department of Radiology
UMD-New Jersey Medical School

Dear Roger:

As Faculty Overseer of the Core Fluorescence Microscope Facility, I can assure you the confocal microscope will be available for your research efforts described in your pending NIH application. As you know, the Facility charges UMDNJ faculty members a fee for the unlimited use of the machine ($2500/yr). This fee includes all instructional technical training and support you may require during your use of the microscope.

Sincerely,

Jeffrey Gardner

Jeffrey Gardner, Ph.D.
Assistant Professor
Chairman and Faculty Overseer, Core Fluorescence Microscope Facility
UMD-New Jersey Medical School

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

Center for Laboratory Investigation
Division of Allergy, Immunology and Infectious Diseases
Departments of Pediatrics and Pathology
Phone: (973)972-5066 Lab: (973)972-7502
Fax: (973)972-6443

185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

January 28, 1999

Roger W. Howell, Ph.D.
UMD-New Jersey Medical School
Department of Radiology
185 South Orange Avenue
Newark, NJ 07103

Dear Dr. Howell,

It has been a pleasure discussing application of cell sorting techniques to the studies outlined in your proposal entitled "*Effects of nonuniform distributions of radioactivity*". As we have discussed, the use of Rhodium 123 appears to be the best choice for your application which entails nondestructive sorting of V79 cells that contain the dye from those that do not. I will be happy to serve as a consultant on your project to assist you in carrying out these studies. The Center maintains a Beckton-Dickinson FACStar dual laser cell sorter capable of simultaneous four-color identification and discrimination of fluorescent labeled cells. It also has both forward angle and 90° light scattering capability. Thus, I am confident that this sorter can support your studies. I look forward to working with you on this project.

Sincerely,

Thomas N. Denny
Assistant Professor of Pathology, Laboratory
Medicine and Pediatrics
Director of Research Administration and Laboratory Services

Printed on recycled paper at UMDNJ

MICHIGAN STATE UNIVERSITY

DEPARTMENT OF PEDIATRICS/HUMAN DEVELOPMENT
B240 LIFE SCIENCES

EAST LANSING • MICHIGAN • 48824-131[illegible]
(517) 353-5042 FAX: (517) 353-8464

October 7, 1999

Dr. Roger W. Howell
Division of Radiation Research
MSB-F453
New Jersey Medical School
185 South Orange Ave.
Newark, NJ 07103-2714

Dear Dr. Howell:

As I am extremely interested in this proposed research grant, I'm writing this note to confirm my willingness to send you several of my well-characterized rat liver epithelial cells for your research proposal, "Effects of nonuniform distributions of radioactivity" to be used to delineate the mechanism of the "Bystander effects" you have observed. These rat liver epithelial cells were derived from a normal rat liver (WB- F344). The parental cells are normal, diploid, non-tumorigenic and have functional gap junctional intercellular communication (GJIC) via connexin43. A mutant clone, isolated for its inability to perform any functional GJIC, expresses the connexin43 message, makes the connexin43 protein and even has gap junction plaques on the membrane. However, it has absolutely no functional GJIC.

Dr. J. Little of Harvard is now using these cells for his studies on the "Bystander Effects" he is observing (as he presented at the International Radiation Research meetings in Dublin).

I am willing to assist you in anyway I can, as a "gap junction" consultant, on your grant proposal.

Most sincerely,

James E. Trosko

James E. Trosko, Ph.D.
246 Natl. Food Safety and Toxicology Center
Dept. Pediatrics and Human Development
Phone: 517-353-6346
Fax: 517-432-6340
E-mail: trosko@pilot.msu.edu
Home page: http://www.phd.msu.edu/trosko

MSU is an Affirmative Action/Equal Opportunity Institution

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☐ NEW application. *(This application is being submitted to the PHS for the first time.)*

☒ REVISION of application number 1 R01 CA83838-01
(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

INVENTIONS AND PATENTS *(Competing continuation appl. only)*

☐ COMPETING CONTINUATION of grant number: _____ ☐ No ☐ Previously reported
(This application is to extend a funded grant beyond its current project period.) ☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT to grant number: _____
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.
Name of former principal investigator/program director: _____

☐ FOREIGN application or significant foreign component.

1. ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications begin on page 27 of Section III. If unable to certify compliance where applicable, provide an explanation and place

• Human Subjects; • Vertebrate Animals; • Debarment and Suspension; •Drug-Free Workplace (applicable to new [Type 1] or revised [Type 1] applications only); • Lobbying; • Delinquent Federal Debt; • Research Misconduct; • Civil Rights (Form HHS441 or HHS 690); • Handicapped Individuals (Form HHS 641 or HHS 690); • Sex Discrimination (Form HHS 620 A or HHS 690); • Age Discrimination (Form HHS 680 or HHS 690); •

2. PROGRAM INCOME *(See instructions, page 19.)*

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is nticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

3. FACILITIES AND ADMINISTRATION COSTS (F&A)

Indicate the applicant organization's most recent F& A rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal Agency, it should, immediately upon notification that an award will be made, develop a tentative F & A rate

most recently completed fiscal year in accordance with the principles set forth in the pertinent DHHS Guide for Establishing Indirect Costs Rates, and submitted to the appropriate DHHS Regional Office or PHS Agency Cost Advisory Office. F&A costs will not be paid on foreign grants, construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Awards, and

☒ DHHS Agreement dated: 06/14/99 ☐ No Facilities and Administration Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office

☐ No DHHS Agreement, but rate established with _____ Date _____

'ALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information. Supplying the following information on F& A costs is optional for forprofit organizations.)*

a. Initial budget period: Amount of base: $ 147,500 x Rate applied 57.00 % = F & A costs (1) $ 84,075

b. Entire proposed project period: Amount of base: $ 847,500 x Rate applied 57.00 % = F & A costs (2) $ 483,075

(1) Add to total direct costs from form page 4 and enter new total on FACE PAGE, item 7b.
(2) Add to total direct costs from form page 5 and enter new total on FACE PAGE, item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*
☐ Off-site, other special rate, or more than one rate involved *(Explain)*

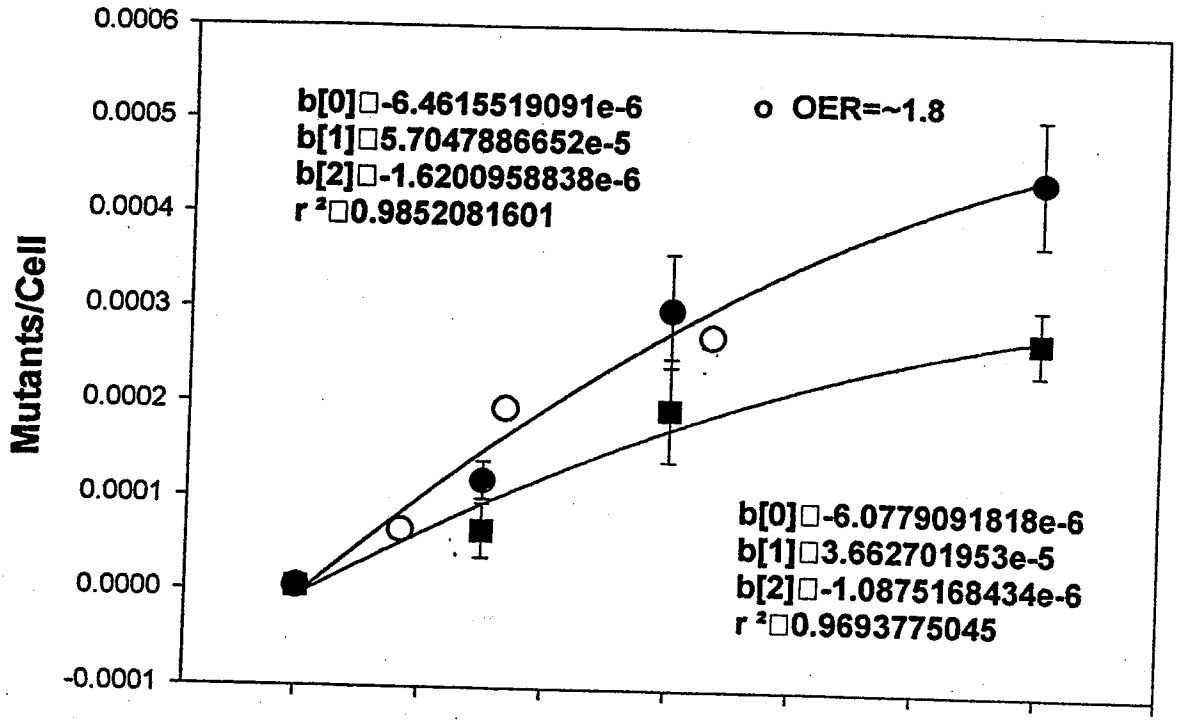
Explanation *(Attach separate sheet, if necessary.)*:

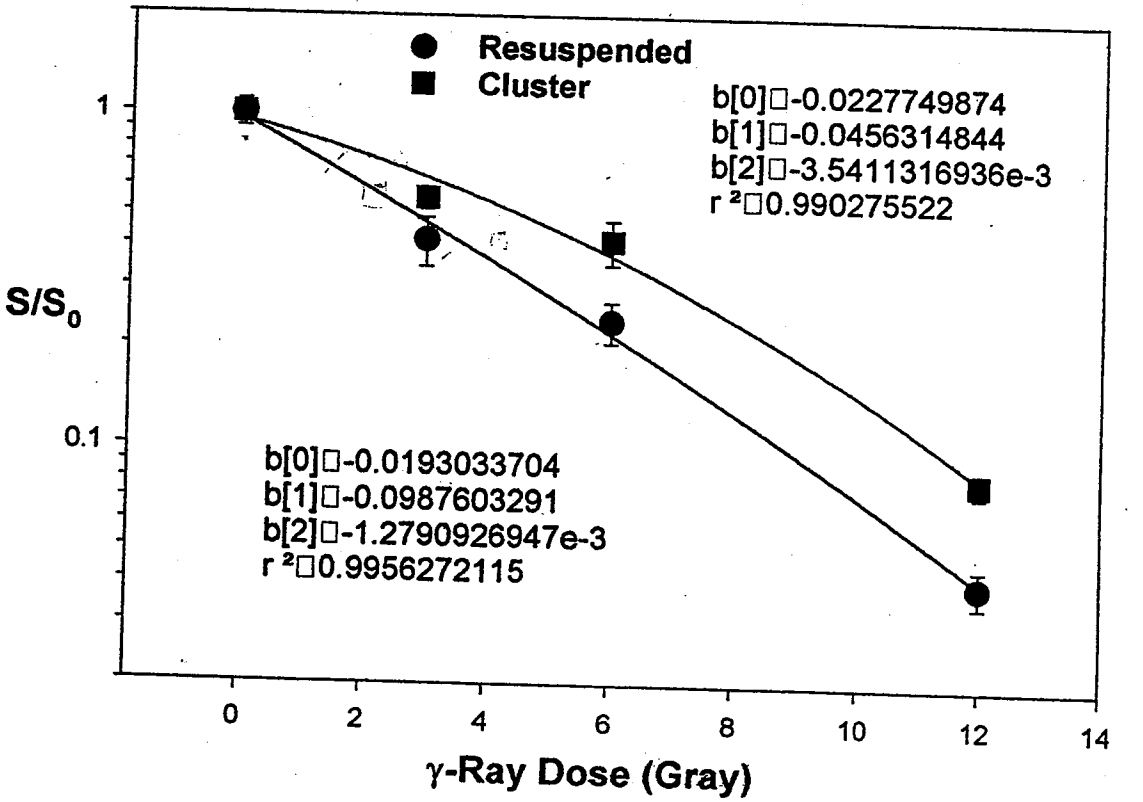
4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workplace and/or promote the nonuse of tobacco products or have plans to do so?

☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*

V79 HPRT Mutants/Cell
Hypoxic versus Aerobic Clusters



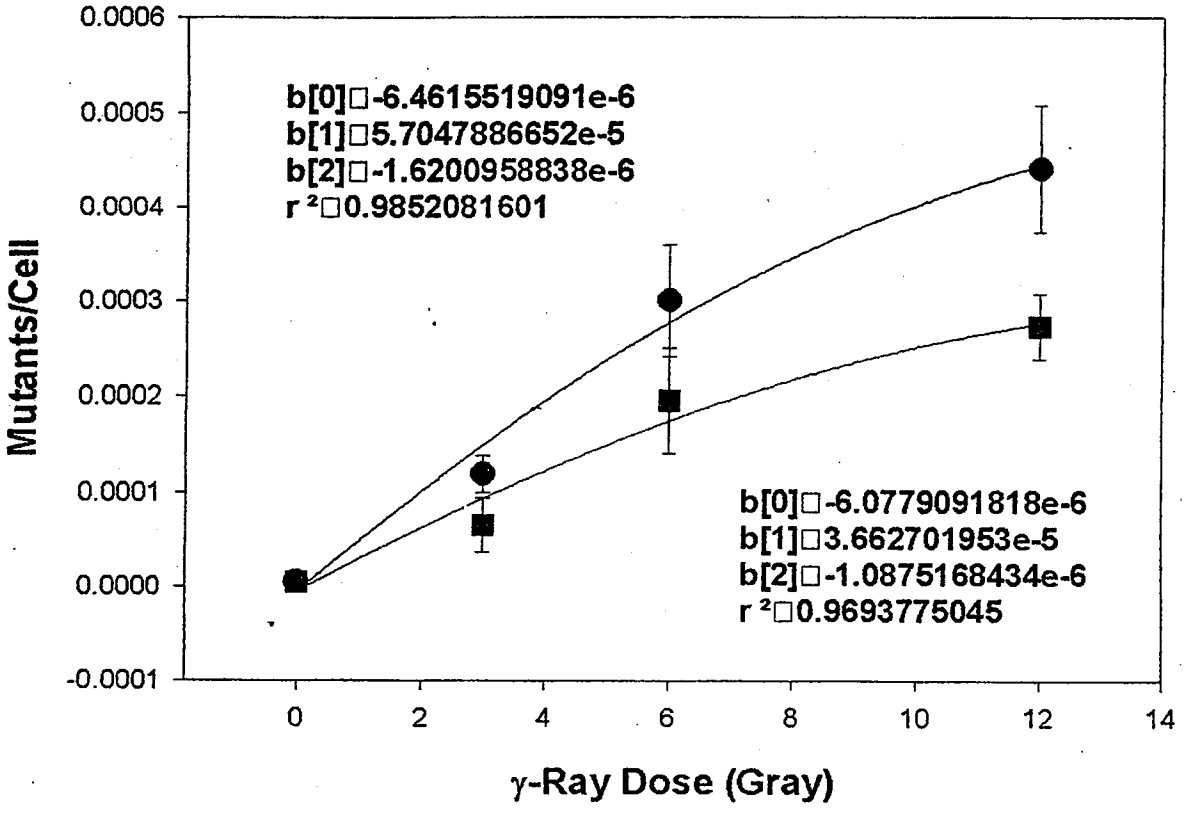


EXHIBIT

tabbies

5

V79 HPRT Mutants/Cell
Hypoxic versus Aerobic Clusters



	colonies			av col	std col	mutants					av muts	std mut	mut/cell	std mut/cell	Survival			PE	S/S0	StdS/S0
1	150	166	149	155	9.53939	1	2	1	1	1	0.8	0.632456	5.16129E-06	4.08036E-06	140	166	152	0.7	1	0.0752
2	137	129	152	139.33	11.6762	0	1	0	1	0					135	149	157			
3	121	137	145	134.33	12.2202	12	17	19	15	17	16	2.645751	0.000119107	1.96954E-05	52	63	73	0.3	0.4182	0.0701
4	152	130	119	133.67	16.8028	38	45	29	50	39	40.2	7.918333	0.000300748	5.92394E-05	36	41	31	0.2	0.2403	0.03337
5	117	125	139	127	11.1355	60	62	59	41	58	56	8.514693	0.000440945	6.70448E-05	5.3	6	6.8	0	0.0403	0.00501
6	165	147	155	155.67	9.0185	2	0	0	1	0	0.7	0.823273	4.49679E-06	5.28869E-06	121	115	139	0.7	1	0.09369
7	141	159	139	146.33	11.0151	2	1	1	0	0					137	152	144			
8	129	125	138	130.67	6.65833	11	6	5	7	14	8.6	3.781534	6.58163E-05	2.89403E-05	69	75	82	0.4	0.5594	0.04342
9	147	152	118	139	18.3576	29	31	19	37	20	27.2	7.628892	0.000195683	5.48841E-05	47	56	66	0.3	0.4183	0.06343
10	167	145	149	153.67	11.7189	36	39	47	40	48	42	5.244044	0.000273319	3.41261E-05	10	11	12	0.1	0.0817	0.00867

Date	Cell Doublings Counts			Vol(ml)		Doublings	Sample	Total Doublings
9/24/99	0 589	598	571	2	4688000			
	611	627	631		4984000			
	541	559	561		4429333			
	629	642	629		5066667			
	667	656	672		5320000			
	542	561	559		4432000			
	620	635	642		5058667			
	529	549	557		4360000			
	607	598	622		4872000			
	511	509	507		4072000			
9/29/99	411	431	435	6	1E+07	3.35276	1	11.12182
	471	461	459		1.1E+07	3.47612	2	10.91191
	389	362	372		8984000	3.16736	3	10.93538
	332	321	341		7952000	2.99132	4	10.62649
	441	458	465		1.1E+07	3.44573	5	11.37441
	432	444	456		1.1E+07	3.41359	6	11.02331
	409	422	436		1E+07	3.34142	7	10.95364
	381	392	401		9392000	3.23143	8	10.74782
	356	365	369		8720000	3.12433	9	10.93045
	403	372	385		9280000	3.21412	0	11.03198
10/1/99	772	761	756	6	1.8E+07	4.19472		
	666	655	677		1.6E+07	3.99856		
	701	711	722		1.7E+07	4.09356		
	656	631	634		1.5E+07	3.94186		
	732	745	739		1.8E+07	4.14796		
	741	756	762		1.8E+07	4.17568		
	635	659	662		1.6E+07	3.96791		
	672	657	659		1.6E+07	3.99132		
	713	732	742		1.7E+07	4.12895		
	699	710	729		1.7E+07	4.09626		
10/4/99	499	488	502	6	1.2E+07	3.57434		
	436	456	462		1.1E+07	3.43723		
	522	532	542		1.3E+07	3.67446		
	536	542	539		1.3E+07	3.69332		
	561	572	585		1.4E+07	3.78073		
	437	452	462		1.1E+07	3.43403		
	501	533	529		1.3E+07	3.64432		
	490	471	478		1.2E+07	3.52507		
	522	535	542		1.3E+07	3.67717		
	531	555	563		1.3E+07	3.72159		

V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (acute, cluster, suspension); **Exp. # : 2;**
Experiment performed by: A. Bishayee **Date:** 09/20/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB for each flusk, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~4,000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : cells/ml)
3. Transfer 1 ml of cell suspension into ten 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 16 h at 37°C, 5% CO_2 **Date/Time:** 09/20/99 ; 6-00 pm
5. After ~16 h incubation period, remove tubes, add 8 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (*precooled centrifuge*). **Date/Time:** 09/21/99; 9-30 a.m.
6. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
9. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
10. Centrifuge tubes for 5 min at 1000 rpm, 4°C
11. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/21/99; 11-00 a.m.
12. After 72 h, for tubes 1-5, carefully remove the supernatant, resuspend the pellet in 400 ml MEMA and place all tubes on the perforated plate of Rainin pipet tip box containing ice (to maintain ~10.5°C)

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), two tube (one tube for pellet and one for the suspension) at a time for a single dose-point, while placing onto a Rainin pipet tip box containing ice as per the Table below

Date/Time: 09/24/99;

Tube #	Total Dose (R)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	300	97.3	3.08	X-10
4	600	~~387.6 739.8~~	~~0.81 1.55~~	X-0
5	1200	~~387.6 739.8~~	~~1.62 3.09~~	X-0
6	0	0	0	0
7	0	0	0	0
8	300	97.3	3.08	X-10
9	600	~~387.6 739.8~~	~~0.81 1.55~~	X-0
10	1200	~~387.6 739.8~~	~~1.62 3.09~~	X-0

14. After irradiation, carefully remove the supernatant from the top for tubes 6-10, resuspend pellet in 200 ul wash MEMA and transfer the content from all tubes to ten 14 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
15. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
16. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)
17. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
18. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle

1. Determine cell concentration by transfering 100 µl to Coulter cup
2. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
3. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
4. Incubate petridishes for 1 week
5. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.05% crystal violet
6. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

mutation Assay

Date	Day	
24/99	0	Plate 10^6 cells from each tube in P100 & 10 ml MEM10
2/99	3	Plate 10^6 cells from each plate in P100 & 10ml MEM10
3/1/99	7	Plate 10^6 cells from each plate in P100 & 10 MEM10
3/4/99	10	9) count and plate 200,000 cells from each p100 to another P100 x5 & 10 ml MEM5 & 10 µM sGma
		11) Plate 200 cells in (Con) P60 x3 in MEM5
3/99	17	Colony count

09/24/99

Cells suspended in 2ml MEMA,
For counting, take 100µl in 2ml

589, 598, 571
601, 627, 631
541, 559, 561
629, 642, 629
667, 656, 672
542, 561, 559
620, 635, 642
529, 549, 557
607, 598, 622
511, 509, 507

Cells suspened in 6ml MEMA 09/27/99

0 411, 431, 435
0 471, 461, 459
3 389, 362, 372
6 332, 321, 341
12 441, 456, 465
0 432, 444, 456
0 409, 422, 436
3 381, 392, 401
6 356, 365, 369
12 403, 372, 385

10/01/99

Cells suspended in 6 ml MEM-A

772, 761, 756
666, 655, 677
701, 711, 722
656, 631, 634
732, 745, 739
741, 756, 762
635, 659, 662
672, 657, 659
713, 732, 742
699, 710, 729

10/04/99

Cells suspended in 6 ml of MEM-A

499, 488, 502
436, 456, 462
522, 532, 542
536, 542, 539
561, 572, 585
437, 452, 462
501, 533, 529
490, 471, 478
522, 535, 542
531, 555, 563

Plating Efficiency

10/11/99

200 cells were plated for each tube

Tube #	# of colonies	Avg # of colonies
1	150, 166, 149	147.16
2	137, 129, 152	
3	121, 137, 145	134.33
4	152, 130, 119	133.66
5	117, 125, 139	127
6	165, 147, 155	151
7	141, 159, 139	
8	129, 125, 138	130.6
9	147, 152, 118	139.0
10	167, 145, 149	153.6

Mutant colonies

200,000 cells were plated for each tube

tube #	# of colonies	Avg. # of colonies	Mutants/cell
1	1, 2, 1, 1, 1	0.8	0.00000544
2	0, 1, 0, 1, 0		
3	12, 17, 19, 15, 17	16	0.000119
4	38, 45, 29, 50, 39	40.2	0.000300
5	60, 62, 59, 41, 58	54	0.000425
6	2, 0, 0, 1, 0	0.7	0.0000046
7	2, 1, 1, 0, 0		
8	11, 6, 5, 7, 14	8.6	0.000065
9	29, 31, 19, 37, 20	27.2	0.000195
10	36, 39, 47, 40, 48	42	0.000273

10/23/99

I write this because I suspect that Dr. Anupam Bishayee, a Post-Doc working for Dr. Roger Howell, is fudging data and I do not believe that Roger is taking what I have told him seriously.

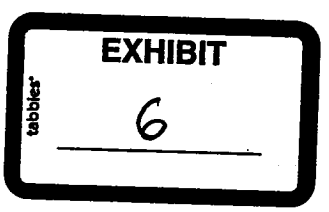
Monday, October 11, 1999: Anupam had started an experiment involving mutagenesis which would end today. If there had been no contamination, there should be 50 P100's ready to stain. I ask him during the day if he will stain them today and he replies that he is busy with other things but that he will stain them later. When I leave around 6 o'clock, I stop in to see if they have been stained and he says that they have not but they he will work late and do them later.

Tuesday, October 12, 1999: When I come in this morning, Anupam has not arrived. I go into the lab to look for the plates. There is no sign of them around the lab or in the trash. I look in the incubator and there is a tray containing 10 stacks of 5 p100's each and marked consecutively from 1 to 10. This is the expected numbering that would result from the experiment. I look at several plates by I-ball and under the microscope. I do not see any colonies. I know from my experience that cells that are not going to form colonies will lyse on about day 4 after plating leaving nuclei – this should be day 7. There are very few nuclei. I worry that these are the plates from the experiment and that Anupam miscounted resulting in too low cell numbers to get colonies. I also figure that he decided not to fix and stain them the night before because it had gotten too late. An extra day would not make any difference. He may also have become aware that they didn't have many, if any, colonies on them When he comes in, I ask him how the experiment went and he says it went very well and he will give me the data later. I ask him what the plates are that are in the incubator and he replies that that is another experiment that he is working on. I know that the other experiments that he does do not utilize the p100's, they use the p60's. In fact, I have supplied the p100's for these experiments. He gives me the data later on and it is as predicted: the data for cells placed in aerobic conditions indicate more mutants than data for cells placed in hypoxic conditions. During the course of the experiment, cells must be plated and replated and the numbers must be carefully recorded in order to calculate the number of cell divisions the cells have undergone before they are challenged with the selection agent 6-TG. I ask Anupam for these data and he tells me he has them and will give them to me later.

Wednesday, October 13, 1999: Again I come in earlier than Anupam. I go to the incubator only to find the mysterious set of 50 p100's is no longer there. Again I search through the trash and around the lab and there is no sign of the plates. Anupam does not give me the missing data.

Tuesday, October 19,1999: Roger has been sick but is now back at work. I relate to him the above events and tell him of my suspicions. He agrees that things sound pretty fishy. He says that he will ask Anupam for the missing data referred to above. I hear him do this later and I hear Anupam reply that he cannot lay his hands on the data because they are at home. Roger scolds him for taking data home.

From now on, I check when I can as to what is going on in Anupam's incubator. There is a set of p60's marked appropriately for the sorts of survival experiments that Anupam does. I don't see anything on these plates but they may be newly plated.



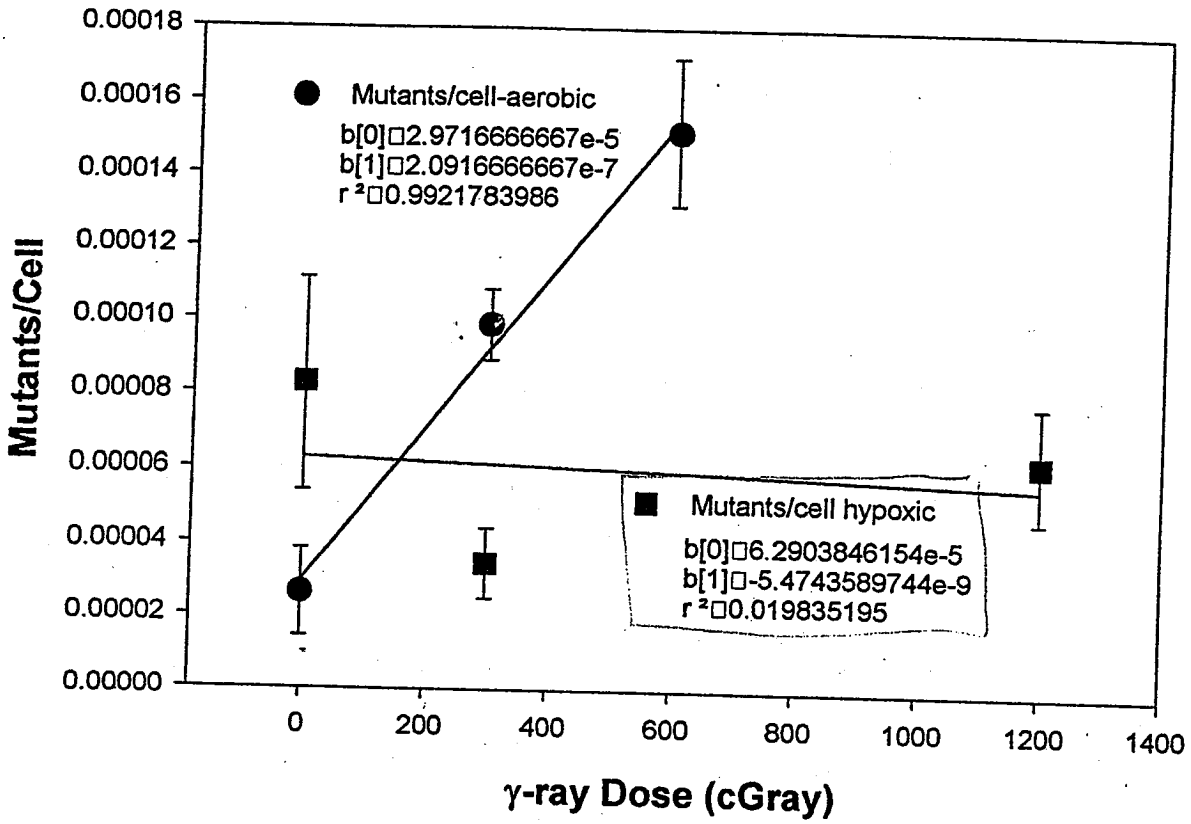
Thursday, October 21, 1999: I discuss the situation with Roger and he poo poos me saying that I never really did like Anupam in the first place. This is not true. I don't dislike him and I don't have it in for him. I do not know if Roger ever got the missing data from Anupam. Roger is pleased with Anupam's work because he faithfully turns out data regularly. He also thinks that Anupam is not smart enough to make up data.

I decide that, as much as possible, I will distance myself from Anupam's work. Until there is a logical explanation for the mysterious set of 50 p100's that should not have been in the incubator, I believe that Anupam made up the data for the mutagenesis arm of the experiment and I do not want to be associated with him.

Friday, October 22, 1999: Roger is analyzing some old data and finds that the RBE for ^{3}H is at least three times greater than it should be. He will repeat these experiments himself to determine whether the data are real or not.

Saturday, October 23, 1999: I look in the incubator for the above mentioned p60's. They are gone. I check the trash. There are plates in the trash that still have pink medium in them which looks a bit cloudy and may be contaminated.

V79 HPRT Mutants/Cell
Hypoxic versus Aeobic Clusters



9/28/99

EXHIBIT

7

V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (acute, cluster, suspension); **Exp. # :** 1;
Experiment performed by: A. Bishayee **Date:** 09/06/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB for each flusk, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~4,000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 3,997,333 cells/ml cells/ml)
3. Transfer 1 ml of cell suspension into ten 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 16 h at 37°C, 5% CO_2 **Date/Time:** 09/06/99; 4-00 p.m.
5. After ~16 h incubation period, remove tubes, add 8 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (*precooled centrifuge*). **Date/Time:** 09/07/99; 10-00 a.m
6. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA,transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
9. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
10. Centrifuge tubes for 5 min at 1000 rpm, 4°C
11. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/07/99; 12-00 noon
9/10 12. After 72 h, for tubes 1-5, carefully remove the supernatant, resuspend the pellet in 400 ~~ml~~ µl MEMA and place all tubes on the perforated plate of Rainin pipet tip box containing ice (to maintain ~ 10.5°C)

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), two tube (one tube for pellet and one for the suspension) at a time for a single dose-point, while placing onto a Rainin pipet tip box containing ice as per the Table below

Tube #	Total Dose (R)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	300	97.3	3.08	X-10
4	600	739.8	0.81	X-0
5	1200	739.8	1.62	X-0
6	0	0	0	0
7	0	0	0	0
8	300	97.3	3.08	X-10
9	600	739.8	0.81	X-0
10	1200	739.8	1.62	X-0

Rinsate

⇒ left in pellet

14. After irradiation, carefully remove the supernatant from the top for tubes 6-10, resuspend pellet in 200 ul wash MEMA and transfer the content from all tubes to ten 14 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
15. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
16. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (*precooled centrifuge*)
17. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
18. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle

21. Determine cell concentration by transfering 100 µl to Coulter cup
22. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
23. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
24. Incubate petridishes for 1 week
25. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.05% crystal violet
26. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

9/10/99

	Dose(Gy)		9/13/99 d3 hemo 70x5= 350	0.5ml Coulter		Av	cells/ml x400	10^6	Rel incr.	x4 Total/P100	#doublings
zeusp.	0	1		8282	8815						
				5987	8221	7701	3.08×10^6/ml	0.32		1.2×10^7	3.6
	0	2		7355					1.00		
				7404		7380	2.95	0.34		1.2	3.6
	3	3		7962							
				7852		7907	3.16	0.32	1.08	1.3	3.6
	6	4		5330							
				5520		5525	2.21	0.45	0.73	0.88	3.1
	~~12~~	~~5~~		5541							
				5494		5518	2.21	0.45	0.73	0.88	3.1
to forum	0	6		8409							
				8165		8287	3.31	0.30		1.3	3.6
	0	7		8760					1.00		
				8755		8758	3.50	0,29		1.4	3.8
	3	8		7748	7688				0.81		
				(5395)	7726	7139	2.86	0.35		1.1	3.5
	~~6~~	~~9~~		5734	5871			0.42	0.71	0.96	3.3
				5987	6426	6004	2.40				
	12	10	67x5= 335	6484	6474						
				6240	6203	5850	2.34	0.43	0.70	0.94	3.2
	Bk		-	~~3539~~ 2273							

9/15 #s 1 & 9 are contaminated. (5)

9/16 #s 5 & 6 " "

9/17 switched to Gibco MEM + 5% FBS (Gem 1567A)

											Total dou
1/17	2	3327 5110	4678 3497	6607	4644 ± 1334	1.86×10^6/ml	0.54	x4	7.4×10^6	2.9	6.5
d7	3	8254 4838	8354 4756	4875 3112	5698 ± 2125	2.28	0.44		9.12	3.2	6.8
	4	7797 4053	6087 4060	5858 7204	5843 ± 1556	2.34	0.43		9.36	3.2	6.3
	7	3752 5577	4007 5504	4013 5640	4748 ± 909	1.90	0.53		7.6	2.9	6.7
	8	8598 4435	4575 6666	4653 6113	5840 ± 1633	2.34	0.43		9.36	3.2	6.7
	10	9784 4144	6314 8415	4755 6735	6358 ± 1968	2.54	0.39		10.16	3.3	6.5
	Bk	11 3 11 6									

9/20 d10 Harvest, dilute $\frac{1}{10}$ ($\frac{0.1}{1.0ml}$ isoton) count by hemocytometer

		#10/4		x 4ml	#doublings	Total doublings
	2	221/4 235/4 (24) 139/4	5.7×10^6/ml	2.3×10^7 cells/P100	4.5	11
	3	144/4	3.5×10^6	1.4×10^7 "	3.8	10.6
	4	121/4 109/4	2.9×10^6	1.2×10^7 "	3.6	9.9
	7	179/4 123/4	4.5×10^6	1.8×10^7 "	4.2	10.9
175/4 196/4	8	214/4 261/4	4.6×10^6	1.8×10^7 "	4.2	10.9
	10	175/4 181/4	4.5×10^6	1.8×10^7 "	4.2	10.9

9/20/99
d10

2	5.7	$\times 10^6$	0.035 →	2×10^5 / 1450	$\times$ 5		
3	3.5		0.057 →				
4	2.9		0.069 →				
7	4.5		0.044 →	0.69			
8	4.6		0.043 →				
10	4.5		0.044 →				

2	5.7	$\times 10^6$	0.0088 / 1 →	5×10^4	0.0046 / 7 →	33	2ml → 66 | P35 X3
3	3.5	$\times 10^6$	0.014 / 1 →		0.0046 / 7 →	33	
4	2.9	$\times 10^6$	0.017 / 1 →		→		
7	4.5	$\times 10^6$	0.011 / 1 →		→		
8	4.6	$\times 10^6$	0.0109 / 1 →		→		
10	4.5	$\times 10^6$	0.011 / 1 →		→		

GTG 2.5mg FW 167.2 Add 25ml MEMS to vial = 100µg/ml

100µg/ml → 5.4ml / 329ml MEMS → 167µg/ml = 10µM

RM 3616
U. S. A.

Plate counts 9/27/99 = day 17

Sample	P35's			Av	std	P100's					Av	std	percell/pl
2	80	74	70	74.7	5.03	3	4	7	10	6	6.0	2.74	2.65E-5 ±
3	65	53	64	60.7	6.66	17	18	20	16	20	18.2	1.79	9.89E-5 ±
4	64	56	65	61.7	4.93	28	35	25	27	27	28.4	3.85	15.2E-5
7	64	67	53	61.3	7.37	11	16	24	15	11	15.4	5.34	8.29E-5
8	74	86	69	76.3	8.74	5	9	8	7	11	8.0	2.24	3.46E-5
10	65	65	65	65.0	0	10	11	10	14	17	12.4	3.05	6.30E-5
		o/all	av	66.61	8.40								

UMDNJ
NEW JERSEY
MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

185 South Orange Avenue, Newark, NJ 07103-2714 • Phone: (973) 972-3421 • Fax: (973) 972-5592

MEMO

Date: 5/22/01

To: Elizabeth Raveché, Ph.D., Professor of Pathology, Chairman, Committee on Scientific Integrity

From: Helene Z. Hill, Ph.D., Professor of Radiology

Re: Experiment performed in September-October, 1999

Please refer to Experiment records delivered to your office on April 16, 2001.

The experiment dated 9/20/99 was performed by Dr. Bishayee. At step 22 (presumably), i.e., after counting the cells, he plated, according to his hand-written notes, 10^6 cells from each sample onto a 100 mm tissue culture dish (p100). He also plated for survival (steps 22-26). In the survival arm – the lower graph -- the cells in suspension (samples 1-5, filled circles) showed a decrease in survival to about 0.04 at 12 Gray (sample # 5) and the cells in clusters (samples 6-10, filled squares) to about 0.08 at 12 Gray (sample #10) (see spread sheet).

The data that now need to be considered are the Coulter counts for the mutation arm on day 3 after plating in p100's (9/27). NB the date 9/29 in the spread sheet is wrong and should read 9/27 – if 9/24 is day 0, 9/29 cannot be day 3).

Consider as follows:

Day 0 (9/24): all samples plated at 10^6 cells/p100
Day 3: See spread sheet 9/29 (should be 9/27) all samples have about the same number of doublings

Is this possible?

Take sample 5: the surviving fraction – from the survival arm of the experiment -- in sample 5 is 0.04. 10^6 cells were plated on day 0. If they had not been irradiated, there would have been about 3 doublings (see samples 1,2,6 and 7). Since they were irradiated, only 4×10^4 cells can replicate ($0.04 \times 10^6 = 4 \times 10^4$). Assume that they replicate at the same rate as the controls, they would reach about 3×10^5 on day 3. Assume further that the remaining 9.6×10^5 cells (from that first 10^6) do not lyse and will remain in the dish to be harvested and counted on day 3*. Adding these to the 3×10^5 that replicated, gives a total of 1.26×10^6 to be harvested on day 3 from

EXHIBIT
tabbies
8

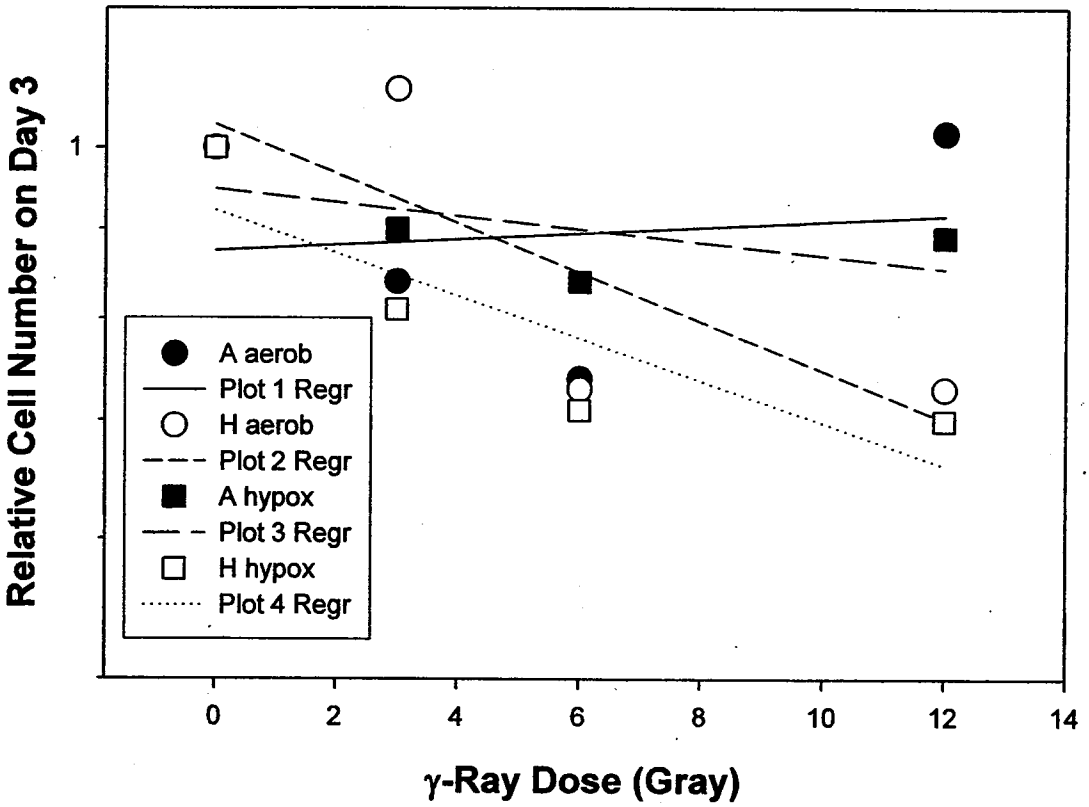
sample 5, yet there were 1.1×10^7 cells as calculated from the recorded data, nearly 10 times the expected. Applying the same calculations to sample 10, the total cells possible on that dish would be 1.5×10^6 on day 3, yet there were 9.3×10^6 about 6 times more than expected. Samples 1,2 and 6,7 are 0 dose. Samples 3, 4, 8 and 9 have progressively fewer survivors according to the survival arm of the experiment and should therefore show a trend to smaller numbers on the p100's on day 3. There is no such trend.

The experiment started on 9/6/99 followed the same protocol. The survival arm of this experiment was done by Dr. Bishayee. He passed 10 p100's to me on 9/10. The number of cells plated is not recorded but it would be in the neighborhood of 10^6 each. Although there was a fair amount of contamination in this experiment, the counts on day 3 do show a decreasing trend from samples 1 and 2 (0 dose) to 5 (12 Gray) and from samples 6 and 7 (zero dose) to 10 (12 Gray).

* Ionizing radiation kills replicative ability of cells, but not their function. As a result, cells that have been lethally irradiated can remain in the dishes for many days after irradiation.

I am well aware of the difficulty in establishing that someone has knowingly performed an experiment incorrectly. However, my earlier notes from October, 1999 indicate my suspicion regarding the experiment that was started on 9/6/99. I would now like to point out that I have noted additional discrepancies with the data from that experiment.

Cell Count as a Function of Dose on Day 3



EXHIBIT

9

Accountability in Research, 9: 75–92, 2002
Copyright © 2002, Taylor & Francis
0898-9621/02 $12.00 +.00
DOI: 10.1080/08989620290009530

Terminal Digits and the Examination of Questioned Data

James E. Mosimann
ABL Associates, Inc.
Rockville, Maryland, USA

John E. Dahlberg, Nancy M. Davidian, and John W. Krueger
Office of Research Integrity,
Department of Health and Human Services,
Rockville, Maryland, USA

Our objective is to illustrate the use of statistical methods to examine the authenticity of data in the investigation of research misconduct. We present examples of statistical analyses of questioned data from several cases that illustrate the experience of the Office of Research Integrity. We show that the statistical examination of numbers that are normally unrepeatable when experiments are repeated, or otherwise are of inconsequential meaning, may reveal substantial clues as to the authenticity of questioned data when compared with numbers in data that are unquestioned. We illustrate the occurrence of the uniform distribution of nonleading (insignificant rightmost) digits in unquestioned numbers, along with examples of deviation from such uniformity for fabricated or falsified numbers. (Most people are unable to choose digits randomly.) We describe several cases in which a variety of anomalies in data sets provided the impetus for the examination of rightmost digits. The anomalous behavior of rightmost digits, when added to testimony and other physical evidence, can greatly enhance or decrease the credibility of witnesses. The cases discussed involve: 1 and 2, Anomalous behavior of terminal digits in published or recorded numbers; 3, Terminal odd digits in event times that should have exhibited only even digits (and why); and 4, Data that were falsified by calculations from computer spreadsheets (detected by the inclusion of an additional digit of accuracy).

Paper presented in November 2000 at the First ORI Research Conference on Research Integrity. The paper will appear in *Investigating Research Integrity: Proceedings of the First ORI Research Conference on Research Integrity, November 2000*, edited by Nicholas H. Steneck and Mary D. Scheetz.

Address correspondence to James E. Mosimann, ABL Associates, Inc., 5 Balmoral Court, Rockville, MD 20850. E-mail: jemosimann@erols.com

EXHIBIT
tabbies
10

KEY WORDS: scientific misconduct, statistical forensics, terminal digits, uniform digits

Allegations of research misconduct[1] often are of the form that a particular experiment was not done as described, or not done at all. In considering such allegations it is often necessary to examine "questioned" data. Such data can establish that the experiment was performed as described. However, if the allegation is true, then these questioned data are necessarily falsified or fabricated.

A useful way to assess questioned data is to examine inconsequential components of data sets that are not directly related to the scientific conclusions of the purported experiment. Thus if the allegation is true and the data are falsified, the falsifier typically devotes attention to numbers that establish the desired scientific outcome. Properties of the numbers that are not directly related to the desired outcome are less likely to receive consideration by the falsifier.

The same principle of examining details inconsequential to the scientific outcome appears valid whether the data are expressed in non-numeric form (images, written descriptions) or as numbers. Here we consider several cases where the data are numeric and lend themselves to immediate statistical description. In all these cases we stress the importance of comparing "questioned" data with similar unquestioned data from the same laboratory or individuals.

RIGHTMOST DIGITS

Consider counts of radioactivity for a biological preparation, for example, 5071. In a recount of the sample, or in a replication of the assay, it is highly unlikely that the rightmost digits will be the same. Thus with two repetitions of the experimental procedure, instead of 5071, one might obtain respectively, 5109 and 4966.

The rightmost, nonleading digits of these three numbers are not the same. Thus _071 differs from _109, and in turn both differ from _966.

Digits are often recorded well beyond the repeatability of the experimental procedure. For such rightmost digits, theoretically[2] there is a tendency to be uniformly distributed as expected in a lottery. For example, a uniform distribution of digits is expected in the Maryland Lottery. Figure 1 shows the frequencies of the digits 0 to 9 found in 5,106 winning Pick-3 numbers (of 3 digits each) for the past ten years.[3] This distribution is not significantly different from uniform. All digits have occurred with nearly the same frequency, as they should in a lottery.

[1] 65 Federal Register 76260, December 6, 2000.

[2] A theoretical discussion is found in J. E. Mosimann and M. V. Ratnaparkhi, "Uniform occurrence of digits for folded and mixture distributions on finite intervals," *Communications in Statistics*, 1996, 25(2), pp. 481–506. Among other issues, this paper discusses approximations to continuous distributions by histogram distributions for which the uniformity of terminal digits up to a specified place is known. Such theoretical issues are important, but our emphasis here is on direct comparison of questioned data with unquestioned data.

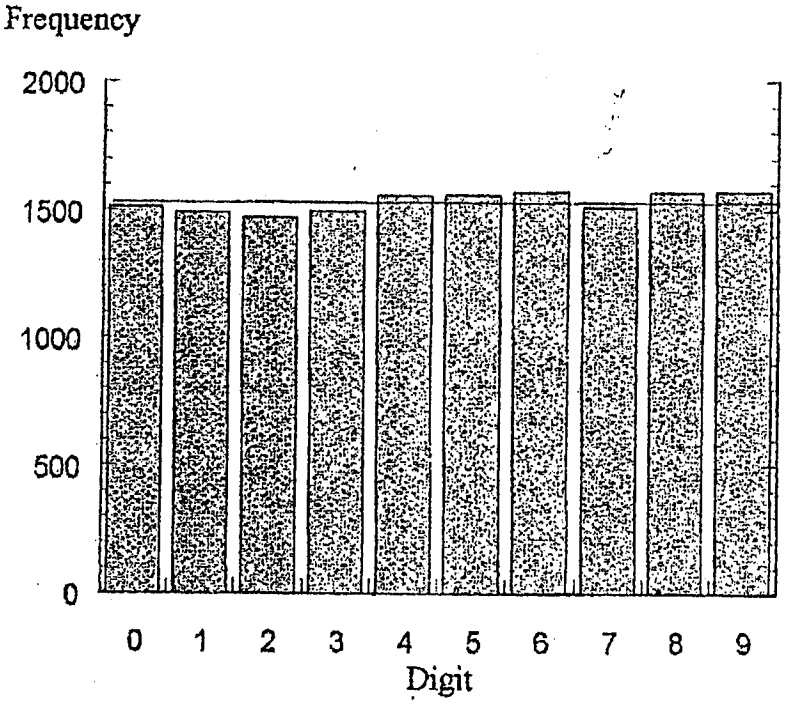


FIGURE 1. Ten Years of Maryland Lottery Pick Three Digits, January 2, 1990 to December 31, 1999; 15,318 Digits

Case 1: Uniformly Distributed Rightmost Digits in Scintillation Counts

In the first case, experimental measurements were known not to have been done because radioactive spots on the experimental sheets had not been excised and hence could not have been counted in the scintillation

[3] On May 1, 1995, the Maryland Lottery initiated a midday pick-3 drawing for weekdays. This is in addition to the nightly drawing. Thus, there are more than 3,650 winning pick-3 numbers over the ten-year period. Maryland Lottery results may be found at the official Web site, *http://www.mdlottery.com*.

TABLE 1: Illustrative falsified and unquestioned counts from the respondent's laboratory notebook.

Falsified counts (Notebook page 145)		Unquestioned counts supported by counter printouts (Notebook page 135)	
Numerator	**Denominator**	**Numerator**	**Denominator**
1078	251183	82267	170679
1770	217763	105584	190994
1091	225853	87592	181133
1434	238995	83341	197822
1247	241139	88426	172062
1131	260074	105068	194570
54350	220938	90707	150614

Numerator (summation of reaction produced counts) and denominator (residual substrate count) are associated with a given clone, and activity is expressed by the ratio, numerator divided by denominator. Note that the 28 counts illustrated each contain from four to six digits.[4]

counter. Yet the respondent's notebook contained (falsified) handwritten counts for that experiment. In this case, faced with the evidence, the respondent admitted to the falsification of the numbers in the notebook.

In addition to the questioned counts, the notebook contained handwritten counts that were supported by counter output, and thus not falsified. Both questioned and unquestioned numbers occur in pairs (a numerator and denominator) and have large numbers of digits. See Table 1.

The following procedure was used to find digits. The rightmost digit of a number was designated as occupying "Place 1," then the digit to its left occupied "Place 2," etc. Digits were examined in four places for each number, except that the leftmost digit was never included in the analysis. Thus by way of example, the following underlined digits would be included in the analysis: 1078, 251183, 735, 62034. It is clear that a three-digit number contributes two digits for analysis and a four-digit number, three digits. Numbers of five or more digits contribute four digits.

Chi-square tests for the uniformity of digit distributions from 252

[4]In all there are 474 counts: 252 admittedly falsified (notebook pages; 141–152); and 222 unquestioned counts that are supported by counter printouts (notebook pages; 104–106,130–131, 134–135). Each count, falsified or unquestioned, contains from three to six digits. Digits were tested in four places, but no digit that was itself the leftmost digit was included in the analysis. Total analyses included 939 digits from 252 falsified numbers and 857 digits from 222 unquestioned numbers.

TABLE 2: Chi-square results for tests of uniformity of digit frequencies from falsified and unquestioned counts.

Chi-square results for falsified and unquestioned counts					
Digits from 252 falsified counts					
	Place 4	**Place 3**	**Place 2**	**Place 1**	**Total**
Number	185	250	252	252	939
Chi-square	34.8	29.3	13.2	27.1	30.94
D. Freedom	9	9	9	9	9
Probability	.00006	.00058	.1521	.0013	.0003
Digits from 222 unquestioned counts					
	Place 4	**Place 3**	**Place 2**	**Place 1**	**Total**
Number	195	218	222	222	857
Chi-square	14.3	9.89	8.72	11.33	11.09
D. Freedom	9	9	9	9	9
Probability	.11	.36	.46	.25	.270

The rightmost place is "Place 1"; the next place to left is "Place 2" etc. (Leftmost digits of numbers were excluded, so there are fewer "Place 4" digits than "Place 3," etc.)

falsified counts from notebook pages 141–152 are presented in Table 2. The distributions are not uniform. Three of the four chi-square values have probabilities less than .05, and when digits from all four places are grouped together, the total distribution is far from uniform (chi-square = 30.94, df = 9, p = .0003).

Chi-square tests for uniformity of the digit distributions from 222 unquestioned counts also are presented in Table 2. The distributions are not significantly different from uniform. All of the four chi-square values have probabilities greater than .05, and when digits from all four places are grouped together, the total distribution is not significantly different from uniform (chi-square = 11.09, df = 9, p = .27).

The unquestioned counts have uniform or nearly uniform rightmost digits, whereas the falsified counts do not.[5]

[5]See "Data fabrication: Can people generate random digits?" J. E. Mosimann, C. V. Wiseman, and R. E. Edelman, *Accountability in Research*, 4, 31–55, 1995. This study shows that many people have difficulty fabricating random digits, even when trying to do so.

TABLE 3: Published Table (Column 5 has questioned data.)

Column 1		Column 2		Column 3		Column 4		Column 5	
Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
17697	1739	17399	1680	15085	1342	18262	2934	27191	1404
20164	3540	16746	1171	19397	1133	17889	3919	26999	7107
23323	3861	24154	722	19094	1340	28763	3373	28611	967
24474	4042	18918	4459	14224	828	24596	6327	29152	1407
29711	1519	21855	8458	23840	1695	29669	3222	28765	7104
24752	1455	22498	4591	21639	1347	32825	3063	70714	2106
32683	8535	26321	2753	20015	2020	34030	3917	68177	7155
43411	4682	41980	1705	34026	3906	47703	1894	66004	3924
26535	2349	41592	5699	31262	2796	54588	5065	74316	2192
33216	3762	37036	2071	27513	5062	32033	8307	71117	6817

TABLE 4: Chi-square tests of rightmost digits for, respectively, Columns 1 to 5 of the Published Table, and for Columns 1–4, together.

Tests of Uniformity of Digits for the Columns of the Published Table						
	Column 1	Column 2	Column 3	Column 4	Column 5	Columns 1–4
Number	70	69	69	70	69	278
Chi-square	8.57	5.93	8.54	9.14	26.22	4.45
D. freedom	9	9	9	9	9	9
Probability	0.478	0.747	0.481	0.424	0.0019	0.880

Case 2: Unlikely Patterns in Rightmost Digits

In this case, we again demonstrate the ability of uniformly distributed digits to distinguish questioned from unquestioned data. However, the digit analyses lead further to the identification of unlikely patterns in numbers that should not be related, given the purported experiment.

Table 3 reproduces the means and standard deviations from a published table that was challenged by a co-worker. Lipopolysaccharide (LPS) extracts were purified from endotoxin from various bacteria. The five rows in each half represent, respectively, different bacterial sources for the endotoxin. LPS was added at various concentrations to the cell cultures. Thus the five columns of the table represent different levels of LPS (left to right, respectively: 5,000, 500, 50, 5, and .5 ng/ml). The upper half of Table 3 represents cultures to which endotoxin and stimulator cells were added at the same time. The lower half represents cultures to which endotoxin was added 24 hours prior to the addition of stimulator cells. However, while supporting notebook data could be found for the first four columns, no supporting notebook data could be found for the questioned numbers in column 5.

Of statistical importance is the fact that means and standard devia-

TABLE 5: Vertical Pattern of Digits

Place 4	Place 3	Place 2	Place 1
1	4	0	4
7	1	0	7
	9	6	7
1	4	0	7
7	1	0	4

TABLE 6: Similar Rightmost Digits in Three Publications

Journal 1 Trauma patients		Journal 2 Cancer Patients		Book Trauma Patients	
26428	406	6428	406	116428	3406
7824	376	7824	376	17824	3761
24840	1107	24840	1107	124840	7107
26660	345	6501	355	116660	34511
7791	407	7906	348	17791	407
9276	1498	12016	1476	9276	1498

tions in this table are reported to several places. Thus numbers are recorded with greater precision than the repeatability that the biological experiment allows, permitting a digit analysis.

The treatment of rightmost digits is the same as that for the previous case. Digits are analyzed in four places with no leftmost digit included in the analysis. See Table 4.

Only the digits of the questioned column 5 are significantly different from uniform ($p = .0019$). Columns 1 to 4 separately are not different from uniform (the probability ranges from .424 to .747). In the aggregate, columns 1 to 4 are again not different from uniform ($p = .88$).

Based on the contrast between the digit distributions for the questioned column 5 and the unquestioned columns, the complainant's assertion that the experiment for column 5 was not done is strengthened.

Furthermore, examination of the standard deviations in the upper half of column 5 of Table 3 reveals a remarkable "vertical" pattern. These numbers should be statistically independent from row to row. However moving vertically downward at each digit place reveals a symmetrical recurrence of digits: 1,7, blank, 1, 7; 4, 1, 9, 4, 1; then 0, 0, 6, 0, 0; and finally, 4, 7, 7, 7, 4. (See Table 5.)

The vertical pattern does not appear consistent with the five presumably statistically independent experiments depicted by the separate rows of Table 3. Such a pattern is consistent with the formation of the numbers after the outline of the published table had been established.

Finally, to check for the possible existence of a pattern, three publications by the respondent (two journal articles and a book chapter) were examined. Examination of these publications reveals patterns of digits that are inconsistent with biological observations. Consider Table 6, which contains numbers from tables in three different publications by the author, all for a similar experimental procedure.

In these three publications, rightmost digits that should not be reproducible are the same in the first and third rows, and they would be the same in the second row except for the arbitrary addition of a "1" after the "376" in the last column. Further, in the fifth row two of the standard deviations are "407" while the corresponding means are "7791" and "17791." Note that the standard deviation 7107 occurs in the book chapter and also in column 5 of the published table already discussed.

The respondent in this case agreed that the data were "flawed" and retracted the relevant articles.

Case 3: Banker's Rounding and "Odd" Terminal Digits

For the purposes of a genetic study, electro-physiological measurements of spontaneous "firings" (action potential spikes) of isolated muscle fibers were made. Initially, a firing was determined to occur whenever a peak on the recording of current equaled or exceeded 10 picoAmps. Since the spontaneous "firings" were infrequent, the continuous record of the electrical signal was not retained. Instead, an "event detection" device sent the time and the amplitude of the firing to Excel spreadsheets as a permanent record of the experiment.

To graph the activity of muscles from different genetic crosses, the firings of various amplitudes were accumulated into bins of 5-picoAmp width (10–15, 15–20, 20–25, etc.), with accumulation continuing until some bin contained 100 firings.[6] The resulting frequency distribution represented the pattern of firings (for Experiment 1, see Figure 2, in which there are just over 100 events in the 20–25 bin).

Prior to publication, the respondent's co-workers thought that firings should only be defined as those peaks 20 picoAmps or greater. Thus they asked the respondent to prepare a new graph like that of Figure 2, but sampling only peaks 20 picoAmps or greater (i.e., resampling the Excel spreadsheet until some bin contained 100 such firings).

The respondent submitted a new frequency graph that appeared like the first, but truncated at 20 rather than 10. Since one would expect the shape of the new graph (above 20 picoAmps) to differ, the co-workers questioned the result.

The respondent asserted that the new graph was not simply a truncated version of the first, but represented a fresh sampling of peaks greater than 20 picoAmps. He asserted that he had properly sampled the peaks in an Excel spreadsheet by counting beyond the initial 321 records on which the first graph (Figure 2) was based. The respondent furnished an

[6] "Inverse" sampling until a certain number of a particular event occurs has a long history, particularly where rare events are to be studied. (For example, see J. E. Mosimann, "On the compound negative multinomial distribution and correlations among inversely sampled pollen counts," 1963, *Biometrika, 50,* 47–54).

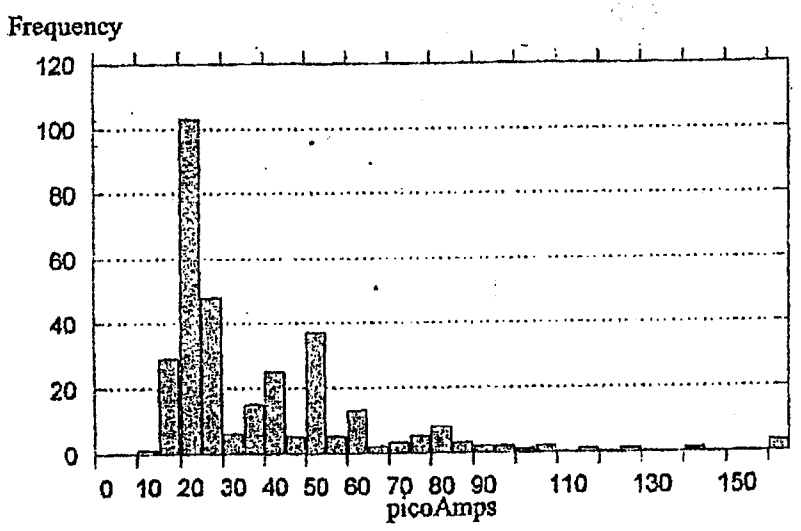


FIGURE 2. Binning of amplitudes into bins of 5-picoAmp width. (Initial 321 records of Experiment 1)

Excel worksheet, "Experiment 1," of 551 records in support of the new graph. This worksheet contained the initial 321 records along with 230 additional records.

In addition to the Excel worksheet for Experiment 1, the respondent also provided a worksheet of unquestioned data "Experiment 2" with 1026 records.

For Experiment 1 and the 10 picoAmp peaks, the initial 321 records of Experiment 1 are largely determined since the initial Figure 2 is known. Thus the last 230 records of Experiment 1 are more questionable. Since all 551 records were provided after the allegation, the opportunity existed to falsify or fabricate time points, but if falsifications occur, most would be expected in the last 230 records.

Table 7, presents the first 12 records of Experiment 1. It is interesting to note that all of the time values in Table 7 terminate in an even digit. The occurrence of only even time values can be explained by a long-used[7] practice sometimes known as "Banker's Rounding.[8]"

A simple explanation of the even terminal digits for time values is

[7]"It is conventional to round off to the nearest even digit when the number to be rounded is exactly half way between two successive digits." pp. 13–14, Paul S. Dwyer, *Linear Computations*, 1951, John Wiley & Sons, Inc. (See also the next two footnotes.)

[8]"PowerBASIC always rounds towards the closest even number. For example, both 1.5 and 2.5 would be rounded to 2. This is called banker's rounding...." p. 169, *User's Guide*, 1997, PowerBASIC, Inc. 316 Mid Valley Center, Carmel, California, *i–vi*, 1–318.

TABLE 7: The Fi 2 Records of Experiment 1

Experiment 1—First 12 records		
Time in minutes	**Amplitude in picoAmps**	**Terminal digit of time**
0.0648	16.1	8
0.4904	22.7	4
0.4952	33.2	2
0.5398	19.8	8
0.9454	36.1	4
1.7182	44.4	2
2.6950	20.5	0
3.3626	19.3	6
3.7294	17.6	4
3.8586	14.9	6
4.3494	12.9	4
4.3712	45.4	2

Note that amplitudes include values less than 20, as expected. Also note that the terminal digit of the time is an "even" number for all 12 records.

that two successive time values are used in determining a peak, and the mid-point of the two is recorded.

Thus when successive time values are added and divided by 2, the resulting terminal digit is 5 and would be rounded to an even digit, for example,

$$\frac{1000 + 1001}{2} = 1000.5 \Rightarrow 1000$$

$$\frac{108.7 + 108.8}{2} = 108.75 \Rightarrow 108.8$$

Thus if numbers ending in 5 are rounded, only even numbers occur. The rounding of terminal 5's to the nearest even digit is the ANSI/IEEE standard[9] for rounding terminal 5's in computers.

Examination of the terminal digits of the 1026 time values of the unquestioned data in Experiment 2 reveals no times ending in an odd

[9]ANSI/IEEE Std 854-1987, October 5, 1987, "ANSI" denotes the American National Standards Institute and "IEEE" denotes the Institute of Electrical and Electronic Engineers, Inc.
"**4.1 Round to Nearest**....if the two nearest representable values are equally near, the one with its least significant digit even shall be delivered."
"**5.4 Round Floating Point Number to Integral Value**....when rounding to nearest, if the difference between the unrounded operand and the rounded result is exactly one half, the rounded result is even."

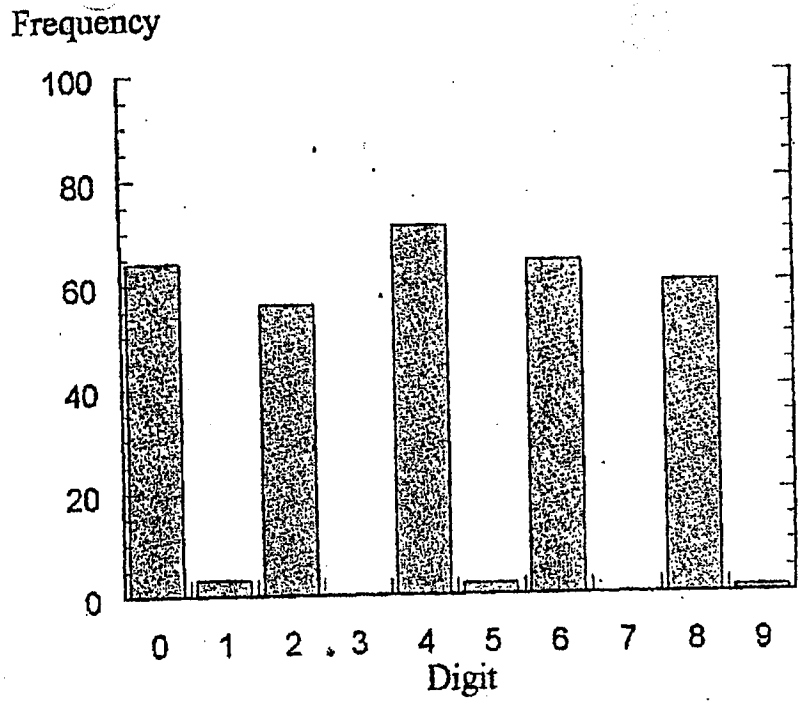


FIGURE 3. Experiment 1: First 321 Time Points 321 Terminal Digits from 321 Numbers (Note presence of six odd digits.)

digit. (The distribution of the 1026 penultimate digits of the times for Experiment 2 is not different from uniform (chi-square = 14.6, df = 9, p = .10).) In contrast, the questioned Experiment 1 contains time values that end in odd digits, reflecting insertions and alterations. In the initial 321 time points, six terminate in an odd digit (see Figure 3). (The distribution of the 315 penultimate digits from the potentially unaltered even times is not different from uniform [chi-square = 8.14, df = 9, p = .52].)

Examination of the graph (see Figure 4) of the final 230 records of Experiment 1 reveals many more (58) time values with odd terminal digits[10] than Figure 3. (The distribution of the 172 penultimate digits from the even, potentially unaltered, times is not different from uniform [chi-square = 12.3, df = 9, p = .20], whereas the distribution of 58 penultimate digits from falsified times ending in an odd digit deviates significantly from uniform [chi-square = 33.0, p = .00013].)

[10]46 of these 58 time values that terminate in odd digits occur with amplitudes greater than 20 picoAmps. In the initial 321 records of Experiment 1, 6 of 6 odd time values occur with amplitudes greater than 20 picoAmps.

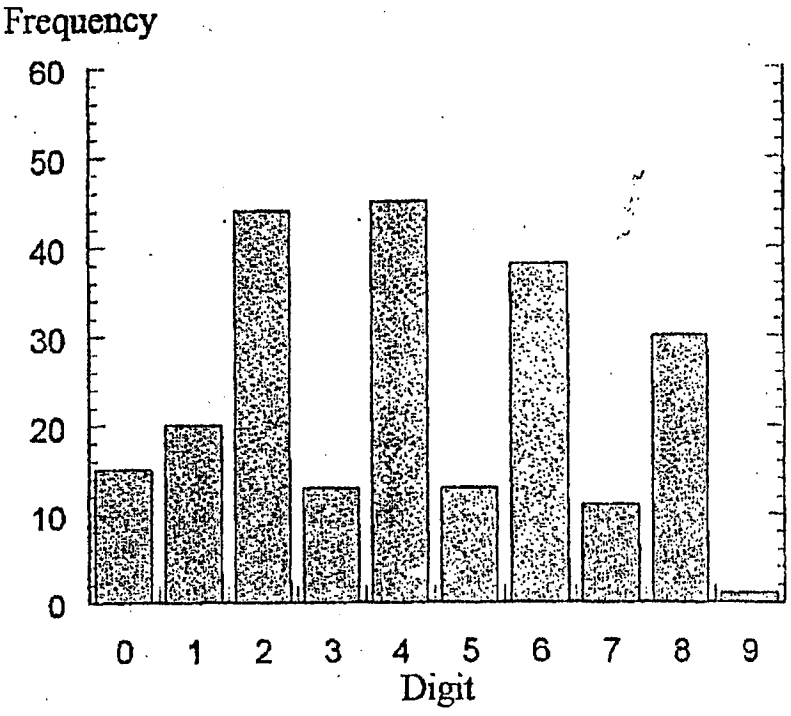


FIGURE 4. Experiment 1: Last 230 Time Points 230 Terminal Digits from 230 Numbers (Note presence of 58 odd digits.)

Many more time values terminate in odd digits in the final portion of Experiment 1, as expected if falsification occurred. The occurrence of time values ending in odd digits, mostly in the latter part of Experiment 1 (and the lack of uniformity of their penultimate digits) indicates data falsification.

The timing of the occurrence of the minutes ending in odd digits is illustrated in Figures 5 and 6. From Figure 6 it can be seen that not only do most of the odd time values occur in the last part of Experiment 1 (after minute 137.3006), but it also appears from the denseness of the plot in the latter that the values immediately after this time point are quite close together. Further statistical tests of the intervals between events confirms the increased density in the latter part of Experiment 1, indicating the insertion of fabricated firing events.

Case 4: One Terminal Digit Too Many

An investigator conducted studies on the effect of rhythmic contrac-

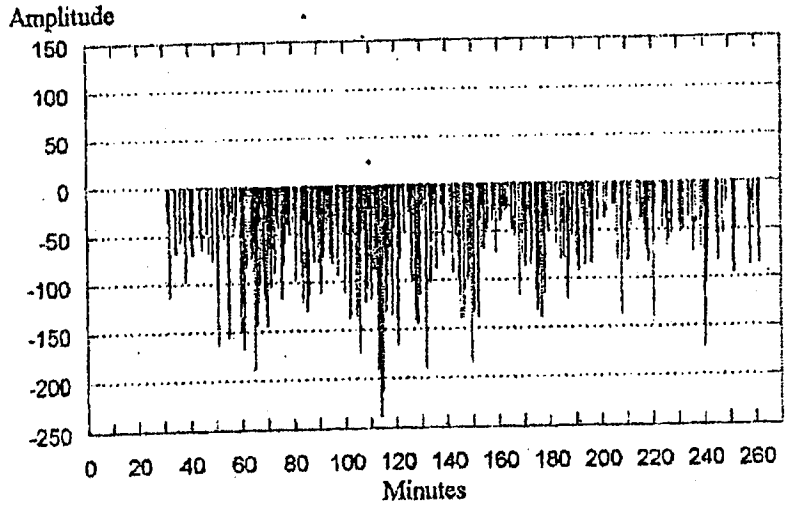


FIGURE 5. Experiment 2, unquestioned, 699 amplitudes (abs > 20). (No amplitude is associated with an odd minute.)

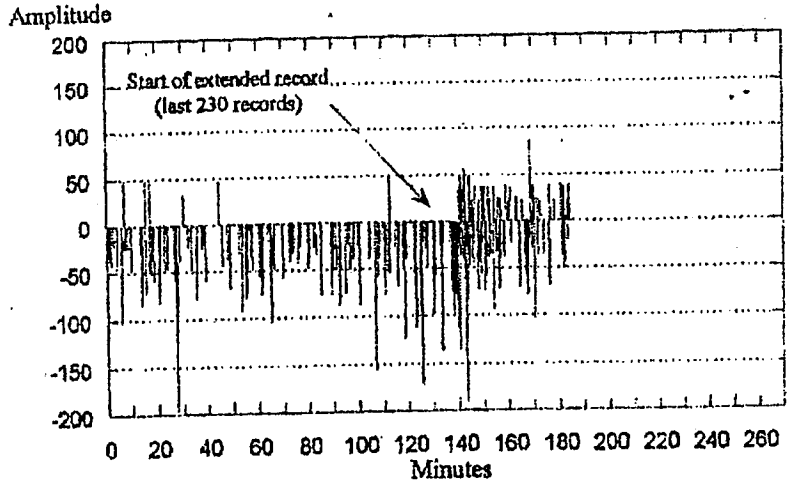


FIGURE 6. Experiment 1, questioned. 371 amplitudes with abs > 20, 52 with odd minutes. (Negative values, even minutes; positive values, odd minutes.)

TABLE 8: Portion of Excel spreadsheet with weights of muscles of rats 1–7.

	Rats					
	Weights-1	Weights-2	Weights-3	Weights-4	Weights-5	Weights-6
M-1	2.495	3.008	2.7515	4.631	2.250	3.4405
M-2	1.695	2.272	1.9835	3.019	0.702	1.8605
M-3	0.738	1.495	1.1165	1.768	0.843	1.3055
M-4	0.780	0.231	0.5055	0.394	0.085	0.2395
M-5	0.276	0.122	0.199	0.155	0.205	0.180
M-6	4.128	3.413	3.7705	2.261	1.187	1.724
M-7	1.131	1.224	1.1775	2.805	0.726	1.7655

Note that some entries for columns Weights-3 and Weights-6 have four decimal digits and end in 5, whereas other entries have at most three decimal digits.

tions of skelctal muscle on blood flow using the hind limbs of rats. Blood flow was measured at rest and during nerve stimulation. In addition to measurements of blood flow, weights of individual skeletal muscles were recorded on data sheets. The experimental results for six rats were presented in a laboratory seminar. Sometime later a co-worker discovered that two of six data sheets were blank, and became suspicious that the measurements (blood flow/weights) had not been made for those rats. Suspicions were confirmed when frozen rat carcasses were checked. Although four had the hind limb muscles dissected, two were still intact and undissected. When confronted, the investigator (now respondent) admitted to falsifying data for two experimental animals. However, he subsequently withdrew the admission and denied the charges. The respondent stated that there was no evidence to support the claims that the research was falsified,[11] and that the university had not followed timely procedures.

The respondent presented to university officials blood flow and weight data for six rats on an Excel spreadsheet, as well as purportedly original data sheets with handwritten entries for the muscle weights for six rats. Weights of 28 muscles and three other body parts for six rats extracted from the Excel printout are presented in Table 8.[12] Further weights as found in handwritten entries on separate data recording sheets for six rats are presented in Table 9.

[11]It is only after the respondent denied the charges and findings of the institution that the ORI demonstrated which two rats on the spreadsheet represented falsified data, and the manner of falsification.

[12]The spreadsheet also contains columns of numbers representing blood pressure measurements and radioactive counts, some of which the university committee regarded as falsified. These are

TABLE 9: A portion of rat muscles weights from handwritten entries on six data recording sheets.

	Rats					
	314-1	314-2	315-1	315-2	316-1	316-2
M-1	2.495	3.008	2.725	3.859	3.479	3.440
M-2	1.695	2.272	1.984	2.087	1.881	1.861
M-3	0.738	1.495	1.117	1.464	1.320	1.306
M-4	0.780	0.231	0.506	0.269	0.242	0.240
M-5	0.276	0.122	0.199	0.202	0.182	0.180
M-6	4.128	3.413	3.771	1.933	1.743	1.724
M-7	1.131	1.224	1.178	1.980	1.785	1.766

Note that all numbers have a precision of three decimal places.

In Table 8, columns Weights-1, Weights-2, Weights-3, and Weights-6 correspond, respectively, to columns 314-1, 314-2, 315-1, and 316-2 in Table 9.

Thus the handwritten "original" data on the four data recording sheets (314-1, 314-2, 315-1, and 316-2) correspond to the columns labeled, respectively, Weights-1, Weights-2, Weights-3, and Weights-6 on the Excel spreadsheet. The columns Weights-4 and Weights-5 do not correspond to two additional data recording sheets labeled 315-2 and 316-1.

When values within a spreadsheet are calculated, rather than transcribed, the numbers may display more digits of accuracy than the original numbers that are the source of the calculated values. Therefore, looking for enhanced precision in spreadsheet numbers can indicate that certain numbers have been calculated or randomly generated by the spreadsheet software.

Since data are presented for six rats, and at most four allegedly were measured, the spreadsheet was evaluated for signs that some of the columns contained calculated values rather than valid data entered from experimental records. The columns Weights-3 and Weights-6 in the Excel spreadsheet (Table 8) contain a number of entries that are recorded to one more decimal accuracy than the other columns (Weights-1, Weights-2, Weights-4, Weights-5). Additionally, these same entries for Weights-3 and Weights-6 contain one more digit than the purported original handwritten data as recorded on the sheets labeled 315-1 and 316-2 (Table 9). This extra precision could not occur from manual entry of the weights from the raw data sheets.

Instead, the presence of an extra digit indicates the possibility that these two columns represent calculated data. Further, where the extra digit occurs, it is always a "5." This indicates the calculation may have

TABLE 10: A Portion of the Weights for Rat 3 and Rat 6

	Rat-3					Rat-6			
	Mean 1,2	Weights-3	315-1	Difference		Mean 4,5	Weights-6	316-2	Difference
vf-1	2.7515	2.7515	2.725	0.0265		3.4405	3.4405	3.440	0.0005
vf-2	1.9835	1.9835	1.984	-0.0005		1.8605	1.8605	1.861	-0.0005
vf-3	1.1165	1.1165	1.117	-0.0005		1.3055	1.3055	1.306	-0.0005
vf-4	0.5055	0.5055	0.506	-0.0005		0.2395	0.2395	0.240	-0.0005
vf-5	0.199	0.199	0.199	0		0.18	0.18	0.180	0
vf-6	3.7705	3.7705	3.771	-0.0005		1.724	1.724	1.724	0
vf-7	1.1775	1.1775	1.178	-0.0005		1.7655	1.7655	1.766	-0.0005

The weights for Rat 3 are precisely the means of the respective weights for Rats 1 and 2. Additionally, the weights for Rat 3 correspond to three decimals to the handwritten weights for Rat 315-1. (The only exception is the weight for M-1 where the rounded 2.752 is transcribed as 2.725. Correspondingly, the weights for Rat 6 are precisely the means of the respective weights for Rats 4 and 5. Additionally, the weights for Rat 6 correspond to three decimals to the handwritten weights for Rat 316-2, without exception.

involved division by "2," suggesting that those numbers could be the means of two columns. (When the sum of the two numbers is even, there is no increase of the nonzero digits; however, when the sum is odd, division by 2 produces an additional "5" digit.)

In fact, the column Weights-3 is precisely the mean of columns Weights-1 and Weights-2 (see Table 10). Correspondingly, the column Weights-6 is the mean of columns Weights-4 and Weights-5 (Table 10).

Since these two columns are calculated on the spreadsheet, the "original" data on the recording sheets 315-1 and 316-2 are copied, respectively, from the spreadsheet-calculated columns Weights-3 and Weights-6. The only modification is that the "original" copied data are only transcribed to three-decimal accuracy as found on the (presumably) valid sheets labeled 314-1 and 314-2.

Lacking muscle-weight data for two rats, the respondent generated weights by twice forming means of measurements of other rats. The presence of the extra digit in the Excel spreadsheet provided the needed clue. When the respondent was shown that the two rats' weights were clearly produced as means, not measures, he accepted the finding of scientific misconduct.

Accountability in Research, Vol. 4, pp. 31–55
Reprints available directly from the publisher
Photocopying permitted by license only

©1995 OPA (Overseas Publishers Association)
Amsterdam B.V. Published under license by
Gordon and Breach Science Publishers SA
Printed in Malaysia

Data Fabrication: Can People Generate Random Digits?

James E. Mosimann,[†] Claire V. Wiseman,[‡] and Ruth E. Edelman[§]

[†]Senior Biostatistician, Office of Research Integrity, Public Health Service, Rockville, Maryland, and Department of Mathematics and Statistics and Department of Psychology, the American University, Washington, D.C., USA
[‡]Department of Psychology, Yale Center for Eating and Weight Disorders, New Haven, Connecticut, USA
[§]Ph.D. candidate, Clinical Psychology program, the American University, Washington, D.C., USA

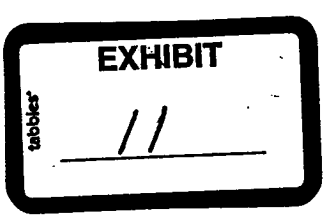
Many people have difficulty in generating random numbers. This difficulty suggests that potentially fabricated numbers encountered in investigations of scientific misconduct be examined for nonrandom behavior. The present paper shows that even with a conscious effort to construct random digits, many subjects are unable to produce digits with a uniform distribution. For this study, subjects were directed to try to produce random digits in three places in order to fabricate a series of "pick 3" lottery numbers. Subjects were most successful at producing a random (uniform) distribution of digits for the leftmost place; however, success at one place was not associated with success at another. In addition, subjects did not select all digits with equal frequency. Of 8,280 digits chosen in this study, the order from most to least chosen was 1, 2, 3, 6, 4, 9, 7, 0, 8, 5. Finally, no strong correlations among subjects' digit choices were found. The conscious effort by these subjects to produce random digits stands in contrast with the usual case of data fabrication in which the fabricator must devote a conscious effort to choose leftmost digits so the number has the magnitude desired and pays little or no attention to the fact that the rightmost digits should be random. The results of the present paper indicate that even if a data-fabricator were aware that error digits would be examined for uniformity, success in constructing uniform error distributions is not guaranteed. The difficulty that people have in creating random error digits supports the utility of examining such digits in investigations of scientific misconduct.

Keywords: scientific misconduct, scientific fraud, data fabrication, random digits, uniform distribution, multinomial distribution, Dirichlet distribution, Dirichlet-multinomial distribution

INTRODUCTION

The present paper reports the results of a study in which subjects were directed to make a conscious effort to produce a series of random digits in three places in order to fabricate a series of numbers from a "pick 3" lottery. This situation is in contrast with the usual case of data fabrication in which the fabricator must devote a conscious effort to choose leftmost digits so the number has the magnitude de-

Requests for reprints: James E. Mosimann, Office of Research Integrity, Suite 700, 5515 Security Lane, Rockville, MD 20852.



sired, and pays little or no attention to the fact that the rightmost digits should be random. The present study shows that even when subjects are directed to make a conscious effort to construct random digits, many subjects are unable to do so; that is, their digits do not follow a uniform distribution. The difficulty that people have in creating random error digits supports the utility of examining such digits in investigations of scientific misconduct. Even if a data fabricator consciously attempts to construct numbers with suitably random error digits, there is no guarantee that the attempt will succeed. The utility of the examination of error digits is not necessarily diminished by a fabricator's knowledge that digits are susceptible to such an examination.

SCIENTIFIC MISCONDUCT AND DATA FABRICATION

Accountability in Science

Since 1980, and continuing unabated at the present time, the scientific community in the United States has been beset by highly-publicized cases of "scientific fraud"[1] in which scientists have been alleged to report falsely, and in some instances fabricate completely, their research results. Books (e.g. Broad and Wade, 1982; Rao, 1989; Miller and Hersen, 1992; Sarasohn, 1993); Congressional hearings (e.g. House Committee on Energy and Commerce; 1988, 1989, 1990); Institutional reports (e.g. National Academy of Sciences, 1992); a new scientific journal, *The Journal of Accountability in Research*; as well as numerous newspaper articles all attest to the current widespread public interest in the area of scientific accountability.

The Public Health Service's Definition of Scientific Misconduct

Scientific misconduct in biomedical research supported by the United States Public Health Service (PHS) is of particular concern to the public: biomedical research often has major impact on the public health and practice of medicine in this country, and large amounts of public funds are used for such research. For example, support for health research and development by the National Institutes of Health (an agency of the Public Health Service) in 1992 was 8.4 billions of dollars (NIH Data Book 1992, Table 2).

The Office of Research Integrity (ORI), located in the Office of the Assistant Secretary for Health, Public Health Service, Department of Health and Human Services, is responsible for the investigation of scientific misconduct in research involving PHS funds. The definition of scientific misconduct that currently is applicable to research grant recipients and applicants for PHS funds appears in the Federal Register of August 8, 1989:

> "'Misconduct' or 'Misconduct in Science' means fabrication, falsification, plagiarism, or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting, or reporting research. It does not include honest error or honest differences in interpretations or judgments of data." (p. 32499)

Prominently featured in this definition is the word "fabrication." The results of the present paper relate to "fabricated data" which purport to represent the

results of an experiment or some other careful observations, but which, in truth, the "observer" has simply made up. For example, in this study subjects were asked to fabricate random digits to appear as if they had been produced by a lottery.

Data Fabrication Destroys Scientists' Collegiality as Well as the Public's Respect

A distinguishing mark of science, as opposed to philosophy or mathematics, is the reliance upon observation of the phenomena under study and the recording of these observations as "data." If two scientists disagree on the interpretation or conclusions derived from a given set of data, nonetheless, their point of departure is that same set of data. The fabrication of research data is a particularly egregious kind of scientific misconduct because it strikes at the distinguishing mark of the scientist; namely, a trust in observation as a way of arriving at knowledge. Further, the entire atmosphere of scientific debate and dispute, which is not necessarily damaged by even acrimonious or heated debates, is completely undermined by data fabrication. The very "collegiality" of science is based on a trust that the individuals are honestly attempting to report observations. This collegiality is destroyed in instances of data fabrication. But, one need not be scientifically trained to know that to "make up" data and to represent these "data" as the result of scientific observation is simply wrong; to present such "data" as the result of one's observation is particularly heinous when policy decisions concerning health care may be based on them. When instances of fabrication are revealed, scientific collegiality is not only diminished, but the public perception of scientists as honest seekers of knowledge is damaged as well.

Detection of Fabricated Data May Be Possible Because People Have Difficulty Making Up Random Numbers

Investigations of scientific misconduct often must address the question, "Are these data fabricated?" Because the usual form in which data are presented is as numbers, the question may be rephrased, "Could these numbers have been produced by the experiment claimed?" A closely-related question is, "Do these numbers show evidence of arbitrary human choice?", which might be paraphrased as, "Did somebody make up these numbers?"

One technique used by the ORI's Division of Research Investigations (DRI) is based on the uniform distribution of error digits. For example, consider a count like 398,717 obtained from a scintillation counter, where because of the experimental procedure only the leftmost two or three digits contain information. The rightmost digits can be discarded, and the count rounded to 390,000 or 400,000 without loss of information. In such a case, the rightmost digits simply indicate "experimental error," and may be referred to loosely as "error digits." It has long been known that error digits, in theory, tend to be uniformly distributed. Yule (1927) and Preece (1981) discuss the situation in which observers are reading a scale, and consider departures from uniformity, in many cases, to indicate personal preference for digits. Yule also noted approximate conditions for error digits to be uniform, the

basic requirement being that the dispersion of the numbers be sufficiently broad.[2] For example, digits may be recorded beyond the ability of an observer to read a scale, beyond the ability of a measuring device to be precise, or beyond the point at which because of experimental error, results may be meaningfully repeated. Some conditions for digits to be uniformly distributed, or approximately so, may be determined mathematically (Diaconis, 1977; Mosimann & Ratnaparkhi, 1994). However, a more direct approach is to compare the rightmost digits of unquestioned control data (from the same kind of experiment, laboratory, investigator, and time period as the questioned data) with those of the questioned data. If the rightmost digits of the questioned data depart significantly from a uniform distribution, while those of the control data do not, then there is evidence of some selective factor applying to the questioned numbers. Specifically, the selection may be due to conscious or unconscious human choice in making up numbers.

For example, in a closed investigation in which scientific misconduct was found (ORI #93-02)[3], the pattern of distribution of the rightmost four digits from fabricated numbers is distinctly non-uniform, while for non-fabricated data from the same investigator (supported by printed tapes from a scintillation counter) comparable digit distributions are not significantly different from uniform (see Table 1). The distributions for all four decimal places are shown in Figure 1 which is taken from Appendix 10 of the ORI's Final Report for this case. In this case of known fabrication, the fabricator did not make up appropriately random error digits. (A brief discussion of these findings is given in the ORI Newsletter (1993), Volume 1, No. 4, page 4, bottom of first column and second column).

Not only this investigator, but people in general, have difficulty generating random numbers. In the course of fabricating data, an investigator may attempt to create data to appear as close to the results of a legitimate experiment as possible. Detection of such fabricated data may be difficult; however, by testing for randomness in those aspects of the data that represent error, (particularly where corroborated data from the same laboratory, type of experiment, and investigator are available for comparison) it may be possible to detect whether or not a given set of data has been created by an individual.

TABLE I. Chisquare values for tests of uniform distribution of digits for fabricated data and unquestioned data from ORI Case #93-02.

	Fabricated data			Unquestioned data		
	N	Chisquare	p	N	Chisquare	p
All places	939	30.94	.0003	857	11.09	.27
Place 4 (leftmost)	185	34.84	<.0001	195	14.28	.11
Place 3 (middle)	250	29.28	.0006	218	9.89	.36
Place 2 (middle)	252	13.24	.15	222	8.72	.46
Place 1 (rightmost)	252	27.13	.001	222	11.33	.25

Digits were tested in four places, but no digit that was itself the first (leftmost) digit of a number was included in the analysis.

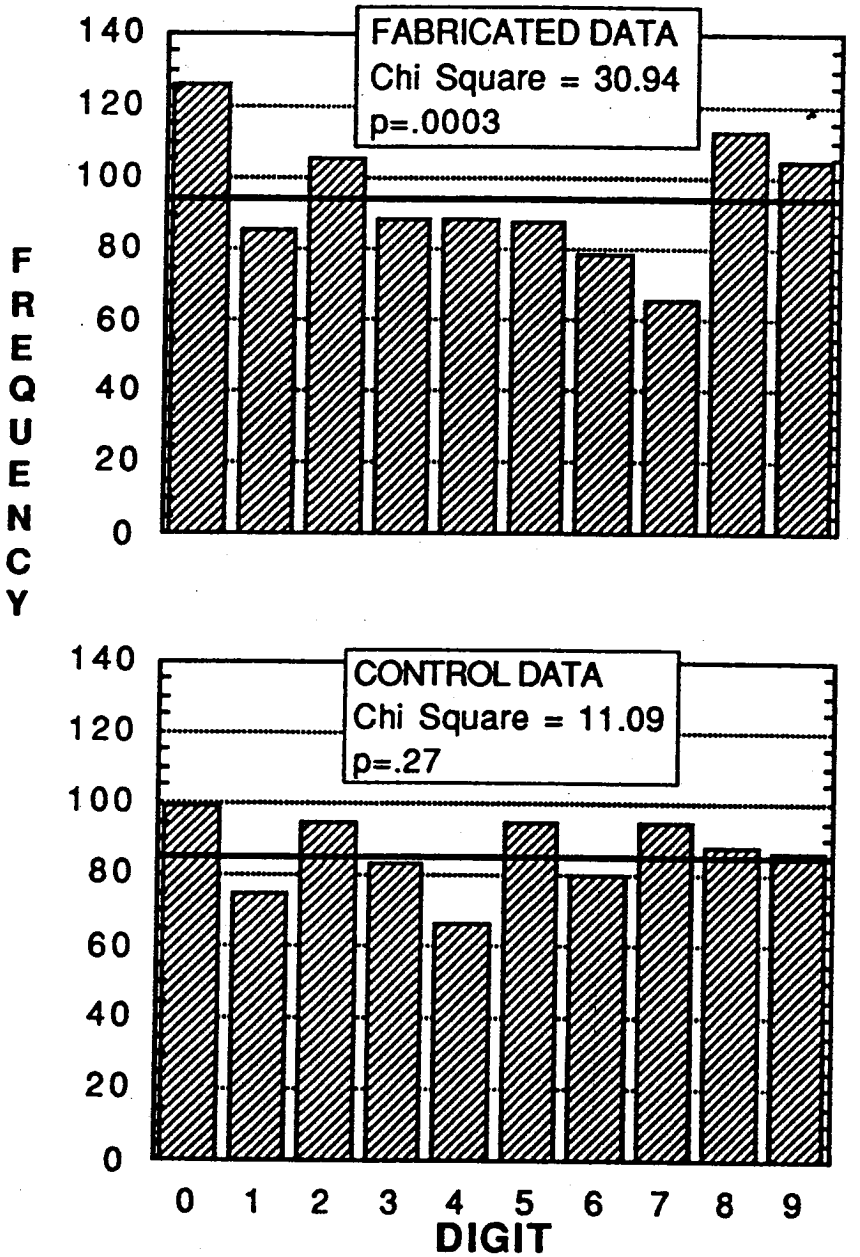


Figure 1. Digit distributions (all four places).

STUDIES ON THE ABILITY OF HUMANS TO GENERATE RANDOM NUMBERS

Generally, people cannot generate random series of numbers (see Tune, 1964a, 1964b and Wagenaar, 1972 for reviews), although "sophisticated" subjects who have some knowledge of the laws of probability and statistics may be more likely to generate more random series than unsophisticated subjects (Chapanis, 1953). The ability of subjects to generate random series of numbers may depend on cognitive status and on informational processing ability. For example, Rosenberg, Weber,

Crocq, Duval, and Macher (1990) found that normal subjects performed better on a randomization task than did schizophrenics, who, in turn, performed better than alcoholics (although it appeared that the alcoholic and normal groups employed similar cognitive processes). Other processes, such as the level of sophistication of the subject, the number of items from which selections were chosen, time constraints, and the age of the subjects may also effect performance on randomization tasks (Tune, 1964a).

In studies of people attempting to generate numbers, the first digit chosen may depend on the experimental procedure. In a spontaneous number-choice situation, Winick (1962) tabulated the number of digits recorded on an adding machine on the sidewalk in New York City. There were 55,375 digits recorded on a special machine. The order of frequency from most to least chosen was 1, 2, 4, 3, 5, 0, 9, 6, 7, 8, demonstrating a preference for lower digits. This higher probability of selection of lower digits is reminiscent of Benford's Law (1938) in which the first (nonzero) digit of a number in miscellaneous tables is more likely to be low than high. Under Benford's law the probability that the first digit is 4 or less is .7; see, for example, Feller (1966, pages 62–63) and also Diaconis (1977). However, when Hill (1988) asked each of 742 students to write down a six-digit number, of 740 responses, the initial digit chosen from most to least frequent was 6, 1, 4, 7, 3, 2, 5, 8, 9, and Benford's Law was not followed (for example, the observed frequency of 4 or less was .48). The most frequently chosen digit in Hill's experiment, 6, may have been associated with the instruction to write a six-digit number. Heywood (1972) found 7 to be the most frequently chosen first digit (49 of 189), and also found preferences for the second digit choice. He noted, based on data from five studies which included Winick's cited above, ". . . that experimental procedure may affect preference . . ." Similarity in the numbers generated by some subjects may remain constant across time. Melendez (1966) asked subjects to generate two series of random numbers. The same subjects were given the exact task 2 days later. Subjects' series were more similar to each other than they were to random series.

People often cannot judge or detect randomness in a series of numbers (Budescu, 1987). People seem to have the expectation that chance mechanisms will behave in a self-correcting manner to produce representative frequencies. This is reminiscent of the representativeness heuristic, or gambler's fallacy. In a coin toss, or similar game of chance, after "heads" has appeared ten times in a row, people will not continue to bet on it.

There are various hypotheses on the non-randomness of subjects' choices. One explanation is that subjects try to use their intuitive concept of randomness to guide their responses, but that this concept is faulty, and, therefore, subjects' responses reflect their misconceptions (Chapanis, 1953; Ross & Levy, 1958). Another explanation is based on limitations of the subjects, e.g., limited memory (Tune 1964b) or limited attention (Weiss, 1964). A third explanation is that subjects focus on local regularity, not global regularity (Kahneman & Tversky, 1972; Falk, 1981; Lopes, 1982).

The present study has attempted to determine the extent to which human subjects are capable of creating numbers randomly. Tests of randomness were limited to the hypothesis that the subjects would not consistently create numbers such that the digits, 0, 1, . . . , 9 are uniformly distributed.

METHODS FOR THE PRESENT STUDY

Subjects

The subject pool consisted of 27 undergraduate and graduate students at The American University and 22 professional scientists and non-scientific administrative staff in an office in the Washington, DC area. Three subjects were discarded due to missing data, leaving 46 subjects in the final sample. Human subject approval was given by the Institutional Review Board of The American University, and each subject read and signed a consent form before participating in this experiment.

Materials

The survey consisted of a four page questionnaire. The questionnaire included a one page demographic questionnaire with specific questions regarding age, education, and professional experience with regard to statistics and mathematics. In the remaining three pages, subjects were asked to simulate the generation of lottery numbers for 90 days.

Procedure

After reading and signing a consent form, subjects were given the following task:

"Suppose you are in charge of a state lottery and you are required to keep a record of the numbers that were selected in the past year. The Governor of the state requests a list of the past year's numbers from the lottery's 'Pick-3' game in which a 3-digit number was picked at random each day by a machine. You note that the numbers are missing for thirty consecutive days in the records for the past year. You decide to make up thirty random numbers to replace the thirty missing numbers. You try to make them look as much like random Pick-3 numbers as possible. Use any digits 0–9. A number may begin with the digit '0' and you may repeat numbers."

Subjects then generated a series of thirty 3-digit numbers. Subjects were asked to write the numbers out as if they were writing numbers out for each weekday for six weeks in a row in the process of generating the numbers for each set of 30 numbers. Subjects were instructed that they may begin the numbers with the digit "0" and that they may repeat digits within a number sequence as well as repeat numbers.

After this task was completed, subjects were given similar instructions and asked to generate thirty 3-digit numbers using only the digits 0–2. Finally, subjects were again asked to write down thirty 3-digit numbers using the digits 0–9. Subjects were asked not to return to the previous sections when selecting each set of 30 numbers.

Statistical Analysis

Programs written in Turbo Basic and in SAS were used to prepare the data for analysis. Maximum Likelihood estimation of the parameters of the Dirichlet-

Multinomial distribution (Mosimann, 1962, 1963 and Appendix 1) was accomplished using an iterative procedure written in Turbo Basic. Most statistical analyses were performed using SAS. All calculations used a Dell 486D personal computer. For future use by others, the frequencies of digits for the 46 subjects are listed in Appendix 2.

VARIATION AMONG SUBJECTS' ABILITY TO PRODUCE RANDOM DIGITS

Successful Production of Random Digits Was Measured by a Chisquare Probability

The randomness of the triples of numbers produced by a subject was examined by means of the frequency of the digits 0, 1, 2, . . . , 9. Each subject produced 60 triples. The respective frequencies of the digits were determined for the 60 digits in the third (leftmost) place, the second (middle) place and the first (rightmost) place. Additionally, the overall frequency for all 180 digits was found (illustrated for three subjects in Figure 2). These frequencies were tested for uniformity by means of a Chisquare goodness of fit test with 9 degrees of freedom. The probability of each Chisquare statistic (four for each subject) was recorded. These probabilities themselves were used as a measure of a subject's ability to produce random numbers.

The Successful Production of Random Digits Did Not Differ by Sample Nor by Other Attributes

There were no differences between subjects in the two samples (university versus office) in their ability to generate series of random numbers (see Table 2). For this comparison, failure to generate random numbers was defined by a significant difference from the uniform distribution (Chisquare, $p < .05$). For the first sample and based on all 180 digits per subject, 14 of 24 subjects produced nonrandom digit distributions; for the second sample, 12 of 22 subjects produced nonrandom distributions. There was no difference in the proportion of nonrandom distributions for the two samples ($p > .8$). Three similar analyses, based, respectively, on the 60 digits for a subject's 1st, 2nd, and 3rd place choices, showed no difference in the proportion of subjects producing nonrandom distributions, all three p's > 0.52.

Corresponding analyses of the overall frequency distribution of 180 digits for each subject revealed no differences between those subjects with scientific training and those without scientific training ($p > .26$), or among those who had or had not completed a college level math class ($p > .88$) or completed a college level statistics class ($p > .51$). No differences were revealed between those subjects currently taking, or not taking, a math class ($p > .95$) or a statistics class ($p > .99$) at the time of the experiment. In addition, those subjects who had completed a graduate degree were no more likely to generate a random series of digits than were those subjects who had completed an undergraduate or high school degree ($p > .34$). Thus, the level of sophistication of the subjects in this sample did not affect their

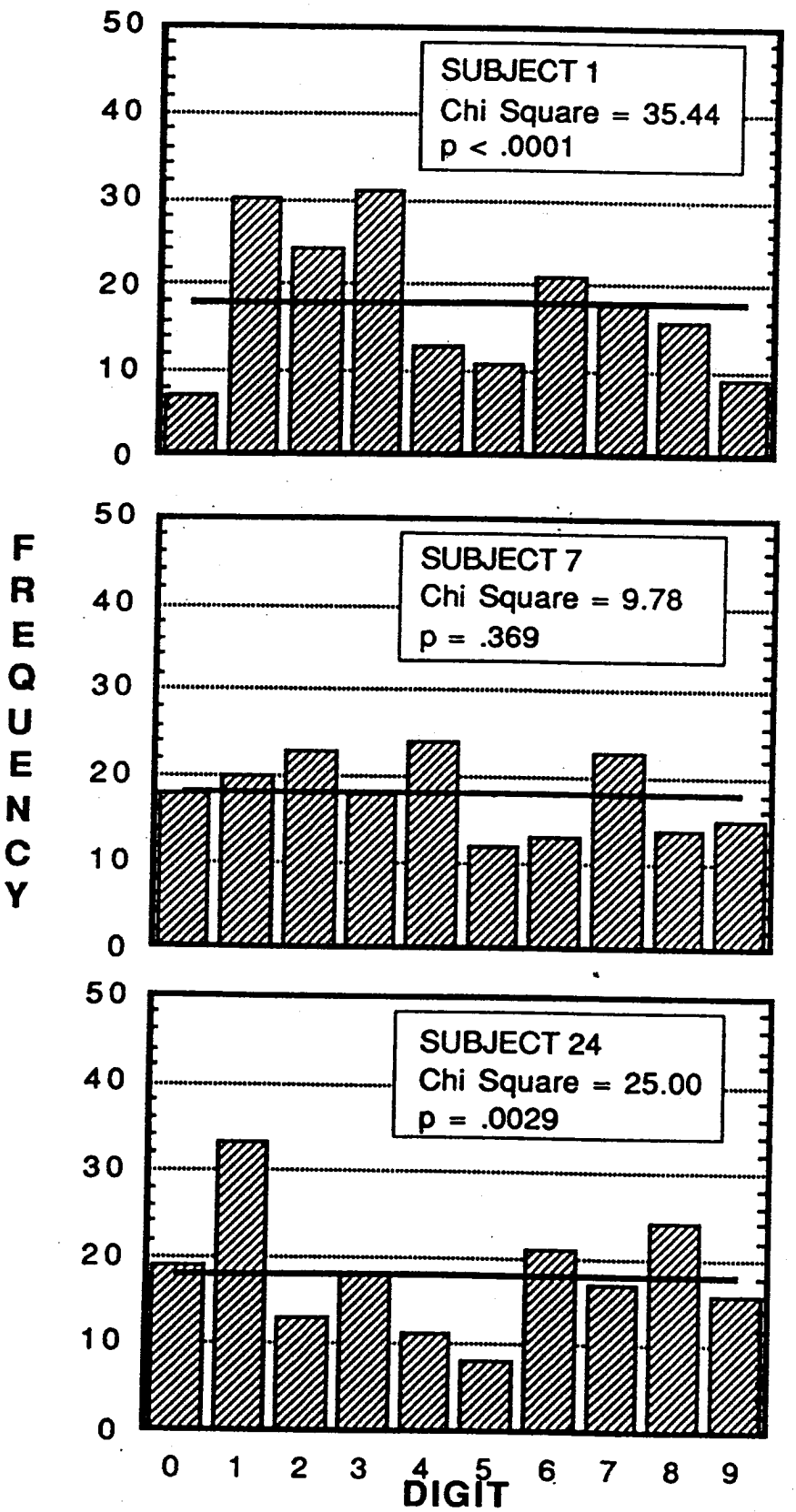


Figure 2. Digits chosen (all places) for three selected subjects.

TABLE II. Chisquare probabilities for the overall frequencies (180 digits for each of the 46 subjects) for the two samples.

	Sample 1 (n = 24)	Sample 2 (n = 22)
$p \geq .05$	10	10
$p < .05$	14	12

Note: Chisquare (1df) = 0.067, $p > .8$; Sample 1, undergraduate and graduate students at The American University; Sample 2, professional scientists and non-scientific administrative staff in an office in the Washington, DC area.

performance on the randomization task. (Again, none of the three associated analyses based, respectively, on the 60 digits for a subject's 1st, 2nd, and 3rd place choices, showed a significant difference between the two samples.)

Subjects Were Most Successful for the Leftmost Digit

The nature of the task assigned to subjects implied not only that the overall digits in the triples of numbers should be uniformly distributed, but also that the digits in each of three places should be uniformly and independently distributed. Because of the decimal system, digits to the left determine the magnitude of the resulting number (triple) more than digits to the right. This fact influenced the subjects' abilities to generate uniform distributions of digits.

Under the null hypothesis that each subject's sample of digits is a sample from a uniform distribution on 0, 1, . . . , 9, the resulting goodness of fit statistic has, approximately, a Chisquare-distribution with 9 degrees of freedom. Further, the corresponding Chisquare probability has, approximately, a rectangular distribution on the interval [0,1]. Given the independence of the goodness of fit statistics over subjects, the 46 probabilities are, under the null hypothesis, a sample of 46 independent observations from a rectangular distribution on the interval [0,1]. Briefly, if all 46 individuals were generating random digit distributions, the corresponding Chisquare probabilities should be distributed smoothly across the interval [0,1].

Figure 3 shows what could already be inferred from Table 2: the null hypothesis that all 46 subjects are producing uniform overall digit distributions is not tenable. Far too many subjects have low Chisquare probabilities indicating digit distributions departing from uniform, and the distribution is clearly not rectangular. Next, Figure 3 reveals the effect of digit place upon the ability to produce uniform distributions. The third (leftmost) place has a distribution that is much closer to rectangular than the other two places.

The results illustrated in Figure 3 are consistent with subjects being most attentive to producing a uniform distribution of digits in the leftmost place. (As noted above, the digit in this place is the most significant determinant of the magnitude of a three digit number.) The results indicate further that subjects were unable to maintain this attentiveness beyond a single place. The distributions for the middle

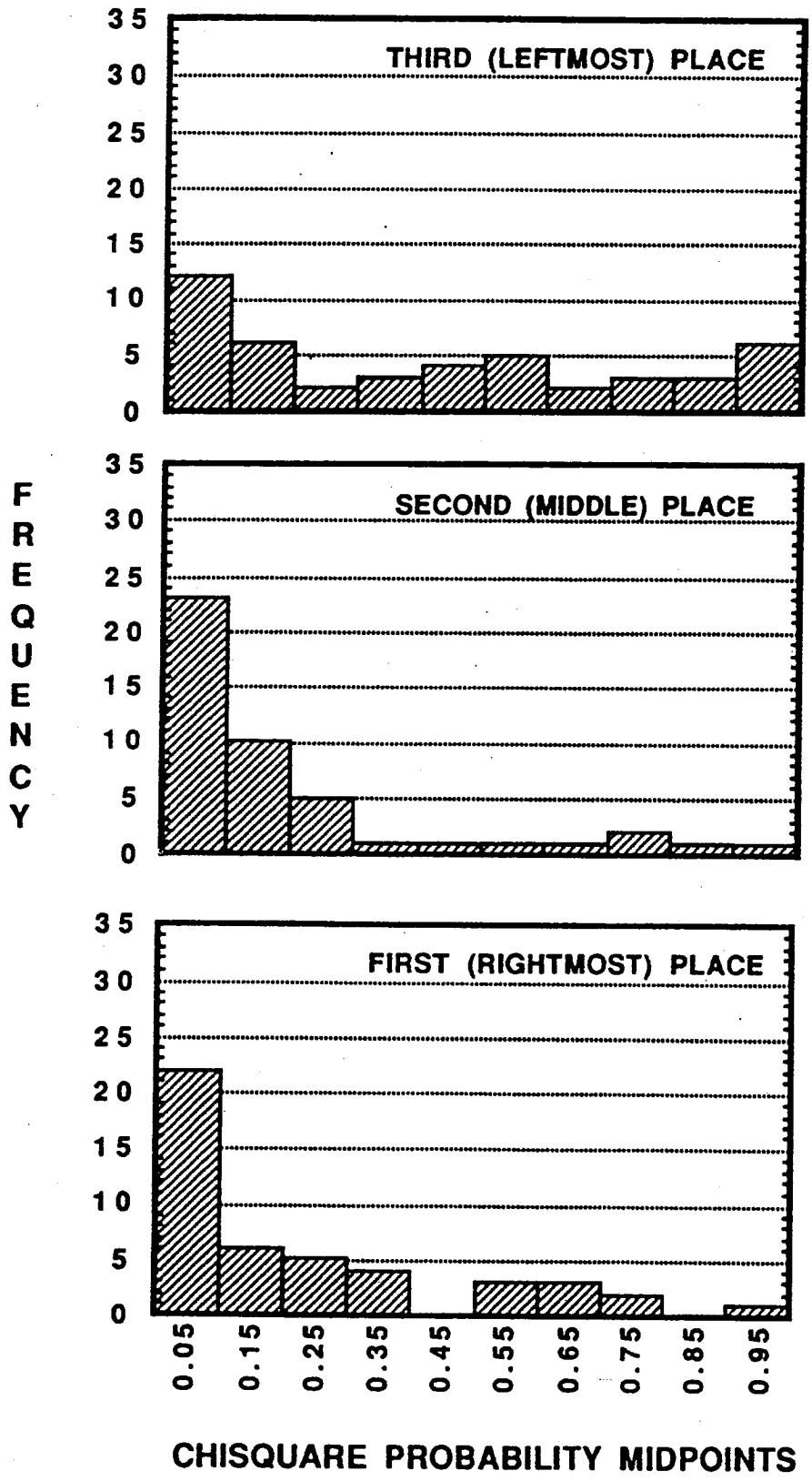


Figure 3. Variation among subjects' ability to produce random digits.

TABLE III. Frequencies of Chisquare probabilities for places 3 to 1 (60 digits for each of the 46 subjects).

	$p < .05$	$.05 \geq p < .1$	$p \geq .1$
Place 3 (leftmost)	8	4	34
Place 2 (middle)	19	4	23
Place 1 (rightmost)	18	4	24

and rightmost digits both deviate in the same way from rectangularity. Each exhibits a preponderance of subjects with low Chisquare probabilities (distributions departing from uniform). Table 3 gives a summary of the distribution of subjects by Chisquare probability.

The three respective probabilities for 3rd, 2nd, and 1st place digits, for each subject were used in statistical tests of differences in the Chisquare probabilities. Three differences were obtained: place 3 minus place 2; place 3 minus place 1; and place 2 minus place 1. These differences were then tested to see if subjects performed better for place 3 than the other two places. For example, if subjects perform better for the third place than the second place, then the difference place 3 minus 2 would be significantly positive. Nonparametric sign tests (based simply on positive or negative differences) gave the following results: 3-2, $z = 3.24$, $p = .0006$; 3-1, $z = 3.24$, $p = .0006$; 2-1, $z = 0$, $p = .5$). Paired t-tests (based on the magnitude of the differences, but whose underlying normal assumptions are less likely met here) give comparable results: 3-2, $t = 3.89$, $p = .0003$; 3-1, $t = 3.09$, $p = .0034$; 2-1, $t = -.54$, $p = .59$. These results show that subjects were significantly better in producing random digits in the third (leftmost) place than in the other two places, and that there was no difference in such production for those two.

Success at One Place Was Not Associated with Success at Another

Finally, there was no evidence that subjects who did well at one place did consistently well, or consistently poorly, at another place. In fact, the subjects' Chisquare probabilities for the three places are not correlated: 3 with 2, $r = .17$, $p = .26$; 3 with 1, $r = .004$, $p = .98$; 2 with 1, $r = .03$, $p = .83$. Specifically, successful production of a random distribution for the third place was not associated with successful production at either remaining place. This supports the notion that subjects were able to be attentive to the digits they were writing for at most one place.

VARIATION AMONG THE DIGITS CHOSEN BY SUBJECTS

The Unequal Selection Of Digits

The subjects did not select all digits equally. For the 180 digits produced by a subject, the number of times each digit (0, 1, . . ., 9) was selected was recorded. For

TABLE IV. Descriptive statistics for the frequency with which each digit was selected.

Digit	N	Minimum	Maximum	Mean	Std Dev
0	46	3	34	16.7	7.06
1	46	10	46	24.7	7.24
2	46	10	33	21.5	5.53
3	46	8	31	19.0	4.82
4	46	1	27	17.3	5.70
5	46	6	22	13.9	4.80
6	46	7	29	17.4	4.94
7	46	6	26	16.9	4.34
8	46	6	24	15.4	4.41
9	46	8	33	17.1	5.83

example, the number of times 0 was recorded is denoted by "Freq0", and similarly for the other digits. Across the 46 subjects there are then 46 values of Freq0, etc. Descriptive statistics for these variables are given in Table 4.

For example, in Table 4 it is seen that some subject chose 0 only three times out of 180 possible times. It is also noted that Digits 1, 2, and 3 were chosen most often, and that at least one subject used digit 1 forty-six times. There were 8,280 digits chosen in this study. From Table 4 the order of digits from those chosen most to those chosen least was: 1, 2, 3, 6, 4, 9, 7, 0, 8, 5. Overall, only the digits 1, 2 and 3 had means exceeding 18. As noted above, in Winick's (1962) relatively unstructured experiment, the order was 1, 2, 4, 3, 5, 0, 9, 6, 7, 8. Our experiment was more structured and produced similar results.

Table 5 gives the mean frequencies over the 46 subjects for the three separate places. In both the rightmost and leftmost places the digit zero has a low mean, indicating that subjects tended to avoid numbers beginning or ending with zero.

TABLE V. Means over 46 subjects for the frequency with which each digit was selected.

Digit	Leftmost Place	Middle Place	Rightmost Place	All Places
0	4.6	7.2 x	4.9	16.7
1	7.3 x	8.5 x	8.8 x	24.7 x
2	7.5 x	7.4 x	6.7 x	21.5 x
3	6.9 x	5.5	6.6 x	19.0 x
4	5.9	5.8	5.6	17.3
5	5.3	4.4	4.2	13.9
6	6.5 x	5.4	5.5	17.4
7	5.6	5.0	6.3 x	16.9
8	4.8	5.1	5.5	15.4
9	5.5	5.7	5.9	17.1

For the first three columns, values above 6 (= 60/10) are indicated by an "x", for the last column, values above 18 (= 180/10) are similarly indicated.

The three most frequently chosen digits are: Leftmost, 1,2,3; Middle, 0,1,2; Rightmost, 1,2,3. Thus excluding beginning or ending zeros, subjects chose the three lowest available digits with higher frequencies than the others. In only two other instances were other digits chosen above the average (6 in the leftmost place, and 7 in the rightmost place). The choice of the lowest available numbers is consistent with the natural manner of counting upwards; 1, 2, 3, . . . rather than "backwards", 9, 8, 7, ...

The results in both Tables 4 and 5 are consistent with Winick's (1962) experiment in which lower digits were chosen more frequently than higher digits.

No Strong Correlations Among Subjects' Digits Choices Were Found

The question of correlations among subjects' choices of digits was examined. To do this with this type of data, it is necessary to consider that under reasonable null hypotheses the expected correlations of the numbers of digits chosen are not zero, but negative. Suppose a subject chooses 180 digits, as done in our experiment. Further, suppose the total number of times (denoted T) that the subject chose one of the first nine digits (either 0 or 1 . . . or 8) is known. Then, necessarily, the number of times the remaining digit 9 was chosen is the difference 180-T.

Patterns of correlation across subjects were evaluated by first estimating the parameters of a Dirichlet-multinomial mixture distribution (Null model) and then using Fisher's z-transformation (Dixon & Massey, 1983, p. 224) to test an observed correlation from the estimated null model correlation (for an explanation of the method see Appendix 1). Eight of the 45 pairwise comparisons gave significant results at the .05 level of significance. The most marked difference was a positive association for choosing 4 and 7. Based on the pairwise differences, the possible general pattern was a negative association of choosing 0 with choosing digits 4, 6, and 7, negative of digit 5 with 4 and positive among 6, 7, and 8. These associations may be driven by the relatively large variation in subjects' willingness or not to choose the digit 0. The digit 0 has, relatively, the largest variance across subjects (ratio 3.3 in Table A2), and the second largest sample variance (49.8) in absolute terms across subjects.

DISCUSSION

In this study subjects were asked to fabricate triples of random numbers to appear as "pick 3" numbers from a lottery. Randomness was evaluated only in terms of the resulting digits being uniformly distributed. Serial properties of the numbers, such as a preference for a particular digit to occur after another specified digit, were not examined. Many subjects were unable to produce uniformly distributed digits. Subjects, however, were most successful at producing uniform digit distributions for the leftmost place. (The leftmost place most determines the magnitude of the number represented by the triple of digits, and relative success in that place may reflect a conscious or unconscious attentiveness to magnitude.) Success at one place was not associated with success at either of the two other places.

No strong correlations among subjects' digit choices were found, but those that were found are possibly explained by subjects' willingness to choose or not to choose the digit 0. Subjects did not select all digits with equal frequency. Of the total 8,280 digits generated, the order of digits from those chosen most to those chosen least was: 1, 2, 3, 6, 4, 9, 7, 0, 8, 5. Overall, only the digits 1, 2, and 3 had means exceeding 18, the number expected under a uniform distribution. For each of three places 2,760 digits were chosen. For the leftmost place the three most frequent digits, from most to least, were 1, 2, 3. This same order was found for the rightmost place; however for the middle place the three digits chosen most frequently were, in order, 0, 1, 2. Thus, subjects tended to avoid leading (leftmost) or terminal (rightmost) zeros. Winick's (1962) relatively unstructured experiment also showed an avoidance of zero, in spite of the fact that the machine he used had keys favoring zero (with one keystroke it was possible to enter either two or three zeros simultaneously). Our results are consistent with those of Winick, although our experiment was more structured and encompassed a specific scenario of fabrication.

Our results indicate that many subjects cannot generate uniform distributions of digits even when consciously attempting to do so. They also demonstrate that more-educated, including statistically or mathematically sophisticated, subjects did no better than other subjects in generating such distributions. In conclusion, even if a data-fabricator were aware that seemingly unimportant error digits would be examined for uniformity, successful construction of a uniform digit distribution may not be achieved. Additionally, if subjects cannot product uniform digits when attempting to do so, how would a data fabricator succeed in producing uniform "error" digits, where the fabricator's attention is concentrated on producing numbers with desired magnitudes (i.e. leftmost digits) in order to represent the empirical result to be falsely reported? The difficulty that people have in creating uniform error digits supports the utility of examining such digits in investigations of scientific misconduct.

APPENDIX 1

For a single subject the expected correlations of counts under a model of "no interesting association" are those of the multinomial distribution. Let the probability that a subject chooses one of the digits (0, 1, . . . , 9) be, respectively (p_0, p_1, . . . , p_9). If the latter "probability vector" remains the same for each of the 180 choices made by the subject, and if the 180 choices are independently made, then the numbers of times each digit was chosen, denoted (x_0, x_1, . . . , x_9) would follow a multinomial distribution (Figure A1), and the expected correlations among counts would be negative (Figure A2). Thus, even in this "null" situation where there is no change in a subject's probability vector from the first choice to the 180th choice, and there is no dependence from choice to choice, the expected correlations are negative.

A multinomial distribution of digits can arise whether or not a subject is choosing digits at random. A subject whose probability vector (p_0, . . . , p_9) = ($\frac{1}{10}$, . . . , $\frac{1}{10}$) has the same probability of choosing each digit, but a subject who avoids 0 but

Probability mass function and moments of the multinomial distribution; the within-subject distribution:

$$p(x_1,\ldots,x_k|p_1,\ldots,p_k,n) = n!\prod_{j=1}^{k}\frac{p_j^{x_j}}{x_j!} \quad ; p_j > 0,\ x_j = 0,1,\ldots,n\ ;\ j = 1,\ldots,k;\ \sum_{j=1}^{k} p_j = 1,\ \sum_{j=1}^{k} x_j = n;$$

$$E(X_j) = np_j\ ,\quad \mathrm{Var}(X_j) = np_j(1-p_j) \quad ;\ j = 1,\ldots,k;$$

$$\mathrm{Cov}(X_m, X_j) = -np_m p_j \quad ;\ j = 1,\ldots,k;\ \ m = 1,\ldots,k;\ \ m \neq j.$$

Density function and moments of the (null-model) Dirichlet distribution; the mixing or between-subject distribution:

$$f(p_1,\ldots,p_k|\lambda_1,\ldots,\lambda_k) = \Gamma\left(\sum_{j=1}^{k}\lambda_j\right)\prod_{j=1}^{k}\frac{p_j^{\lambda_j-1}}{\Gamma(\lambda_j)} \quad ;\lambda_j > 0,\ p_j > 0;\ j = 1,\ldots,k;\ \sum_{j=1}^{k} p_j = 1\ ;$$

$$E(P_j) = \frac{\lambda_j}{\left(\sum_{j=1}^{k}\lambda_j\right)},\qquad \mathrm{Var}(P_j) = E(P_j)(1-E(P_j))C_D \quad ;\ j = 1,\ldots,k;\ \ C_D = \frac{1}{1+\left(\sum_{j=1}^{k}\lambda_j\right)};$$

$$\mathrm{Cov}(P_m, P_j) = -E(P_m)E(P_j)C_D \quad ;\ j = 1,\ldots,k;\ \ m = 1,\ldots,k;\ \ m \neq j.$$

Probability mass function and moments of the Dirichlet-multinomial distribution, the resulting null-model mixture distribution:

$$p(x_1,\ldots,x_k|\lambda_1,\ldots,\lambda_k,n) = \left[\frac{n!\,\Gamma\left(\sum_{j=1}^{k}\lambda_j\right)}{\Gamma\left(n+\sum_{j=1}^{k}\lambda_j\right)}\right]\prod_{j=1}^{k}\frac{\Gamma(x_j+\lambda_j)}{x_j!\,\Gamma(\lambda_j)};\quad \lambda_j > 0, x_j = 0,1,\ldots,n;\ j = 1,\ldots,k;\sum_{j=1}^{k} x_j = n;$$

$$E(X_j) = n\frac{\lambda_j}{\left(\sum_{j=1}^{k}\lambda_j\right)} = nE(P_j),\quad \mathrm{Var}(X_j) = nE(P_j)(1-E(P_j))C_M \quad ;j = 1,\ldots,k;\ C_M = \frac{n+\left(\sum_{j=1}^{k}\lambda_j\right)}{1+\left(\sum_{j=1}^{k}\lambda_j\right)};$$

$$\mathrm{Cov}(X_m, X_j) = -nE(P_m)E(P_j)C_M \quad ;\ j = 1,\ldots,k;\ \ m = 1,\ldots,k;\ \ m \neq j.$$

$\Gamma(\cdot)$ denotes the Gamma function, see Figure A3.

Figure A1. Probability mass or density functions and moments for the multinomial, Dirichlet, and Dirichlet-multinomial distributions.

favors 1 has $p_0 < 1/10$ and $p_1 > 1/10$. For example, such a subject's probability vector might be (1/20, 3/20, 1/10, . . . , 1/10). Whatever a subject's probability vector, if it remains the same for the 180 choices and those choices are independently made then the multinomial distribution applies.

But different subjects avoid or prefer different digits, and therefore the under--lying probability vector will vary from subject to subject. Subjects (equivalently,

The correlation of counts X_m and X_j under the multinomial model:

$$Corr(X_m, X_j) = -\sqrt{\frac{p_m p_j}{(1-p_m)(1-p_j)}} \quad ; \quad j=1,...,k; \quad m=1,...,k; \quad m \neq j.$$

The correlation of counts X_m and X_j under the Dirichlet-multinomial model is the same if the Expected probabilities over subjects replace the constant probabilities of the multinomial model:

$$Corr(X_m, X_j) = -\sqrt{\frac{E(P_m)E(P_j)}{(1-E(P_m))(1-E(P_j))}} \quad ; \quad j=1,...,k; \quad m=1,...,k; \quad m \neq j.$$

Note that this last expression, in terms of the parameters $\lambda_1,...\lambda_k$, is:

$$-\sqrt{\frac{E(P_m)E(P_j)}{(1-E(P_m))(1-E(P_j))}} = -\sqrt{\frac{\lambda_m \lambda_j}{\left[\left(\sum_{j=1}^{k}\lambda_j\right)-\lambda_m\right]\left[\left(\sum_{j=1}^{k}\lambda_j\right)-\lambda_j\right]}}; \quad j=1,...,k; \quad m=1,...,k; \quad m \neq j.$$

Figure A2. Correlation coefficients of counts X_m *and* X_j *under the multinomial and Dirichlet-multinomial models*

probability vectors) may be thought of as sampled independently from some underlying statistical distribution. For example, the 46 subjects in this paper could be considered as a random sample of 46 probability vectors from an underlying distribution (the "mixing distribution") that describes the "between subject" variation in digit-preferences; that is in probability vectors, (p_0, p_1, . . . , p_9). If, subsequently, each subject's 180 digit-choices follow a multinomial distribution (so that the "within subject" variation is that of the multinomial distribution) then, across subjects, the distribution of numbers of digits is a mixture of multinomial distributions.

The Dirichlet-multinomial distribution (Figure A1) occurs when the distribution of the subjects' proportion vectors follows a Dirichlet distribution[4]. The Dirichlet-multinomial affords a "null model" for correlation coefficients where the variation across subjects is essentially uninteresting (Mosimann, 1962, 1963, 1970; James and Mosimann, 1980). All the pairwise correlations are negative, and are the same as those of the corresponding multinomial distribution where the constant multinomial probability vector, (p_0, p_1, . . . , p_9), is replaced by the vector ($E(P_0)$, $E(P_1)$, . . . , $E(P_9)$) of expected values of the probabilities across subjects (Figure A2).

Maximum likelihood estimation of the Dirichlet-multinomial parameters was performed using an iterative procedure (see, for example, Campbell and Mosimann (1987) who used a similar method with other Dirichlet models). An approximate procedure, using the z-transformation (Dixon & Massey, 1983, p. 224), was used to compare observed correlation coefficients with those of the Dirichlet-multinomial (the latter based on the maximum likelihood estimates). The maximum likelihood estimates of the parameters of the Dirichlet-multinomial distribution are given in Table A1. It is seen that the estimated proportions under the Dirichlet-multinomial model are very close to the observed proportions. Under the Dirichlet-

TABLE A1. Maximum likelihood estimates of the Dirichlet-multinomial parameters (lambda's) and corresponding proportions.

	Lambda estimate	Estimate's proportion	Observed proportion
FREQ0	17.94	.091	.093
FREQ1	26.88	.136	.137
FREQ2	23.65	.120	.120
FREQ3	20.95	.106	.106
FREQ4	18.89	.096	.096
FREQ5	15.25	.077	.077
FREQ6	19.17	.097	.097
FREQ7	18.69	.095	.094
FREQ8	16.95	.086	.085
FREQ9	18.71	.095	.095

multinomial model, each variance is the same multiple (by the constant CM of Figure 1A) of its "multinomial-type" variance. The estimated constant is 1.904. Table A2 contrasts the observed variance with estimates of the multinomial-type variance and the Dirichlet-multinomial variance.

Figure A3 contains the equations for the likelihood and its derivatives. Tables A2 and A3 contrast the observed covariance matrix and the estimated covariance matrix under the Dirichlet-multinomial model.

TABLE A2. Comparison of observed variance with the corresponding multinomial-type variance based on the mean proportions over subjects.

	Observed variance (O)	Multinomial-type variance (M)	Ratio O/M	Dirichlet-multinomial variance (D) (D/M) = 1.9
FREQ0	49.8	15.0	3.3	28.4
FREQ1	52.5	21.2	2.5	40.4
FREQ2	30.6	19.0	1.6	36.2
FREQ3	23.2	17.1	1.4	32.6
FREQ4	32.5	15.6	2.1	29.7
FREQ5	23.1	12.9	1.8	24.5
FREQ6	24.4	15.8	1.5	30.1
FREQ7	18.9	15.5	1.2	29.4
FREQ8	19.5	14.2	1.4	26.9
FREQ9	34.0	15.5	2.2	29.4

The common ratio under the Dirichlet-multinomial model based on the maximum likelihood estimates is 1.9 (D/M) in the table (D/M here is denoted C_M in Figure A1). To the accuracy shown, 1.9 is also the average of the ratios in the column labeled (O/M). The ratios in this latter column are the same, to the accuracy shown, whether estimates of the multinomial-type variances, column (M), were based on the observed proportions or on the estimated lambdas (Table A1).

TABLE A3. Observed covariance matrix for digits (N = 46).

	FREQ0	FREQ1	FREQ2	FREQ3	FREQ4	FREQ5	FREQ6	FREQ7	FREQ8	FREQ9
FREQ0	49.78									
FREQ1	3.93	52.45								
FREQ2	−1.24	−10.67	30.61							
FREQ3	−5.28	−5.33	2.39	23.22						
FREQ4	−19.91	−10.35	−6.11	−5.72	32.54					
FREQ5	3.30	−1.03	1.43	−2.49	−0.57	23.06				
FREQ6	−13.41	−7.62	−0.71	−1.48	−0.17	−3.38	24.38			
FREQ7	−11.80	−13.62	−0.21	−0.60	10.56	−5.27	3.10	18.86		
FREQ8	−7.99	−9.31	−4.98	1.61	2.94	−8.99	4.84	3.98	19.48	
FREQ9	2.62	1.56	−10.49	−6.34	−3.20	−6.06	−5.55	−4.99	−1.58	34.03

TABLE A4. Dirichlet-multinomial covariance matrix as determined from the maximum likelihood estimates of the parameters $\lambda_1, \ldots, \lambda_{10}$.

	FREQ0	FREQ1	FREQ2	FREQ3	FREQ4	FREQ5	FREQ6	FREQ7	FREQ8	FREQ9
FREQ0	28.36									
FREQ1	−4.25	40.36								
FREQ2	−3.74	−5.61	36.19							
FREQ3	−3.32	−4.97	−4.37	32.55						
FREQ4	−2.99	−4.48	−3.94	−3.49	29.69					
FREQ5	−2.41	−3.62	−3.18	−2.82	−2.54	24.4				
FREQ6	−3.03	−4.55	−4.00	−3.54	−3.19	−2.58	30.09			
FREQ7	−2.96	−4.43	−3.90	−3.45	−3.11	−2.52	−3.16	29.42		
FREQ8	−2.68	−4.02	−3.54	−3.13	−2.82	−2.28	−2.87	−2.79	26.93	
FREQ9	−2.96	−4.44	−3.90	−3.46	−3.12	−2.52	−3.16	−3.08	−2.80	29.44

Dirichlet-multinomial probability mass function for n choices by the ith subject

$$p(x_{i1},\ldots,x_{ik}|\lambda_1,\ldots,\lambda_k,n)=\left[\frac{n!\,\Gamma\left(\sum_{j=1}^{k}\lambda_j\right)}{\Gamma\left(n+\sum_{j=1}^{k}\lambda_j\right)}\right]\left[\prod_{j=1}^{k}\frac{\Gamma(x_{ij}+\lambda_j)}{x_{ij}!\,\Gamma(\lambda_j)}\right];\qquad x_{ij}=0,1,\ldots,n;\quad \lambda_j>0;\quad j=1,\ldots,k;\quad \sum_{j=1}^{k}x_{ij}=n;\quad i=1,\ldots N.$$

Natural logarithm of the Likelihood function for N subjects:

$$\ln L=\left[\sum_{i=1}^{N}\sum_{j=1}^{k}\ln\Gamma(x_{ij}+\lambda_j)\right]+N\left[\ln\Gamma\left(\sum_{j=1}^{k}\lambda_j\right)-\ln\Gamma\left(n+\sum_{j=1}^{k}\lambda_j\right)-\sum_{j=1}^{k}\ln\Gamma(\lambda_j)\right]+\ln(\text{constant}).$$

Partial derivatives, and second order partial derivatives, of the likelihood function:

$$\frac{\partial \ln L}{\partial \lambda_l}=\left[\sum_{i=1}^{N}\Psi(x_{il}+\lambda_l)\right]+N\left[\Psi\left(\sum_{j=1}^{k}\lambda_j\right)-\Psi\left(n+\sum_{j=1}^{k}\lambda_j\right)-\Psi(\lambda_l)\right];\quad l=1,\ldots,k;$$

$$\frac{\partial^2 \ln L}{\partial^2 \lambda_l}=\left[\sum_{i=1}^{N}\Psi'(x_{il}+\lambda_l)\right]+N\left[\Psi'\left(\sum_{j=1}^{k}\lambda_j\right)-\Psi'\left(n+\sum_{j=1}^{k}\lambda_j\right)-\Psi'(\lambda_l)\right];\quad l=1,\ldots,k;$$

$$\frac{\partial^2 \ln L}{\partial \lambda_m \partial \lambda_l}=N\left[\Psi'\left(\sum_{j=1}^{k}\lambda_j\right)-\Psi'\left(n+\sum_{j=1}^{k}\lambda_j\right)\right];\qquad l=1,\ldots,k;\quad m=1,\ldots,k;\quad m\neq l.$$

Here *ln* denotes the natural logarithm, $\Gamma(\cdot)$ denotes the Gamma function, $\Psi(\cdot)$ denotes the Digamma function, and $\Psi'(\cdot)$ denotes the Trigamma function. Definitions and properties of the last three functions may be found in Davis (1964, p. 253 et seq.).

Figure A3. Expressions used in the maximum likelihood estimation of the parameters $\lambda_1, \ldots \lambda_k$, of the Dirichlet-multinomial distribution

APPENDIX 2. Digit selection by 46 subjects for the leftmost place.

SUB	FREQ0	FREQ1	FREQ2	FREQ3	FREQ4	FREQ5	FREQ6	FREQ7	FREQ8	FREQ9
1	3	8	9	10	4	3	11	0	8	4
2	3	5	8	6	11	6	6	2	6	7
3	2	11	6	7	6	6	8	6	3	5
4	4	6	8	10	5	5	11	3	2	6
5	6	5	6	5	8	8	5	2	6	9
6	3	5	7	6	10	8	7	7	5	2
7	6	7	5	5	6	6	5	6	8	6
8	2	6	11	7	5	5	9	6	5	4
9	4	10	10	9	2	2	6	9	3	5
10	5	9	11	7	6	1	8	5	2	6
11	3	8	10	5	5	3	7	6	8	5
12	14	9	8	1	11	1	5	3	2	6
13	2	10	11	5	2	3	7	7	11	2
14	7	7	7	9	2	4	10	6	4	4
15	2	9	5	7	6	6	8	5	4	8
16	4	6	2	3	6	7	4	7	8	13
17	7	6	12	5	6	8	3	5	3	5
18	7	6	8	7	6	4	8	6	3	5
19	5	5	7	7	9	7	7	5	2	6
20	4	9	15	10	1	3	12	1	2	3
21	5	6	3	10	6	4	7	9	3	7
22	5	7	5	6	6	5	9	5	6	6
23	7	8	6	6	6	7	3	6	7	4
24	5	14	5	4	5	6	3	10	5	3
25	2	9	6	2	6	7	11	5	9	3
26	7	8	4	4	6	5	7	4	4	11
27	5	6	12	5	5	4	7	7	4	5
28	7	12	2	5	6	4	4	8	2	10
29	2	8	5	7	5	9	6	6	6	6
30	3	8	7	11	6	3	6	5	4	7
31	10	11	5	7	3	6	6	2	5	5
32	2	1	5	8	11	6	9	5	8	5
33	5	5	11	7	11	6	2	2	3	8
34	0	4	5	10	5	7	6	10	8	5
35	3	9	11	9	6	5	5	6	3	3
36	2	3	2	9	13	7	5	8	4	7
37	4	4	14	5	6	9	5	8	2	3
38	2	11	14	10	3	8	5	2	2	3
39	5	5	6	10	7	4	9	4	5	5
40	7	10	7	8	5	5	3	5	6	4
41	4	6	7	8	6	5	5	7	5	7
42	1	6	10	9	4	5	7	6	7	5
43	4	5	7	5	5	7	5	11	4	7
44	5	7	6	7	6	4	7	7	7	4
45	12	9	3	10	2	4	5	4	5	6
46	4	6	10	6	5	6	7	7	4	5

(Subject ID numbers differ from their study numbers, and the order of listing bears no relation to membership in sample 1 or 2)

APPENDIX 2. Digit Selection by 46 Subjects for the Middle Place.

SUB	FREQ0	FREQ1	FREQ2	FREQ3	FREQ4	FREQ5	FREQ6	FREQ7	FREQ8	FREQ9
1	1	8	6	15	6	5	4	7	6	2
2	5	7	9	13	6	2	3	6	5	4
3	6	6	8	8	11	5	2	7	4	3
4	7	12	9	5	5	6	5	6	1	4
5	9	10	4	2	5	1	9	5	5	10
6	7	8	5	7	3	6	6	5	5	8
7	7	9	3	3	14	5	5	6	3	5
8	6	6	8	6	8	4	6	3	3	10
9	4	9	10	8	7	3	4	2	7	6
10	5	5	9	10	10	2	3	6	5	5
11	8	11	4	7	5	3	8	2	3	9
12	8	8	4	3	6	7	6	7	3	8
13	6	6	12	9	8	7	2	3	5	2
14	8	12	5	8	2	10	2	5	2	6
15	1	8	12	6	5	5	5	9	6	3
16	7	5	2	5	12	2	3	11	8	5
17	12	7	11	4	1	4	1	4	5	11
18	11	9	7	5	2	6	3	4	9	4
19	8	20	3	1	5	4	5	2	4	8
20	5	6	12	5	0	5	13	3	6	5
21	7	5	10	4	7	7	7	2	9	2
22	9	8	9	5	10	1	3	9	5	1
23	4	8	7	5	5	4	5	6	7	9
24	10	10	7	1	3	1	11	2	9	6
25	7	18	6	8	4	5	4	5	2	1
26	8	5	1	6	6	4	9	8	5	8
27	6	12	3	7	6	2	7	3	6	8
28	11	13	4	4	9	3	1	4	5	6
29	5	12	10	6	8	1	9	3	3	3
30	7	4	9	4	5	3	8	4	8	8
31	16	12	7	2	5	10	3	1	0	4
32	7	1	5	7	5	2	7	10	9	7
33	2	21	4	4	4	5	4	5	2	9
34	2	10	2	3	15	5	12	5	5	1
35	4	11	4	7	6	2	9	4	9	4
36	7	4	13	3	5	8	5	4	4	7
37	3	6	11	2	8	7	6	9	6	2
38	12	16	11	3	2	9	1	2	3	1
39	8	5	8	7	1	6	7	6	5	7
40	4	9	10	3	4	1	7	5	9	8
41	4	11	8	9	4	4	2	8	7	3
42	18	6	5	6	4	3	5	1	7	5
43	24	3	8	8	1	4	4	5	1	2
44	5	2	8	3	11	2	10	9	4	6
45	4	3	19	4	3	5	5	2	3	12
46	5	5	8	3	5	6	4	7	5	12

APPENDIX 2. Digit Selection by 46 Subjects for the Rightmost Place.

SUB	FREQ0	FREQ1	FREQ2	FREQ3	FREQ4	FREQ5	FREQ6	FREQ7	FREQ8	FREQ9
1	3	14	9	6	3	3	6	11	2	3
2	9	5	8	4	5	5	5	6	9	4
3	1	7	5	5	7	11	3	6	5	10
4	3	5	2	7	11	8	5	4	9	6
5	7	16	9	1	0	0	5	6	5	11
6	3	7	9	5	5	5	7	9	6	4
7	5	4	15	10	4	1	3	11	3	4
8	1	9	8	7	11	6	5	8	2	3
9	4	10	3	8	7	1	6	9	7	5
10	3	16	7	5	4	4	2	9	6	4
11	4	15	7	6	7	1	4	6	2	8
12	7	18	4	7	1	2	5	6	3	7
13	15	6	8	8	5	3	1	5	4	5
14	10	10	5	6	4	7	4	6	4	4
15	1	7	8	4	8	7	8	6	4	7
16	9	8	6	8	4	7	4	3	4	7
17	3	12	10	6	6	4	9	4	4	2
18	5	10	9	2	2	6	9	7	2	8
19	5	9	5	3	3	9	6	5	4	11
20	10	10	1	4	0	4	4	11	11	5
21	4	4	12	4	11	3	3	6	7	6
22	5	7	6	3	8	1	11	7	10	2
23	3	7	8	6	5	2	3	8	9	9
24	4	9	1	13	3	1	7	5	10	7
25	2	19	6	2	6	6	6	1	3	9
26	8	2	7	7	4	4	4	7	12	5
27	5	8	3	9	2	5	3	7	9	9
28	3	10	4	10	10	1	3	8	3	8
29	2	7	7	5	9	10	7	6	4	3
30	5	2	7	9	3	3	12	4	5	10
31	5	9	4	10	6	6	3	5	9	3
32	4	12	9	4	8	2	8	3	5	5
33	6	6	5	4	6	8	7	3	8	7
34	1	9	7	10	7	3	8	7	4	4
35	2	7	5	9	7	5	7	9	5	4
36	4	9	8	4	4	4	7	5	9	6
37	5	0	8	3	12	2	12	9	6	3
38	19	8	6	8	4	5	1	2	3	4
39	8	13	7	10	3	4	5	3	4	3
40	4	9	5	6	10	0	4	8	6	8
41	2	7	7	10	6	4	5	7	8	4
42	3	5	2	10	9	6	5	5	6	9
43	6	6	11	7	5	8	7	7	1	2
44	2	13	11	5	5	1	5	8	6	4
45	5	9	6	11	2	1	4	6	1	15
46	1	12	7	11	6	5	3	7	2	6

NOTES

1. "Misconduct in science is commonly referred to as fraud. But most legal interpretations of the term 'fraud' require evidence not only of intentional deception but also of injury or damage to victims. Proof of fraud in common law requires documentation of damage incurred by victims who relied on fabricated or falsified research results. Because this evidentiary standard seemed poorly suited to the methods of scientific research, 'misconduct in science' has become the common term of reference in both institutional and regulatory policy definitions" (National Academy of Sciences, 1992, p. 25).
2. Yule stated (1927, page 572), "It is obvious that the measurements available must cover a range considerable as compared with the unit represented by the final digit, or the frequency distribution of final digits will be affected by the form of the distribution of the measurements themselves."
3. The respondent in this case admitted to fabricating these data. Additionally, physical evidence proves that the counts were fabricated. The critical spots were not cut out from the experimental plates, and therefore were not counted in the scintillation counter. Scientific misconduct was found, and the respondent signed a voluntary exclusion from receiving federal funds for two years.
4. The Dirichlet distribution (cf. Figure A1) was referred to as the "multivariate beta distribution" in Mosimann (1962, 1963), and there the multinomial distribution "compounded" with the multivariate beta distribution refers to a Dirichlet-multinomial "mixture" in today's terminology. This mixture is also a form of the negative hypergeometric distribution (Patil, Boswell, Joshi and Ratnaparkhi, 1984, page 106).

REFERENCES

Benford, F. (1938). The law of anomalous numbers. *Proc. Am. Philos. Soc., 18,* 551–572.

Broad, W., & Wade, N. (1982). *Betrayers of the Truth: Fraud and Deceit in the Halls of Science.* New York: Simon and Schuster.

Budescu, D. V. (1987). A markov model for generation of random binary sequences. *Journal of Experimental psychology: Human Perception and Performance, 13,* 25–39.

Campbell, G., & Mosimann, J. E. (1987). Multivariate analysis of size and shape: Modelling with the Dirichlet Distribution. In R. M. Heiberger (Ed.), *Computer Science and Statistics: Proceedings of the Nineteenth Symposium on the Interface,* 93–101.

Chapanis, A. (1953). Random-number guessing behavior. *American Psychologist, 8,* 332. (abstract)

Davis, P. J. (1964). Gamma function and related functions. In *Handbook of Mathematical Functions.* Abramowitz, M. and Stegun, I. (Eds.) National Bureau of Standards, Applied Mathematics Series 55, 253–293.

Diaconis, P. (1977). The distribution of leading digits and uniform distribution mod 1. *The Annals of Probability,* 5, 72–81.

Dixon, W. J., & Massey, F. J. Jr. (1983). *Introduction to Statistical Analysis,* Fourth Edition. New York: McGraw-Hill.

Falk, R. (1981). The perception of randomness. In *Proceedings of the Fifth International Conference of the Psychology of Mathematical Education.* Grenoble, France. pp. 222–229.

Federal Register, Vol. 54, No. 151, Tuesday August 8, 1989. p. 32449.

Feller, W. (1966). *An Introduction to Probability Theory and its Applications, Vol II.* New York: John Wiley & Sons, Inc.

Heywood, S. (1972). The popular number seven or number preference. *Perceptual and Motor Skills,* 34, 357–358.

Hill, T. P. (1988). Random number guessing and the first digit phenomenom. *Psychological Reports,* 62, 967–971.

House Committee on Energy and Commerce, Subcommittee on Oversight and Investigations (1988). *Fraud in NIH grant programs: Hearing before the Subcommittee on Oversight and Investigations of the Committee on Energy and Commerce, House of Representatives,* 100th Cong., 2nd sess., April 12, 1988. Washington DC: United States Congress.

House Committee on Energy and Commerce, Subcommittee on Oversight and Investigations (1989). *Scientific fraud: Hearings before the Subcommittee on Oversight and Investigations of the Committee on Energy*

and Commerce, House of Representatives, 101st Cong., 1st sess., May 4 and 9, 1989. Washington DC: United States Congress.

House Committee on Energy and Commerce, Subcommittee on Oversight and Investigations (1990). *Maintaining the integrity of scientific research: Summary of a hearing before the Subcommittee on Oversight and Investigations transmitted to the Committee on Science, Space, and Technology, House of Representatives*, 101st Cong., 2nd sess. Washington DC: United States Congress.

James, I. R. & Mosimann, J. E. (1980). A new characterization of the Dirichlet distribution through neutrality. *Annals of Statistics, 8*, 183–189.

Kahneman, D. & Tversky, A. (1972). Subjective probability: A judgment of representativeness. *Cognitive Psychology, 3*, 430–454.

Lopes, L. L. (1982). Doing the impossible: A note on induction and the experience, of randomness. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 8*, 626–636.

Melendez (1966). Number preference as a variable influencing human Ss inability to generate a truly random series of numbers. *Perceptual and Motor Skills, 23*, 1201–1202.

Miller, David J., & Hersen, M. (1992). *Research Fraud in the Behavioral and Biomedical Sciences*. New York: John Wiley & Sons.

Mosimann, J. E. (1962). On the compound multinomial distribution, the multivariate Beta Distribution and correlations between proportions. *Biometrika, 49*, 65–82.

Mosimann, J. E. (1963). On the compound negative multinomial distribution and correlations among inversely sampled pollen counts. *Biometrika, 50*, 47–54.

Mosimann, J. E. (1970). Discrete distribution models arising in pollen studies. In Patil, G. P. (Ed.) *Random Counts in Scientific Work*, Volume 3. *Random Counts in Physical Sciences, Geoscience and Business*. The Pennsylvania State University Press, University Park Pennsylvania (pages 1–30).

Mosimann, J. E., & Ratnaparkhi, M. V. (1994). Folded rectangular distributions on finite intervals (unpublished manuscript).

National Academy of Sciences (1992). *Responsible Science: Ensuring the Integrity of the Research Process*, Volume I. Washington DC: National Academy Press.

National Institutes of Health (1992). *NIH Data Book*. Bethesda, MD: NIH. p. 2.

Office of Research Integrity (1993). *ORI Newsletter*, Volume 1, No. 4. Rockville, MD. (p. 4, bottom of first column and second column).

Patil, G. P., Boswell, M. T., Joshi, S. W., & Ratnaparkhi, M. V. (1984). *Dictionary and Classified Bibliography of Statistical Distributions in Scientific Work*, Volume 1, *Discrete Models*. International Co-operative Publishing House, Burtonsville, MD (page 106).

Preece, D. A. (1981). Distributions of final digits in data. *The Statistician*, 30, 31–60.

Rao, C. R. (1989). *Statistics and Truth*. International Co-operative Publishing House, Burtonsville, MD (Lecture 2 and Appendix).

Ross, B. M., & Levy, N. (1958). Patterned predictions of chance events by children and adults. *Psychological Reports, 4*, 87–124.

Rosenberg, S., Weber, N., Crocq, M., Duval, F., & Macher (1990). Random number generation by normal, alcoholics and schizophrenic subjects. *Psychological Medicine, 20*, 953–960.

Sarasohn, Judy, (1993). *Science on Trial: The Whistle-blower, the Accused, and the Nobel Laureate*. New York: St. Martin's Press.

Tune, G. S. (1964a). A brief survey of variables that influence random-generation. *Perceptual and Motor Skills, 18*, 705–710.

Tune, G. S. (1964b). Response preferences: A review of some relevant literature. *Psychological Bulletin, 4*, 286–302.

Wagenaar (1972). Generation of random sequences by human subjects: A critical survey of the literature. *Psychological Bulletin, 77*, 65–72.

Weiss, R. L. (1964). On producing random responses. *Psychological Reports, 14*, 931–941.

Winick, C. (1962). Preference for individual digits. *Journal of General Psychology, 67*, 271–281.

Yule, G. Udny (1927). On reading a scale. *Journal of the Royal Statistical Society, 90*, 570–587.

Analysis of coulter counter counts by Bishayee and Hill

If the numbers had 3 digits, units were analyzed
If the numbers had 4 digits, units and tens were analyzed

Exp		DIGITS	0	1	2	3	4	5	6	7	8	9	Total	Probability that digits are uniform	One chance in _____ that digits are uniform
Bishayee	9/24/99	Coulter-units	2	6	5	0	0	1	1	5	2	8	30		
	9/27/99	Coulter-units	0	9	7	1	1	4	4	0	0	4	30		
	10/1/99	Coulter-units	1	5	8	1	1	3	4	2	0	5	30		
	10/4/99	Coulter-units	2	3	11	2	0	3	3	1	2	3	30		
	12/20/99	Coulter-units	7	6	3	4	3	5	2	2	3	7	42		
	2/28/00	Coulter-units	6	6	7	1	1	4	2	2	1	6	36		
TOTAL			18	35	41	9	6	20	16	12	8	33	198	1.76E-11	56,757,605,284.90
	Control for Bishayee		19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8			
Hill	9/13/99	Coulter-units	4	3	3	2	5	5	2	2	2	2	30		
		Coulter-tens	3	1	5	3	3	3	3	2	5	2	30		
	9/17/99	Coulter-units	3	0	2	4	7	7	2	7	3	0	35		
		Coulter-tens	4	6	1	3	2	8	2	4	2	3	35		
	12/6/99	Coulter-units	0	3	6	3	3	2	3	4	4	2	30		
TOTAL			14	13	17	15	20	25	12	19	16	9	160	0.235	4.25
	Control for Hill		16	16	16	16	16	16	16	16	16	16			
Lenarczyk	12/26/02	Coulter-units	3	3	6	1	1	3	3	4	1	2	27		
		Coulter-tens	1	5	3	2	2	0	3	5	3	3	27		
	2/5/01	Coulter-units	2	4	2	3	2	4	5	3	2	3	30		
		Coulter-tens	7	1	3	2	1	5	5	2	3	1	30		
	1/15/01	Coulter-units	3	4	1	3	3	2	5	4	4	1	30		
		Coulter-tens	4	2	3	2	3	2	2	1	4	7	30		
	6/14/01	Coulter-units	7	0	5	2	3	2	4	2	3	2	30		
		Coulter-tens	2	5	2	5	1	1	2	3	4	5	30		
	RExp3	Coulter-units	3	3	2	1	5	3	4	3	2	4	30		
		Coulter-tens	4	4	2	2	3	2	4	3	2	4	30		
	RExp4	Coulter-units	2	1	1	4	2	3	2	1	5	3	24		
		Coulter-tens	5	1	3	2	1	1	2	2	4	3	24		
	RExp5	Coulter-units	1	5	2	5	5	1	4	4	1	1	29		
		Coulter-tens	4	4	1	3	3	4	3	4	3	0	29		
	A&M ATCC	Coulter-units	0	4	5	2	3	2	5	4	3	1	29		
		Coulter-tens	2	4	3	3	2	1	8	1	0	5	29		
			50	50	44	42	40	36	61	46	44	45	458	0.426	2.34
	Control for Lenarczyk		45.8	45.8	45.8	45.8	45.8	45.8	45.8	45.8	45.8	45.8			

tabbies

EXHIBIT 12

Scintillation Counts

3/24/00	1	2	3	4	5	6	7	8	9	0	
units	4	1	2	1	3	3	2	4	2	2	24
tens	3	4	3	5	4	2	1	1	0	1	24
hundreds	2	1	3	1	1	3	2	0	0	2	15
All	9	6	8	7	8	8	5	5	2	5	63
ctrl	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	
	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	

chi sq	
units	0.888136592
tens	0.337161966
hundreds	0.637119423
All	0.70289624

3/31/00											
units	2	1	1	2	3	2	4	0	2	7	24
tens	2	1	5	2	3	1	2	0	2	3	21
hundreds	0	3	2	2	1	1	1	0	0	2	12
All	4	5	8	6	7	4	7	0	4	12	57
ctrl	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	
	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	
	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	
	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	

chi sq		
units	0.110952168	
tens	0.52935461	
hundreds	0.53414624	
All	0.07102258	1 chance in 14 tries

3/28/00											
units	3	2	0	0	1	3	5	3	4	3	24
tens	4	0	3	1	1	0	4	2	0	0	15
all	7	2	3	1	2	3	9	5	4	3	39
ctrl	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	
	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	

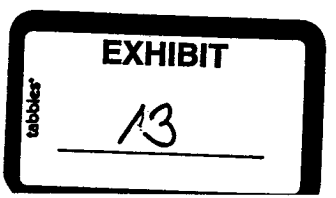
chi sq		
units	0.337161966	
tens	0.060238672	1 chance in 16.6 tries
all	0.119615115	

3/27/00											
units	6	0	3	1	0	2	2	2	4	3	23
tens	0	1	3	2	1	0	1	2	1	1	12
all	6	1	6	3	1	2	3	4	5	4	35
ctrl	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	
	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	
	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	

chi sq		
units	0.158710889	
tens	0.706148716	
all	0.464055201	

Grand Total											
all all	26	14	25	17	18	17	24	14	15	24	194
ctrl all	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	

chi sq all	0.293789705



Coulter Counter replicates sent by Dr. Lenarczyk

Exp Date				Average	Std	sqrt(av)	Ratio std/av
12/26/00	2780	2723	2534	2679.00	128.77	51.76	2.49
	2807	2830	2783	2806.67	23.50	52.98	0.44
	2653	2686	2607	2648.67	39.68	51.47	0.77
	2891	2883	2822	2865.33	37.74	53.53	0.71
	2428	2515	2576	2506.33	74.38	50.06	1.49
	2773	2728	2783	2761.33	29.30	52.55	0.56
	3192	3252	3131	3191.67	60.50	56.49	1.07
	2878	2891	2747	2838.67	79.65	53.28	1.49
	2926	2949	2908	2927.67	20.55	54.11	0.38
average					**54.90**	**52.91**	**1.04**
2/5/01	9086	8946	9076	9036.00	78.10	95.06	0.82
	8917	8917	8867	8900.33	28.87	94.34	0.31
	8917	9114	9002	9011.00	98.81	94.93	1.04
	9448	9392	9363	9401.00	43.21	96.96	0.45
	8884	8632	8699	8738.33	130.52	93.48	1.40
	8078	8171	8017	8088.67	77.55	89.94	0.86
	7868	7929	7830	7875.67	49.94	88.74	0.56
	8354	8632	8271	8419.00	189.07	91.76	2.06
	8760	8515	8415	8563.33	177.51	92.54	1.92
	6490	6666	6773	6643.00	142.90	81.50	1.75
average					**101.65**	**91.92**	**1.11**
1/15/01	8671	8454	8777	8634.00	164.65	92.92	1.77
	8598	8337	8448	8461.00	130.98	91.98	1.42
	6693	6596	6618	6635.67	50.86	81.46	0.62
	5301	5609	5520	5476.67	158.51	74.00	2.14
	2225	2699	2464	2462.67	237.00	49.63	4.78
	6458	6859	6964	6760.33	267.04	82.22	3.25
	6002	6437	6317	6252.00	224.67	79.07	2.84
	9216	9905	9345	9488.67	366.28	97.41	3.76
	7031	7112	7182	7108.33	75.57	84.31	0.90
	6309	6002	6007	6106.00	175.82	78.14	2.25
average					**185.14**	**81.11**	**2.28**
6/14/01	9698	9493	9590	9593.67	102.55	97.95	1.05
	9431	9585	9579	9531.67	87.23	97.63	0.89
	8615	8504	8509	8542.67	62.69	92.43	0.68
	7846	7901	7781	7842.67	60.07	88.56	0.68
	6623	6463	6314	6466.67	154.53	80.42	1.92
					27.15	82.03	0.33

Coulter Counter replicates from Bishayee's Experiments

Exp Date				Average	Std	sqrt(av)	Ratio std/av
12/20/99	545	567	551	554.33	11.37	23.54	0.48
	552	543	541	545.33	5.86	23.35	0.25
	468	455	449	457.33	9.71	21.39	0.45
	649	683	666	666.00	17.00	25.81	0.66
	561	523	550	544.67	19.55	23.34	0.84
	605	590	603	599.33	8.14	24.48	0.33
	588	574	614	592.00	20.30	24.33	0.83
	750	767	739	752.00	14.11	27.42	0.51
	789	772	769	776.67	10.79	27.87	0.39
	721	719	710	716.67	5.86	26.77	0.22
	630	666	639	645.00	18.73	25.40	0.74
	612	631	645	629.33	16.56	25.09	0.66
	565	574	591	576.67	13.20	24.01	0.55
	638	610	580	609.33	29.01	24.68	1.18
Average					**14.30**	**24.82**	**0.58**
Oct 1, 1999	772	761	756	763.00	8.19	27.62	0.30
	666	655	677	666.00	11.00	25.81	0.43
	701	711	722	711.33	10.50	26.67	0.39
	656	631	634	640.33	13.65	25.30	0.54
	732	745	739	738.67	6.51	27.18	0.24
	741	756	762	753.00	10.82	27.44	0.39
	635	659	662	652.00	14.80	25.53	0.58
	672	657	659	662.67	8.14	25.74	0.32
	713	732	742	729.00	14.73	27.00	0.55
	699	710	729	712.67	15.18	26.70	0.57
overall average				**702.87**	**11.35**	**26.50**	**0.43**
Sep 24, 199	589	598	571	586.00	13.75	24.21	0.57
	611	627	631	623.00	10.58	24.96	0.42
	541	559	561	553.67	11.02	23.53	0.47
	629	642	629	633.33	7.51	25.17	0.30
	667	656	672	665.00	8.19	25.79	0.32
	542	561	559	554.00	10.44	23.54	0.44
	620	635	642	632.33	11.24	25.15	0.45
	529	549	557	545.00	14.42	23.35	0.62
	607	598	622	609.00	12.12	24.68	0.49
	511	509	507	509.00	2.00	22.56	0.09
				591.03	**10.13**	**24.29**	**0.42**
Sep 27, 199	411	431	435	425.67	12.86	20.63	0.62
	471	461	459	463.67	6.43	21.53	0.30
				374.33	13.65	19.35	0.71

EXHIBIT 14 Labbies

	6623	6463	6314	6466.67	154.53	80.42	1.92
	6725	6757	6703	6728.33	27.15	82.03	0.33
	6821	6891	6741	6817.67	75.06	82.57	0.91
	6277	6426	6108	6270.33	159.10	79.19	2.01
	6437	6187	6240	6288.00	131.73	79.30	1.66
average					**94.62**	**87.14**	**1.09**
RExp3	8985	8822	8917	8908.00	81.87	94.38	0.87
	9664	9567	9312	9514.33	181.81	97.54	1.86
	9476	9505	9323	9434.67	97.79	97.13	1.01
	7770	7907	7819	7832.00	69.42	88.50	0.78
	7901	7830	7715	7815.33	93.86	88.40	1.06
	7279	7241	7095	7205.00	97.14	84.88	1.14
	7231	7150	7128	7169.67	54.24	84.67	0.64
	7590	7508	7655	7584.33	73.66	87.09	0.85
	6853	6826	6447	6708.67	227.01	81.91	2.77
	7188	6886	6972	7015.33	155.59	83.76	1.86
average					**113.24**	**88.83**	**1.27**
RExp4	9779	9910	9659	9782.67	125.54	98.91	1.27
	9996	10014	10072	10027.33	39.72	100.14	0.40
	9369	9004	8934	9102.33	233.58	95.41	2.45
	9687	9505	9647	9613.00	95.65	98.05	0.98
	9080	9335	9210	9208.33	127.51	95.96	1.33
	9613	9596	9431	9546.67	100.53	97.71	1.03
	8609	8598	8421	8542.67	105.51	92.43	1.14
	8744	8699	8716	8719.67	22.72	93.38	0.24
average					**106.34**	**96.50**	**1.10**
RExp5	4827	4878	4587	4764.00	155.39	69.02	2.25
	5348	5384	5312	5348.00	36.00	73.13	0.49
	4752	4898	4672	4774.00	114.59	69.09	1.66
	5120	4944	4934	4999.33	104.62	70.71	1.48
	5457	5625	5562	5548.00	84.87	74.48	1.14
	5265	5084		5174.50	127.99	71.93	1.78
	4929	4878	4753	4853.33	90.56	69.67	1.30
	5115	4965	5094	5058.00	81.22	71.12	1.14
	4615	4711	4583	4636.33	66.61	68.09	0.98
	4662	4596	4647	4635.00	34.60	68.08	0.51
average					**89.65**	**70.53**	**1.27**
A&M ATTC	4873	4965	4933	4923.67	46.70	70.17	0.67
	4226	4377	4142	4248.33	119.08	65.18	1.83
	4918	4874	5043	4945.00	87.68	70.32	1.25
	4777	4802	4712	4763.67	46.46	69.02	0.67
	4429	4375	4438	4414.00	34.07	66.44	0.51
	3883	3776	3929	3862.67	78.50	62.15	1.26
	4333	4442	4358	4377.67	57.10	66.16	0.86
	3505	3507	3370	3460.67	78.53	58.83	1.33
	4404	4177	4329	4303.33	115.66	65.60	1.76
	3908	*3857*		*3882.50*	*36.06*	62.31	0.58
average					**69.98**	**65.62**	**1.07**

Sep 27, 199	411	431	435	425.67	12.86	20.63	0.62
	471	461	459	463.67	6.43	21.53	0.30
	389	362	372	374.33	13.65	19.35	0.71
	332	321	341	331.33	10.02	18.20	0.55
	441	456	465	454.00	12.12	21.31	0.57
	432	444	456	444.00	12.00	21.07	0.57
	409	422	436	422.33	13.50	20.55	0.66
	381	392	401	391.33	10.02	19.78	0.51
	356	365	369	363.33	6.66	19.06	0.35
	403	372	385	386.67	15.57	19.66	0.79
				405.67	**11.28**	**20.12**	**0.56**
Oct 4,1999	499	488	502	496.33	7.37	22.28	0.33
	436	456	462	451.33	13.61	21.24	0.64
	522	532	542	532.00	10.00	23.07	0.43
	536	542	539	539.00	3.00	23.22	0.13
	561	572	585	572.67	12.01	23.93	0.50
	437	452	462	450.33	12.58	21.22	0.59
	501	533	529	521.00	17.44	22.83	0.76
	490	471	478	479.67	9.61	21.90	0.44
	522	535	542	533.00	10.15	23.09	0.44
	531	555	563	549.67	16.65	23.44	0.71
				512.50	**11.24**	**22.62**	**0.50**

6586	6628	6538	6584.00	45.03	81.14	0.55
6639	6918	6735	6764.00	141.74	82.24	1.72
6245	6282	6161	6229.33	62.00	78.93	0.79
6956	6709	6923	6862.67	134.10	82.84	1.62
6367	6431	6532	6443.33	83.19	80.27	1.04
4597	4525	4148	4423.33	241.15	66.51	3.63
6452	6373	6309	6378.00	71.63	79.86	0.90
6655	6810	6805	6756.67	88.08	82.20	1.07
				98.71	**78.96**	**1.29**

average of ratios of standard deviations to square roots of the averages of Coulter counts by Lenarczyk and by Bishayee

Lenarczyk: **1.28**

Bishayee **0.50**

9431	9585	9579	9531.67	102.55	97.95	1.05
8615	8504	8509	8542.67	87.23	97.63	0.89
7846	7901	7781		62.69	92.43	0.68

620	635	642	632.33	11.24	25.15	0.45
529	549	557	545.00	14.42	23.35	0.62
607	598	622	609.00			

Is There a Bystander Effect for ^{3}H?

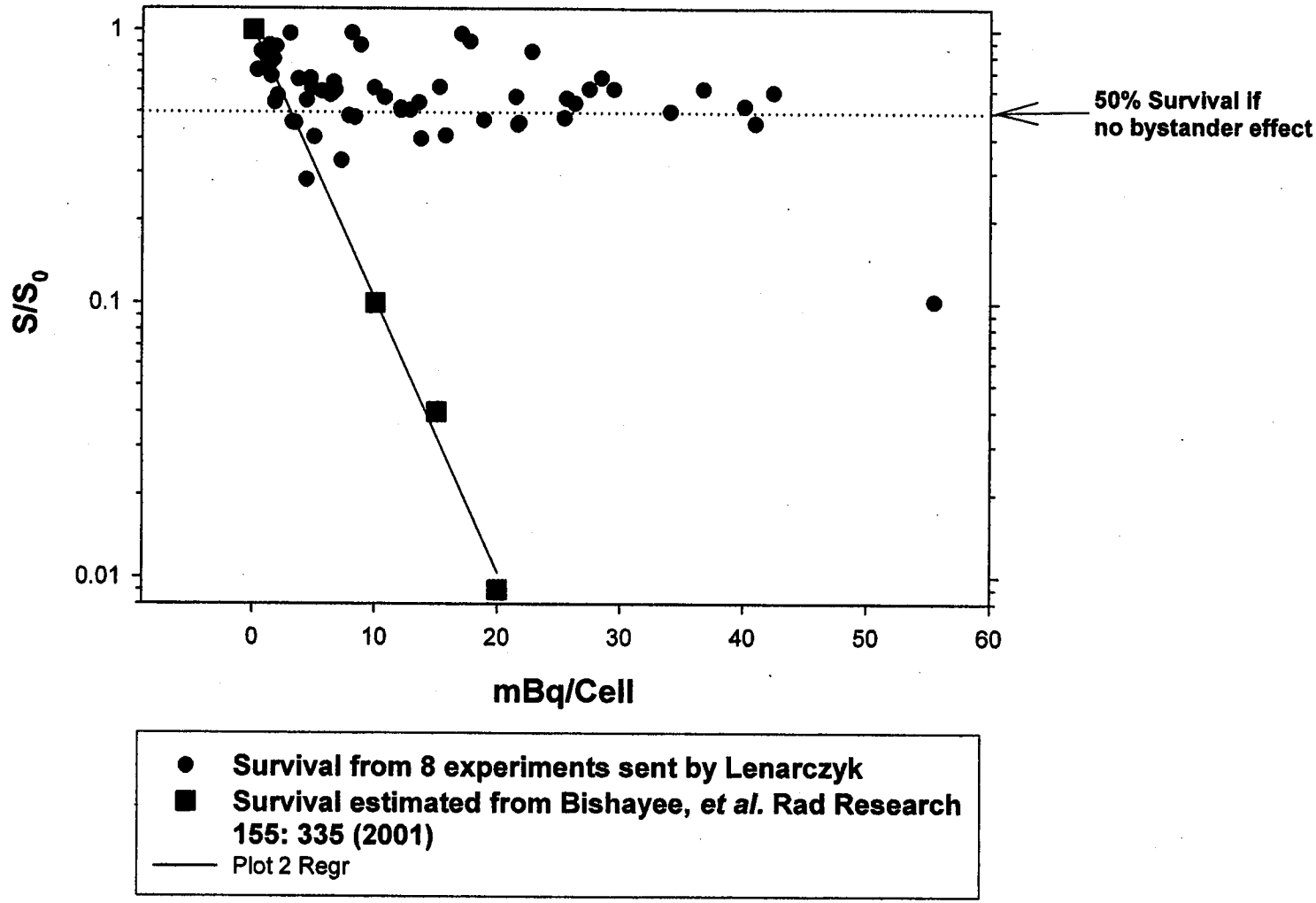


EXHIBIT 15

RADIATION RESEARCH 152, 88–97 (1999)
0033-7587/99 $5.00
© 1999 by Radiation Research Society.
All rights of reproduction in any form reserved.

Evidence for Pronounced Bystander Effects Caused by Nonuniform Distributions of Radioactivity using a Novel Three-Dimensional Tissue Culture Model

Anupam Bishayee, Dandamudi V. Rao and Roger W. Howell[1]

Division of Radiation Research, Department of Radiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103

Bishayee, A., Rao, D. V. and Howell, R. W. Evidence for Pronounced Bystander Effects Caused by Nonuniform Distributions of Radioactivity using a Novel Three-Dimensional Tissue Culture Model. *Radiat. Res.* 152, 88–97 (1999).

A new *in vitro* multicellular cluster model has been developed to assess the impact of nonuniform distributions of radioactivity on the biological response of mammalian cells, and the relative importance of bystander effects compared to conventional radiation effects. Chinese hamster V79 cells are labeled with tritiated thymidine ([^{3}H]dThd), mixed with unlabeled V79 cells, and centrifuged gently to form multicellular clusters about 1.6 mm in diameter. The short range of the ^{3}H β particles effectively allows only self-irradiation of labeled cells and no cross-irradiation of unlabeled cells. The percentage of labeled cells is controlled precisely by varying the number of labeled cells mixed with unlabeled cells prior to assembling the cluster. The clusters are assembled in the absence or presence of 100 μ*M* lindane, a chemical that is known to inhibit formation of gap junctions. After the clusters are maintained at 10.5°C for 72 h, the cells are dispersed and plated for colony formation. In the case of 100% labeling, the survival of cells in the cluster depends exponentially (SF = $e^{-A/1.8}$) on the cluster activity *A* (in kBq), and lindane has no effect on the response. A two-component exponential response is obtained for 50% labeling in the absence of lindane (SF = 0.33 $e^{-A/0.81}$ + 0.67 $e^{-A/11.8}$), and lindane has a marked effect on the response (SF = 0.33 $e^{-A/1.6}$ + 0.67 $e^{-A/41.6}$). These data suggest that bystander effects play an important role in the biological response of V79 cells when the ^{3}H is localized in the cell nucleus and distributed nonuniformly among the cells. In contrast, bystander effects cannot be detected above traditional radiation effects (i.e. direct + indirect) when the ^{3}H is localized in the cell nucleus and distributed uniformly among the cells. These results indicate that this multicellular cluster model is well suited for studying the effects of nonuniform distributions of radioactivity, including bystander and "hot-particle" effects. Furthermore, these results suggest that bystander effects may play an important role in the prediction of the biological effects of radiopharmaceuticals used in medical diagnosis and treatment. © 1999 by Radiation Research Society

INTRODUCTION

Over the past several years there have been several reports that cells that have received no radiation exposure suffer biological consequences when they are in the presence of cells that have been irradiated (*1–5*). This phenomenon has been termed the bystander effect. Nagasawa and Little (*1*) used acute external beams of α particles to irradiate monolayers of Chinese hamster ovary cells with low doses. These exposures led to the formation of sister chromatid exchanges in 30–50% of the cells despite the fact that statistically only about 1% of the cell nuclei could have been traversed by an α particle. They concluded that genetic damage can be imparted to "bystander" cells when cell populations are exposed to low doses from α particles. Similar observations were made by Deshpande *et al.* (*2*). Azzam *et al.* (*5*) studied the mechanisms of the bystander effect by showing that the expression levels of TP53, CDKN1A, CDC2, CCNB1 and RAD51 are significantly modulated when human diploid cell populations are irradiated with low doses of α particles where only a small fraction of the nuclei are actually hit. They also found that the extent of modulation was significantly reduced when lindane, an inhibitor of gap-junction intercellular communication (*6, 7*), was present during the irradiation period. These data suggest that gap-junction intercellular communication may play an important role in the bystander effect.

While the studies described above involve the use of α particles, Mothersill and Seymour (*4*) have irradiated cells with γ rays to study the bystander effect. In this case, the gap-junction inhibitor phorbol myristate acid actually increased killing by the bystander effect. Based on these data, they suggested that signal transduction mechanisms, as op-

[1] Author to whom correspondence and requests for reprints should be addressed.

EXHIBIT
tabbies
16

posed to the release of a factor that is directly cytotoxic, may control death or survival due to the bystander effect.

The studies of Nagasawa and Little (*1*), Deshpande *et al.* (*2*) and Mothersill and Seymour (*4*) raise interesting points regarding the biological effects of ionizing radiation, in particular the bystander effect. As noted by these authors and others (*3, 8, 9*), the bystander effect is particularly relevant to the "hot-particle" problem as well as the biological effects of incorporated radionuclides in general. However, there remain several aspects to be addressed such as: (1) What is the significance of the bystander effect compared to the overall effect to the cell when it experiences damage from both bystander and traditional radiation effects (i.e. direct + indirect)? (2) Can bystander effects be observed in three-dimensional tissue models? (3) Do bystander effects indeed result from nonuniform distributions of radioactivity? (4) If so, what types of ionizing radiation produce significant bystander effects? The present work attempts to address some of these questions using a novel three-dimensional cell culture model and precisely controlled nonuniform distributions of incorporated radionuclides to deliver radiation exposures.

MATERIALS AND METHODS

Radiochemical and Quantification of Radioactivity

Tritiated thymidine ([^{3}H]dThd) was obtained from NEN Life Science Products (Boston, MA) as a sterile aqueous solution at a concentration of 37 MBq/ml and a specific activity of 3000 GBq/mmol. The activity of ^{3}H was measured with a Beckman LS3800 automatic liquid scintillation counter (Fullerton, CA) by transferring aliquots of radioactive culture medium into 6 ml of Aquasol™ liquid scintillation cocktail (NEN Research Products, Boston, MA). The detection efficiency for the 5.7 keV β particles emitted by ^{3}H was 0.65. The radionuclide ^{3}H has a physical half-life of 12.3 years and emits β particles with a mean energy of 5.67 keV (*10*) corresponding to a mean range in water of about 1 μm (*11*).

Cell Line

Chinese hamster V79 lung fibroblasts (kindly provided by A. I. Kassis, Harvard Medical School, Boston, MA) were used in the present study, with clonogenic survival serving as the biological end point. V79 cells are known to exhibit some degree of gap-junction intercellular communication at 37°C (*12, 13*). The cells were cultured in minimum essential medium (MEM) supplemented with 10% heat-inactivated (57°C, 30 min) fetal calf serum with 2 m*M* L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (MEMA). The pH of the culture medium was adjusted to 7.0 with $NaHCO_3$. All media and supplements used in this study were from Life Technologies (Grand Island, NY). Cells were maintained in 175-cm^2 Falcon sterile tissue culture flasks (Becton Dickinson, Lincoln Park, NJ) at 37°C and 5% CO_2, 95% air, and were subcultured twice weekly or as required.

Radiolabeling and Assembly of Multicellular Clusters with 50% of Cells Labeled

V79 cells growing as monolayers in 175-cm^2 Falcon flasks were washed with 10 ml of phosphate-buffered saline, trypsinized with 0.05% trypsin–0.53 m*M* EDTA, and suspended at 2 × 10^6 cells/ml in calcium-free MEM with 10% heat-inactivated (57°C, 30 min) fetal calf serum, 2 m*M* L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (MEMB). Aliquots of 1 ml were placed in two sets of sterile 17 × 100-mm Falcon polypropylene round-bottom culture tubes (10 tubes in each set) and placed on a rocker-roller (Fisher Scientific, Springfield, NJ) for 3–4 h at 37°C in an atmosphere of 95% air and 5% CO_2. After this conditioning period, 1 ml of MEMB containing various activity concentrations (0–296 MBq) of [^{3}H]dThd was added to the first set of culture tubes containing 1 ml of V79 cells. Only 1 ml of MEMB was added to the other set of tubes. All tubes were then returned to the rocker-roller at 37°C, 95% air and 5% CO_2. After a 12-h period of labeling with radioactivity, the first set of tubes were removed and centrifuged at 2000 rpm at 4°C for 10 min. Aliquots of the supernatant were used to check the concentrations of radioactivity added. The cells were washed three times with 10 ml of MEM with 10% heat-inactivated (57°C, 30 min) calf serum, 2 m*M* L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (wash MEMA). The cells in the second set of tubes (unlabeled) were similarly washed and the contents of a given tube transferred to one of the first set of tubes containing radiolabeled cells. Finally, the pooled cells in each tube were suspended in 400 μl of MEMA or 0.58% DMSO (Sigma Chemical Co., St. Louis, MO) in MEMA or 0.58% DMSO–100 μ*M* lindane (hexachlorocyclohexane, γ-isomer from Sigma) and transferred directly to a sterile 400-μl polypropylene microcentrifuge tube with attached cap (Helena Plastics, San Rafael, CA) (Fig. 1). The concentration of lindane (i.e. 100 μ*M*) was selected based on a separate study as described below, and DMSO served as a control for lindane. The 400-μl tubes were centrifuged at 1000 rpm for 5 min at 4°C to form a multicellular cluster ~1.6 mm in diameter. The resulting clusters contained a total of 4 × 10^6 cells, of which 50% were labeled (Fig. 1). The capped

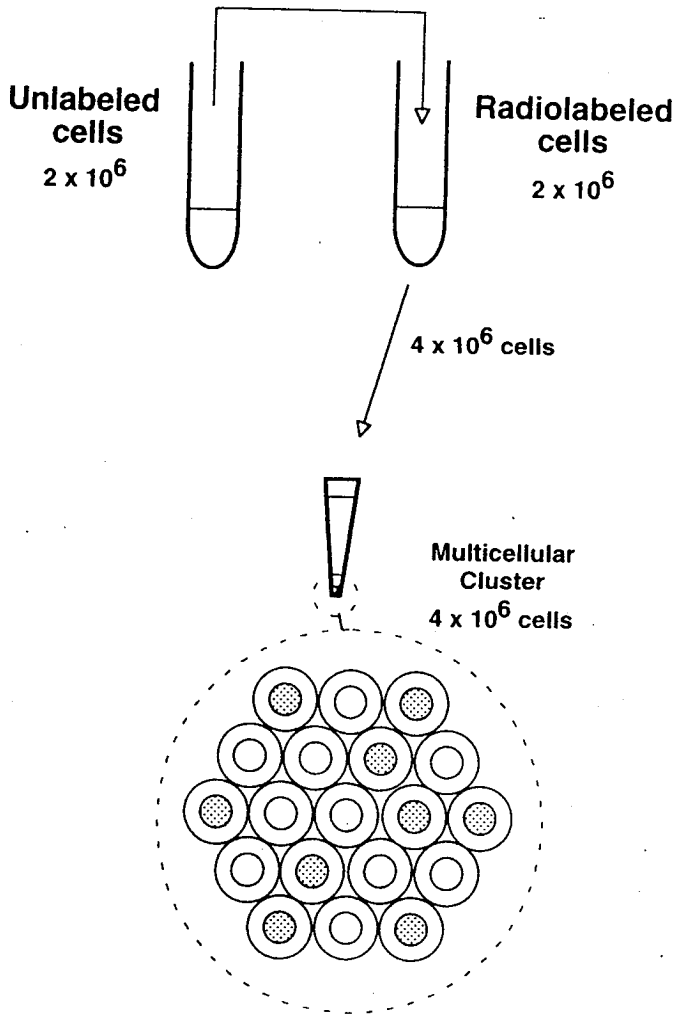


FIG. 1. Assembly of multicellular cluster of V79 cells in which 50% of the cells are radiolabeled with [^{3}H]dThd.

microcentrifuge tubes containing the clusters were placed in a perforated microcentrifuge tube rack and transferred to a refrigerator at 10.5°C. This temperature was selected because V79 cells can remain in the cluster configuration at this temperature for long periods (up to 72 h) without a decrease in plating efficiency. This was also true for V79 cells in suspension culture (*14*). Therefore, the cells accumulate the preponderance of their radioactive decays while in the cluster configuration as opposed to the radiolabeling and colony-forming periods. After 72 h at 10.5°C, the supernatant was carefully removed and the tube was vortexed to disperse the cell cluster. The cells were resuspended in MEMA, transferred to 17 × 100-mm Falcon polypropylene tubes, washed three times with 10 ml of wash MEMA, resuspended in 2 ml of MEMA, passed through a 21-gauge needle five times to disperse cells, and serially diluted (four 10× dilutions), and 1 ml of the appropriate dilutions (approximately 200 cells for control tubes) was seeded in triplicate into 60 × 15-mm Falcon tissue culture dishes. The dishes were then placed in an incubator at 37°C with 95% air and 5% CO_2. Aliquots were taken from each tube before serial dilution, and the mean radioactivity per cell was determined (*15*). The tissue culture dishes were removed from the incubator after 1 week, and the resulting colonies were washed 3 times with normal saline and 2 times with methanol and finally stained with 0.05% crystal violet. The colonies were counted under fluorescent light. A colony count of 25–250 was considered as a valid data point for each tissue culture dish. The surviving fraction compared to the parallel control was determined for each radioactivity concentration employed.

Chemotoxicity and Optimum Concentration of Lindane

Multicellular clusters were prepared wherein 50% of the cells were labeled with a fixed activity concentration of [^{3}H]dThd (148 MBq/ml) as described above. The clusters were maintained at 10.5°C for 72 h in the presence of 20–200 μ*M* of lindane. To achieve this, lindane was first dissolved in DMSO (5 mg/ml), filtered through a Millex®-HV filter (Millipore Corporation, Bedford, MA), and subsequently diluted with MEMA to a final concentration 20–200 μ*M* lindane, 0.58% DMSO. Parallel controls were established where clusters of unlabeled cells were maintained at the same concentrations of lindane with 0.58% DMSO. Thus, for each concentration of lindane, two tubes were prepared—one having a cluster of radiolabeled cells (50%) and one having a cluster of unlabeled cells. After 72 h the cluster was dismantled, the mean activity per cell was determined, and the cell survival was compared to that of its matched control using the procedure outlined above.

Assembly of Multicellular Clusters with 100% Radiolabeled Cells

Multicellular clusters in which 100% of the cells were radiolabeled were assembled using the cells prepared as above. In short, 1 ml of MEMB containing different concentrations of radioactivity was added to culture tubes containing 1 ml of conditioned cells (4 × 10^6 cells). Half of the concentrations used for the 50% labeling experiment were used to maintain approximately the same cluster activity. After an incubation period of 12 h at 37°C in an atmosphere of 95% air and 5% CO_2, the radiolabeled cells (4 × 10^6) were washed as above, suspended in 400 μl of MEMA, 0.58% DMSO in MEMA, or 0.58% DMSO–100 μ*M* lindane in MEMA, and transferred to a 400-μl microcentrifuge tube and centrifuged as described above. The microcentrifuge tubes containing the cell clusters were maintained at 10.5°C for 72 h, after which the surviving fraction of cells was determined as described above.

Response of Multicellular Clusters to Chronic and Acute Exposure to External γ Rays

Microcentrifuge tubes containing multicellular clusters prepared with 4 × 10^6 unlabeled cells as described above were transferred to a refrigerator at 10.5°C. The tubes were placed at different distances from a 370-MBq ^{137}Cs source housed in a small stainless steel capsule. Two control tubes were similarly maintained at 10.5°C without radiation exposure. The cumulated absorbed dose to the irradiated cells was measured using a Thomson-Nielson (Ottawa, Canada) miniature MOSFET dosimeter system. After 72 h of chronic irradiation, the cells were processed as described above to determine the surviving fraction. Cumulated doses of 2.4 to 12.7 Gy were delivered over 72 h at dose rates from 3 to 18 cGy/h, depending on the distance from the source. The response of the multicellular cluster to acute ^{137}Cs γ rays was also studied by maintaining identically prepared multicellular clusters at 10.5°C for 72 h and then irradiating them acutely at the same temperature in a J. L. Shepherd Mark I irradiator (San Fernando, CA). The acute dose rate was ~1–1.7 Gy per minute and total doses ranged from 1 to 12.5 Gy. After the acute irradiation, the cells were processed as above and the surviving fraction was determined compared to that for unirradiated control cells.

Gap-Junctional Intercellular Communication at 10.5°C

The scrape-loading and dye transfer technique of El-Fouly *et al.* (*12*) was used with slight modification. Approximately 4 × 10^6 cells were thawed from a stock of V79 cells maintained at −70°C, washed with MEMA, and immediately plated in a 30-mm Corning tissue culture dish (Corning, NY) with 2 ml of fresh MEMA. The dish was placed in an incubator at 37°C, 95% air and 5% CO_2 for 1 h and was then transferred to a refrigerator at 10.5°C. After 72 h, the confluent cell population was rinsed three times with Ca^{2+}-Mg^{2+}-free PBS. Two milliliters of PBS containing 0.05% Lucifer yellow (Molecular Probes, Inc., Eugene, OR) was added to the dish at room temperature and the monolayer was scraped along three parallel lines using a sterile scalpel blade. The dish was placed in the dark for 5 min to complete dye transfer. As Lucifer yellow is a hydrophilic fluorescent dye with a low molecular weight (mol. wt. 457.2), it can traverse gap junctions and therefore is an efficient means by which to monitor gap-junctional intercellular communication. The dye solution was decanted, the dish was rinsed three times with fresh PBS, and 2 ml of PBS was added to the dish. The plate was observed with an Olympus BX60 epifluorescence phase-contrast microscope illuminated with an Osram HBO 200 W lamp.

RESULTS

Response of Multicellular Clusters to External γ Rays

Figure 2 shows the dose–response curves for multicellular clusters of V79 cells exposed to chronic (3–18 cGy/h) and acute (1–1.7 Gy/min) ^{137}Cs γ irradiation. A least-squares fit of these data to the linear-quadratic model [SF = $\exp(-\alpha D - \beta D^2)$] yielded α(chronic) = 0.0440 ± 0.0183 Gy^{-1}, β(chronic) = 0.00391 ± 0.00231 Gy^{-2}, α(acute) = 0.118 ± 0.025 Gy^{-1}, and β(acute) = 0.00566 ± 0.0042 Gy^{-2}.

Response of Multicellular Clusters to [^{3}H]dThd

Figure 3 shows the surviving fraction of cells in the multicellular cluster as a function of the ^{3}H activity in the cluster when either 50% or 100% of the cells are radiolabeled. The response curve for 100% labeling is exponential, whereas the curve for 50% labeling is two-component exponential. A least-squares fit of these data to a two-component exponential function yields

$$SF = (1 - b)\, e^{-A/A_1} + b\, e^{-A/A_2}, \qquad (1)$$

where SF is the surviving fraction, A is the cluster activity, and b, A_1, and A_2 are the fitted parameters. For 50% labeling, the fitted parameters b, A_1, and A_2 are 0.67 ± 0.12, 0.81 ± 0.56 kBq, and 11.8 ± 3.1 kBq, respectively. In the

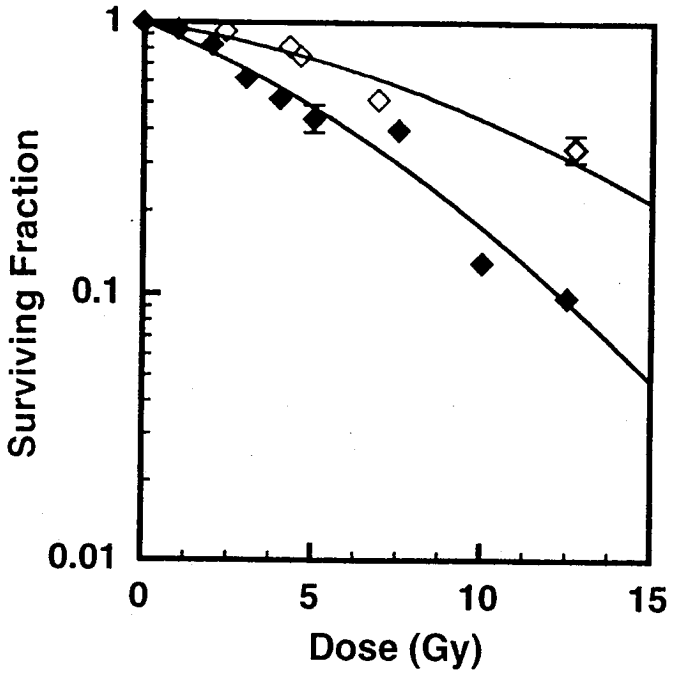


FIG. 2. Survival of V79 cells after acute (◆) and chronic (◇) irradiation of multicellular clusters with ^{137}Cs γ rays. Irradiations were carried out at 10.5°C. The acute dose rate was ~1–1.7 Gy per minute. For chronic irradiation, the tubes containing clusters were placed at different distances from a 370-MBq ^{137}Cs source housed in a small stainless steel capsule. After the acute and chronic irradiation, the clusters were dismantled, cells were processed, and the surviving fraction was determined compared to cells from unirradiated control clusters. Representative standard deviations are indicated by the error bars. Solid curves represent least-squares fits to the linear-quadratic model.

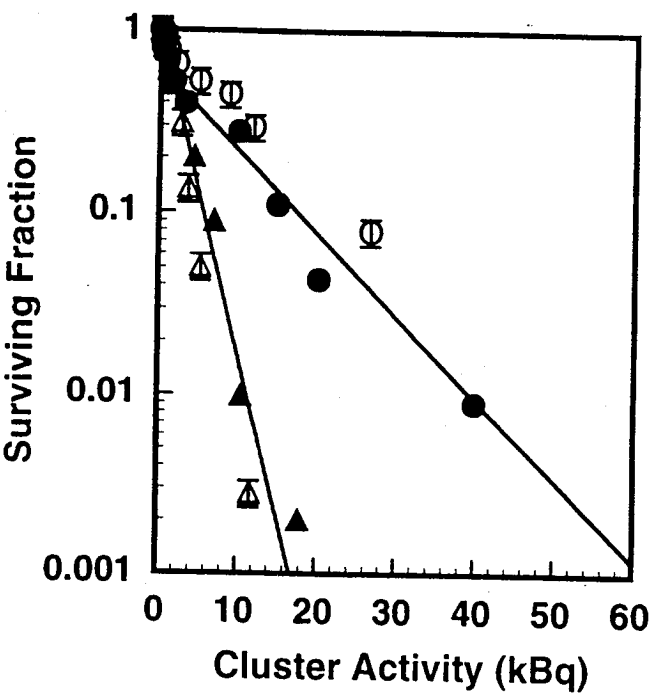


FIG. 3. Survival of V79 cells as a function of cluster activity of [^{3}H]dThd. Data are shown for experiments where 50% (●, ○) or 100% (▲, △) of the cells were radiolabeled in multicellular clusters which were maintained at 10.5°C for 72 h and then the surviving fraction was determined compared to unlabeled cells. Data from two independent experiments are plotted for each labeling condition and are differentiated by open and closed symbols. Representative standard deviations are indicated by the error bars.

case of 100% labeling, with $h = 0$, the fitted value of A_1 is 2.44 ± 0.11 kBq.

Chemotoxicity of Lindane

Figure 4 shows the fraction of surviving cells in multicellular clusters of V79 cells after a 72-h exposure to different concentrations of lindane in the culture medium. The surviving fraction compared to that for untreated controls remains close to unity up to about 100 μ*M* lindane, whereupon a significant decrease is observed. These data suggest that concentrations in excess of 100 μ*M* are not desirable for experiments involving inhibition of gap-junction intercellular communication due to associated cytotoxicity.

Optimum Concentration of Lindane to Inhibit Bystander Effect

Determination of the optimum concentration of lindane to minimize bystander effects is an essential element of the present study. Figure 5 shows the surviving fraction of V79 cells in multicellular clusters as a function of lindane concentration in the cell culture medium. In these experiments, 50% of the cells in the cluster are radiolabeled with approximately 4.8 mBq/cell of [^{3}H]dThd for a total cluster

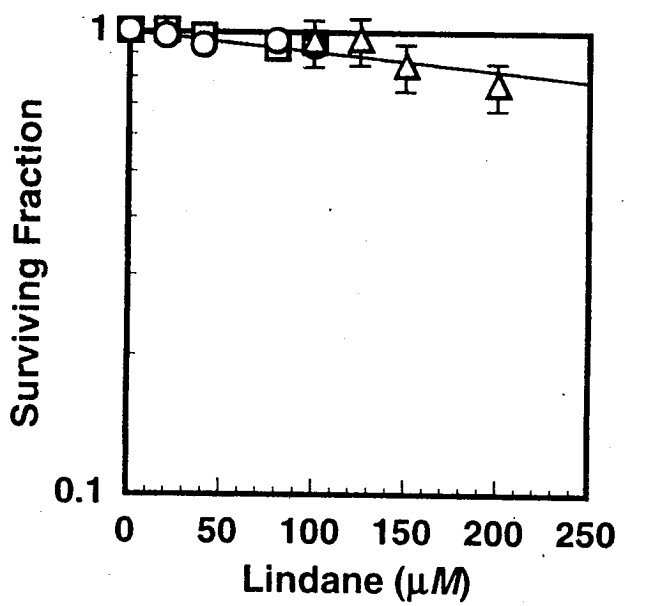


FIG. 4. Chemotoxicity of lindane when V79 multicellular clusters were exposed to the chemical at 10.5°C for 72 h. Representative standard deviations for individual data points are shown. Data from three independent experiments are indicated by different symbols (○, □, △).

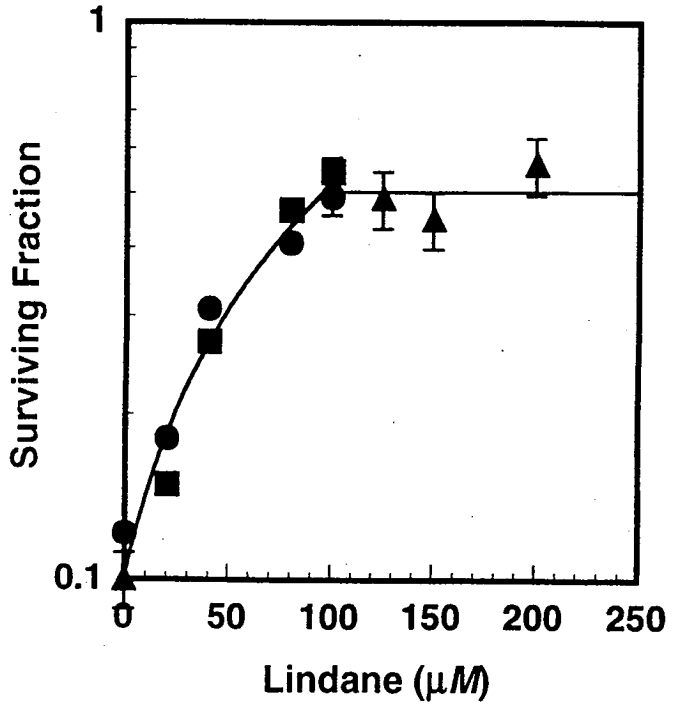


FIG. 5. Effect of lindane concentration on survival of V79 cells from multicellular clusters in which 50% of the cells are labeled with [^{3}H]dThd. The surviving fraction increased steadily with increasing lindane concentration up to 100 μM, after which no additional protective effect was observed. Data from three independent experiments are indicated by three symbols (●, ■, ▲). Representative standard deviations are indicated by the error bars.

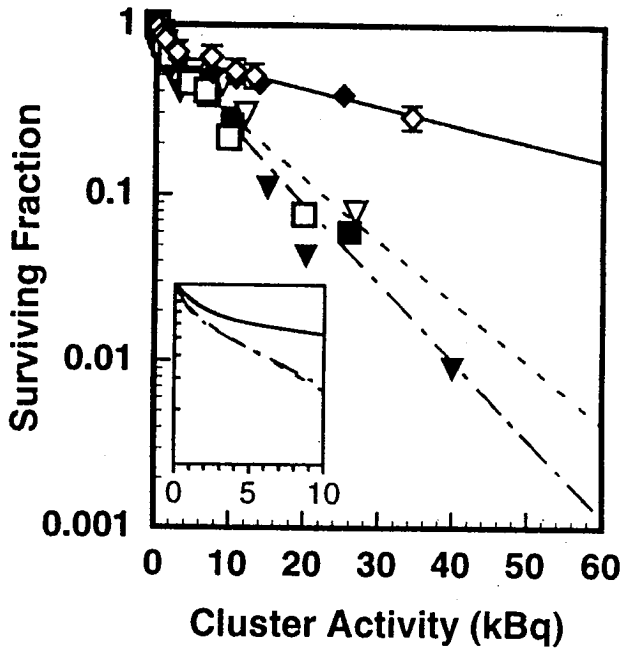


FIG. 6. Survival of V79 cells as a function of cluster activity of [^{3}H]dThd when 50% of the cells were labeled. Multicellular clusters were maintained at 10.5°C for 72 h in the presence of (1) [^{3}H]dThd (▼, ▽; data reproduced from Fig. 3); (2) [^{3}H]dThd + 0.58% DMSO (■, □); or (3) [^{3}H]dThd + 0.58% DMSO + 100 μM lindane (◆, ◇). Data from two independent experiments are plotted for each treatment condition and are differentiated as open and closed symbols. Representative standard deviations are indicated by the error bars. The short-dashed, long-short dashed, and solid curves represent least-squares fits of the data to Eq. (1) for cases 1, 2 and 3, respectively.

activity of about 19 kBq. Multicellular clusters treated with neither [^{3}H]dThd nor lindane served as controls. The concentration of lindane in the culture medium has a marked impact on the surviving fraction of cells in the cluster, elevating the fraction from about 10% at 0 μM lindane to about 50% at 100 μM lindane for multicellular clusters with 50% labeled cells. No further significant increase in surviving fraction was observed at lindane concentrations in excess of 100 μM. These data indicate that 100 μM is the optimum concentration of lindane for carrying out detailed studies of bystander effects in V79 cell multicellular clusters.

Response of Multicellular Clusters to [^{3}H]dThd in the Absence and Presence of Lindane

Figure 6 shows the surviving fraction of cells in the multicellular cluster as a function of the ^{3}H activity in the cluster when only 50% of the cells are radiolabeled. In the absence of lindane, when the cluster activity increases, the surviving fraction decreases sharply to about 50% and then continues to decrease albeit with a more shallow slope. Essentially the same curve is obtained when these clusters are maintained in the presence of 0.58% DMSO. In contrast, clusters that were maintained in the presence of 0.58% DMSO + 100 μM lindane show a similar sharp decrease in the slope of the response curve to about 50% survival and only limited cell killing at higher cluster activities. A least-squares fit of these data to Eq. (1) in the case of 100 μM lindane gives values of 0.67 ± 0.03, 1.6 ± 0.3 and 41.6 ± 5.8 kBq for h, A_1 and A_2, respectively. The fitted parameters for the three irradiation conditions are summarized in Table 1.

The surviving fraction of cells in multicellular clusters assembled with 100% of the cells radiolabeled with [^{3}H]dThd is shown in Fig. 7 as a function of the cluster activity for the three experimental conditions: (1) [^{3}H]dThd, (2) [^{3}H]dThd + 0.58% DMSO, and (3) [^{3}H]dThd + 0.58% DMSO + 100 μM lindane. The fraction of cells surviving compared to untreated controls was calculated in each case. The survival curves in all three cases are single-component exponential, which is commensurate with our earlier studies that examined the radiotoxicity of [^{3}H]dThd in V79 cells maintained in suspension culture (*14*). Least-squares fits of these data to Eq. (1) with $h = 0$ give A_1 values of 2.7 ± 0.1, 2.7 ± 0.2, and 2.8 ± 0.1 kBq for cases 1, 2 and 3, respectively. The fitted parameters for the three irradiation conditions are summarized in Table 1.

Evidence of Gap-Junctional Intercellular Communication at 10.5°C

To verify the capacity of V79 cells to form intercellular communication through gap junctions during maintenance

TABLE 1
Fitted Parameters for Survival Curves for Multicellular Clusters[a]

Treatment	Percentage cells labeled	b	A_1 (kBq)	A_2 (kBq)
[^{3}H]dThd[b]	100	0	2.7 ± 0.1	—
[^{3}H]dThd + 0.58% DMSO[b]	100	0	2.7 ± 0.2	—
[^{3}H]dThd + 0.58% DMSO + 100 μM lindane[b]	100	0	2.8 ± 0.1	—
[^{3}H]dThd[c]	50	0.67 ± 0.12	0.81 ± 0.56	11.8 ± 3.1
[^{3}H]dThd + 0.58% DMSO[c]	50	0.75 ± 0.04	0.70 ± 0.17	9.3 ± 0.7
[^{3}H]dThd + 0.58% DMSO + 100 μM lindane[c]	50	0.67 ± 0.03	1.6 ± 0.3	41.6 ± 5.8

[a] Standard errors are indicated.
[b] Least-squares fit to data in Fig. 7.
[c] Least-squares fit to data in Fig. 6.

at 10.5°C for 72 h, the transfer of Lucifer yellow dye between neighboring cells was studied in cells in monolayers. As shown in Fig. 8, Lucifer yellow was transferred into contiguous cells after the parallel lines were scraped in the monolayer with a scalpel. The highest intensity of Lucifer yellow was noticed in cells at the periphery of the scraped areas, and a gradient of decreasing intensity is evident as the dye spreads further into contiguous cells through gap junctions.

DISCUSSION

Radiopharmaceuticals are used widely in clinical medicine to diagnose and treat a variety of medical conditions. It is well known that when radiopharmaceuticals are administered to the patient, the radioactivity localizes in different tissues in the body and its distribution at the macroscopic and microscopic levels is nonuniform. The degree of nonuniformity can vary widely depending on a variety of factors. The biological consequences of nonuniform distributions of radioactivity in a given tissue can also vary substantially. Despite these well-known facts, current internationally accepted methods for assessing risks from diagnostic nuclear medicine procedures assume that the radioactivity is distributed uniformly in organs and tissues and that the biological response depends principally on absorbed dose, radiation type and tissue radiosensitivity (*16*). Bystander effects and other potential consequences of nonuniform distributions of radioactivity are ignored in these risk estimates. The same assumption is frequently made in assessing risks from environmental (e.g. ^{222}Rn) and accidental (e.g. ^{137}Cs, ^{131}I) exposures to radioactivity. Adelstein *et al.* (*17*) and Makrigiorgos *et al.* (*18*) have raised important concerns regarding the assumption of uniform distribution of radioactivity and their impact on risk estimates. However, one of the major stumbling blocks to predicting the biological response of tissues with nonuniform distributions of radioactivity has been the absence of experimental models that allow tight control over the distribution of the radioactivity.

Multicellular Model

The data in the present work have been obtained with a new three-dimensional tissue culture model that has been designed specifically to quantify the impact of nonuniform distributions of radioactivity in tissues on the biological effect of the incorporated radionuclides. It is demonstrated that multicellular clusters can be assembled by mixing suspensions of radiolabeled and nonradiolabeled cells to achieve a controlled degree of nonuniformity of radioactivity in an *in vitro* multicellular cluster model (Fig. 1). This

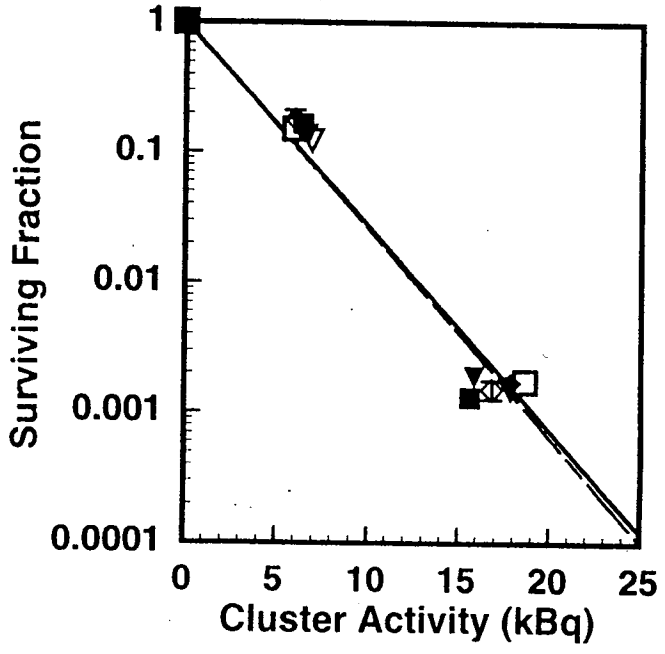


FIG. 7. Survival of V79 cells as a function of cluster activity of [^{3}H]dThd when 100% of the cells were labeled. Multicellular clusters were maintained at 10.5°C for 72 h in the presence of (1) [^{3}H]dThd (▼, ▽), (2) [^{3}H]dThd + 0.58% DMSO (■, □); (3) [^{3}H]dThd + 0.58% DMSO + 100 μM lindane (◆, ◇). Data from two independent experiments are plotted for each treatment condition and are differentiated as open and closed symbols. Representative standard deviations are indicated by the error bars. The short-dashed, long-short dashed, and solid curves represent least-squares fits of the data to Eq. (1) for cases 1, 2 and 3 respectively.

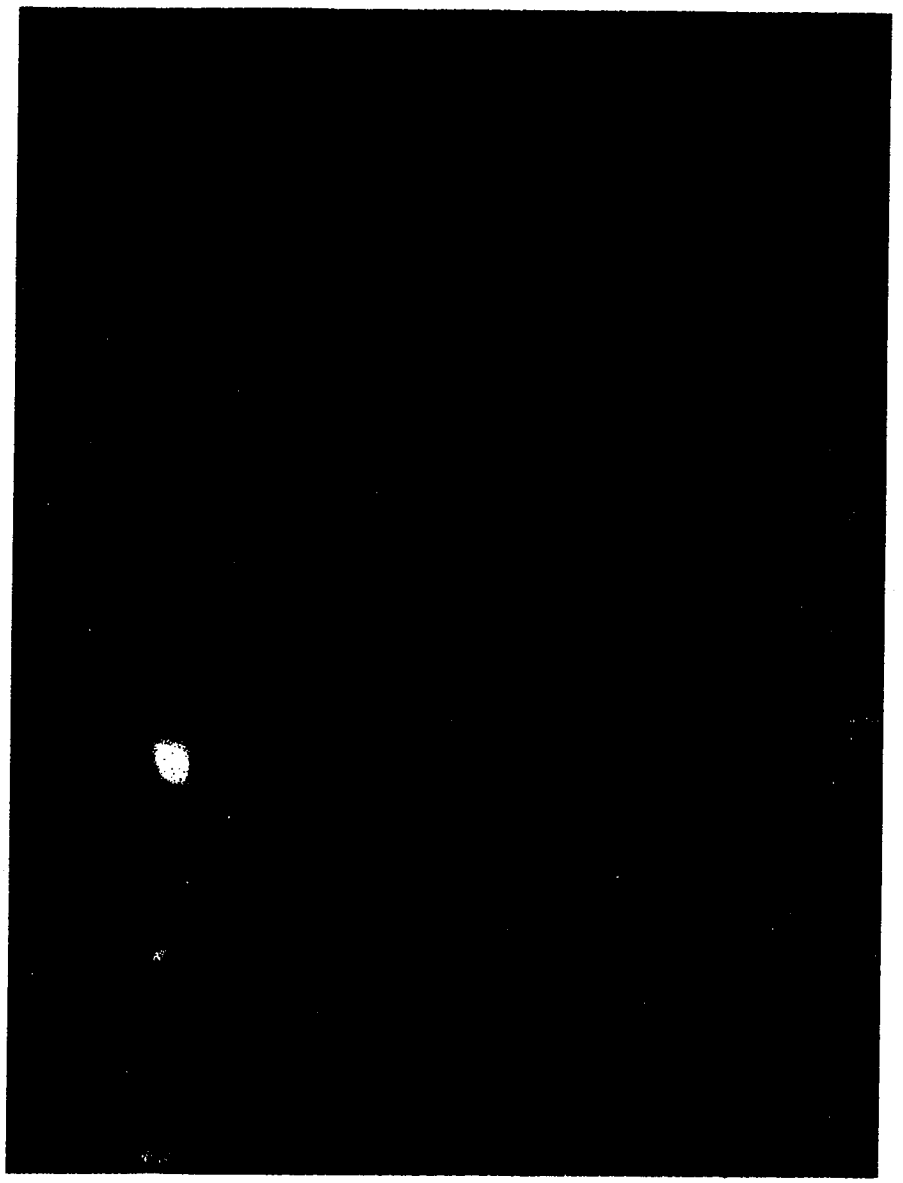


FIG. 8. Transfer of the fluorescent dye Lucifer yellow through gap junctions in V79 cells maintained as a monolayer culture at 10.5 C for 22 h.

new model affords a high degree of control over the percentage of radiolabeled cells in the cluster. The use of different radiochemicals can provide further control over the subcellular distribution of the radioactivity in the labeled cells. These degrees of control over the model are a major departure from past *in vitro* multicellular cluster models wherein multicellular spheroids are prepared prior to treatment with radioactivity, thus leading to a condition where only cells at the periphery of the cluster are effectively labeled (*19, 20*).

The response of the multicellular clusters used in the present work to external beams of ^{137}Cs γ rays is characterized in Fig. 2 for both acute and chronic irradiation. The dose–response curves with shoulders are characteristic of the response of mammalian cells to radiations of low linear energy transfer (LET). The acute doses were delivered at dose rates of 1–1.7 Gy/min, whereas the chronic irradiation was carried out at a dose rate of 3–18 cGy/h. The latter dose rates are more in keeping with those encountered in therapeutic nuclear medicine. Clearly, dose rate has a significant impact on the response.

The survival curves in Fig. 3 correspond to the case where V79 cells are labeled with [^{3}H]dThd. The dose responses for 50% and 100% labeling are markedly different from those observed when the cells are irradiated with external γ rays. In the case of 100% labeling of the cells in the cluster, the dose–response curve is exponential with a value of A_1 of 2.44 kBq. This exponential response is commensurate with the response of suspension cultures of V79 cells labeled with [^{3}H]dThd (*14*). In contrast, the dose–response curve for 50% labeling is two-component exponential, with the second component having a relatively

shallow slope compared to the first component as characterized by the parameters A_1 = 0.81 kBq and A_2 = 11.8 kBq. In other words, for a given amount of ^{3}H radioactivity in the cluster, the two labeling conditions (50%, 100%) yield very different surviving fractions. Therefore, the distribution of radioactivity in the cluster plays an important role in the biological response of the cells in the cluster. This is an important aspect of this new model that can be exploited to obtain quantitative data on the response of multicellular systems to nonuniform distributions of radioactivity.

Another significant feature of this new model is that typical cell survival experiments *in vitro* using radioprotectors or gap-junction inhibitors involve acute radiation exposures in the presence of chemotoxic concentrations of these agents. The cells are usually washed free of the agent immediately after the irradiation and plated for colony formation. When cells are irradiated by incorporated radionuclides, the radiation dose is delivered chronically. To examine the capacity of radioprotectors or gap-junction inhibitors to modify effects caused by chronic irradiation by incorporated radionuclides, the chemical agent should be present throughout the irradiation period (*21*). However, chronic exposure of cultured cells to these chemical agents at 37°C leads to extreme chemotoxicity, particularly when levels sufficient to afford protection are used (*21*). The results in Figs. 4 and 5 show that this problem can be overcome by maintaining the cells at 10.5°C. Under these conditions, the V79 cells did not divide and only minimal chemotoxicity was observed for both control and treated cells when the lindane concentration was maintained at or below 100 μM.

Chinese hamster V79 cells maintained at 37°C have been shown to exhibit intercellular communication through gap junctions. This has been demonstrated by freeze-fracture coupled with quantitative morphology (*13*) as well as the scrape-loading and dye transfer technique (*12*). However, to the best of our knowledge, the capacity of V79 cells to form gap junctions at 10.5°C has not been demonstrated. In the present study, this aspect has been explored by maintaining confluent monolayers of V79 cells at 10.5°C for 72 h and then studying the transfer of the fluorescent dye Lucifer yellow to detect gap-junctional intercellular communication. Figure 8 shows that V79 cells indeed retain their ability to form membrane channels through gap junctions even at 10.5°C, a process that can be seen efficiently through positive dye transfer into contiguous cells.

In view of the versatility and reproducibility of this new multicellular cluster model, it is possible that it may merit consideration for assessing bystander effects as set forth by Mill *et al.* (*8*) in their recent Letter to the Editor. Mill *et al.* (*8*) have argued that to clearly establish the existence of a bystander effect in the case of hot particles (and nonuniform activity distributions in general), "an internationally validated *in vitro* assay together with an internationally validated dosimetry protocol" is needed. The present experimental *in vitro* model, coupled with our theoretical multicellular dosimetry model (*22*), may be considered as a candidate for this purpose. It should be noted, however, that the current experimental protocol uses a maintenance temperature of 10.5°C, which may have an impact on metabolic processes such as DNA repair and cell proliferation. Ward *et al.*, (*23*) have shown that irradiated V79 cells are capable of repairing DNA single-strand breaks at temperatures as low as 10°C, albeit at a reduced rate. Double-strand breaks were not repaired at this temperature. However, despite the dependence of repair on temperature, a 3-h incubation of the irradiated cells at this temperature had no impact on cell survival. Nevertheless, the maintenance temperature in the present model can be increased to 37°C; this will reduce the capacity to introduce adequate concentrations of chemical modifiers such as DMSO and lindane without leading to undesired chemotoxicity.

The Bystander Effect

The β particles emitted by ^{3}H have a spectrum of energies from 0–18.6 keV (*10*) with corresponding ranges in water from 0–7 μm (*24*). The mean energy is only 5.7 keV, which corresponds to a range of only 1 μm in water (*24*). The mean diameter of a V79 cell is 10 μm and its nucleus has a mean diameter of 8 μm (*21*). Since the ^{3}H is incorporated into the DNA of the nuclei of labeled cells, the nuclei in these cells will be efficiently self-irradiated by the low-energy β particles emitted by the radionuclide. However, β particles emitted by ^{3}H decays in the cell nucleus must travel 2 μm (range of 10 keV electron, ref. *24*) just to get from the perimeter of the nucleus of a labeled cell to the perimeter of a nucleus of an unlabeled cell. The distance to the nucleus of the unlabeled cell is considered important because the nucleus presumably contains the primary radiosensitive targets. Since the electrons are emitted by decays occurring randomly throughout the nucleus, nearly all of the β particles will have to travel substantially more than 2 μm just to reach the nucleus of an unlabeled cell. Given that very few of the β particles emitted are in excess of the minimum requirement of 10 keV, the cross-dose received by cells in the cluster is negligible. This premise is supported by the multicellular dosimetry calculations of Goddu *et al.* (*22*) that show that cross-dose for electrons in this energy range is negligible when the radioactivity is localized in the cell nucleus. Therefore, in the absence of bystander effects, one anticipates essentially no killing of unlabeled cells, which should translate into a 50% surviving fraction in the case of 50% labeling at high cluster activities. The steep first component (A_1 = 0.81 kBq) of the two-component dose–response curve in Fig. 3 shows that about 50% of the cells are indeed killed. However, the second component (A_2 = 11.8 kBq) indicates that the unlabeled cells continue to be killed as the activity in the labeled cells is increased even though the unlabeled cells are not significantly irradiated. This suggests that a bystand-

er effect is responsible for the killing of unlabeled cells and, unlike the results of Mothersill and Seymour (*4*), the effect does not saturate with increasing dose (i.e. activity in the labeled cells).

To elucidate the potential mechanisms responsible for the bystander effect observed in Fig. 3, the gap-junction inhibitor lindane was added to the culture medium prior to formation of the multicellular clusters in which 50% of the cells were labeled. Figure 6 shows that 100 μM lindane has a marked impact on the survival of V79 cells with the value of A_2 in Eq. (1) changing from 11.8 kBq to 41.6 kBq. The solvent 0.58% DMSO had no impact on the response of the V79 cells. If the bystander effect blocking factor (BBF) is defined as the ratio

$$\text{BBF} = \frac{A_2 \text{ (with lindane)}}{A_2 \text{ (without lindane)}}, \qquad (2)$$

then the bystander effect blocking factor for 50% labeling of cells in the multicellular cluster with [^{3}H]dThd and maintenance in culture medium with 0.58% DMSO + 100 μM lindane is 3.5 ± 1.0. Since lindane is known to be a gap-junction inhibitor (*6, 7*), and it has been demonstrated in the present study that V79 cells form gap junctions at 10.5°C, it is likely that the bystander effects observed when 50% of the cells in the cluster are labeled with [^{3}H]dThd are due primarily to intercellular communication processes that depend on the formation of gap junctions which connect adjacent cells (*25*).

While lindane is known to be an inhibitor of gap-junction intercellular communication, it is also known to affect other processes that may pertain to its apparent ability to decrease bystander effects. For example, lindane may increase levels of superoxide dismutase and the extent of lipid peroxidation (*26, 27*) and cause alterations in intracellular free calcium and mitochondrial transmembrane potential (*28*). It may also increase the activity of NADPH-cytochrome P450 and the ratio of superoxide anion production/superoxide dismutase activity (*29*) and the formation of reactive oxygen species that result from the metabolism of lindane (*30*). Therefore, it is possible that mitigation of bystander effects by lindane may be due not only to inhibition of gap-junctional intercellular communication but also to these other processes.

Bystander Effects Relative to Conventional Radiation Effects

To assess the relative importance of bystander effects compared to conventional radiation effects (i.e. direct + indirect), the clusters were assembled such that 100% of the cells were again labeled with [^{3}H]dThd. Figure 7 shows the response of multicellular clusters treated with [^{3}H]dThd, [^{3}H]dThd + 0.58% DMSO, or [^{3}H]dThd + 0.58% DMSO + 100 μM lindane. The response of the V79 cells to the three treatment regimens is essentially the same within experimental uncertainties. Each of the cells in the cluster is surrounded by approximately 13 neighbors (*22*). Hence the biological effect imparted to a given target cell in the cluster is due not only to the ^{3}H decays that occur in the target cell but also to the sum of the bystander effects imparted by the neighboring cells. Therefore, since lindane had no impact on cell survival, the contribution of the bystander effect appears to be negligible for this biological end point in the case of 100% labeling, at least over the range of activities considered.

It thus stands to reason that the impact of the bystander effect on cell survival depends on the percentage of cells in the cluster that are labeled, with the effect being most pronounced at low labeling percentages and when cross-irradiation between cells is absent or minimal. Accordingly, it is anticipated that a somewhat smaller bystander effect may be observed when cells are labeled with radionuclides that emit particles with ranges of several cell diameters or more (e.g. ^{125}I) because cross-irradiation plays a more important role in these cases (*22*).

Finally, it should be noted that the arguments made above are based on cell survival data alone. While it is not expected that data based on other biological end points will point toward very different conclusions than those reached above, the importance of examining other end points is recognized.

ACKNOWLEDGMENTS

The authors would like to thank Prof. James E. Trosko, Michigan State University College of Human Medicine, for providing advice and his protocols for the scrape-loading and dye transfer technique. They are also grateful to Dr. Satam Banga, UMDNJ–New Jersey Medical School, for his assistance with the fluorescence microscopy techniques used in this work.

Received: March 31, 1999; accepted: May 6, 1999

REFERENCES

1. H. Nagasawa and J. B. Little, Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* **52**, 6394–6396 (1992).
2. A. Deshpande, E. H. Goodwin, S. M. Bailey, B. L. Marrone and B. E. Lehnert, Alpha-particle-induced sister chromatid exchange in normal human lung fibroblasts—Evidence for an extranuclear target. *Radiat. Res.* **145**, 260–267 (1996).
3. M. Sigg, N. E. A. Crompton and W. Burkhart, Enhanced neoplastic transformation in an inhomogeneous radiation field: An effect of the presence of heavily damaged cells. *Radiat. Res.* **148**, 543–547 (1997).
4. C. Mothersill and C. B. Seymour, Cell–cell contact during γ irradiation is not required to induce a bystander effect in normal kidney keratinocytes: Evidence for release during irradiation of a signal controlling survival into the medium. *Radiat. Res.* **149**, 252–262 (1998).
5. E. I. Azzam, S. M. de Toledo, T. Gooding and J. B. Little, Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiat. Res.* **150**, 497–504 (1998).
6. X. Guan and R. J. Ruch, Gap junction endocytosis and lysosomal degradation of connexin43-P2 in WB-F344 rat liver epithelial cells treated with DDT and lindane. *Carcinogenesis* **17**, 1791–1798 (1996).
7. K. A. Criswell and R. Loch-Caruso, Lindane-induced elimination of

gap junctional communication in rat uterine monocytes is mediated by an arachidonic acid-sensitive cAMP-independent mechanism. *Toxicol. Appl. Pharmacol.* **135**, 127–138 (1995).

8. A. J. Mill, M. W. Charles and P. J. Darley, Enhanced neoplastic transformation in an inhomogeneous radiation field: An effect of the presence of heavily damaged cells. *Radiat. Res.* **149**, 649–651 (1998). [Letter to the Editor]
9. N. E. A. Crompton, M. Sigg and W. Burkart, Enhanced neoplastic transformation in an inhomogeneous radiation field: An effect of exposure to supralethally damaged cells. *Radiat. Res.* **149**, 651–653 (1998). [Letter to the Editor]
10. E. Browne and R. B. Firestone, *Table of Radioactive Isotopes*. Wiley, New York, 1986.
11. ICRU, *Photon, Electron, Proton and Neutron Interaction Data for Body Tissues*. Report 46, International Commission on Radiation Units and Measurements, Bethesda, MD, 1992.
12. M. H. El-Fouly, J. E. Trosko and C. C. Chang, Scrape-loading and dye transfer: A rapid and simple technique to study gap junctional intercellular communication. *Exp. Cell. Res.* **168**, 422–430 (1987).
13. S. B. Yancey, J. E. Edens, J. E. Trosko, C. C. Chang and J. P. Revel, Decreased incidence of gap junctions between Chinese hamster V-79 cells upon exposure to the tumor promoter 12-*o*-tetradecanoyl phorbol-13-acetate. *Exp. Cell Res.* **139**, 329–340 (1982).
14. R. W. Howell, S. M. Goddu, A. Bishayee and D. V. Rao, Radioprotection against lethal damage caused by chronic irradiation with radionuclides *in vitro*. *Radiat. Res.* **150**, 391–399 (1998).
15. A. I. Kassis and S. J. Adelstein, A rapid and reproducible method for the separation of cells from radioactive media. *J. Nucl. Med.* **21**, 88–90 (1980).
16. ICRP, *Radiation Dose to Patients from Radiopharmaceuticals*. Publication 53, International Commission on Radiological Protection, Pergamon Press, Oxford, 1987.
17. S. J. Adelstein, A. I. Kassis and K. S. R. Sastry, Cellular vs. organ approaches to dose estimates. In *Proceedings of Fourth International Radiopharmaceutical Dosimetry Symposium* (A. T. Schlafke-Stelson and E. E. Watson, Eds.), pp. 13–25. National Technical Information Service, Springfield, VA, 1986.
18. G. M. Makrigiorgos, S. J. Adelstein and A. I. Kassis, Cellular radiation dosimetry and its implications for estimation of radiation risks. Illustrative results with technetium-99m-labeled microspheres and macroaggregates. *J. Am. Med. Assoc.* **264**, 592–595 (1990).
19. V. K. Langmuir, J. K. McGann, F. Buchegger and R. M. Sutherland, The effect of antigen concentration, antibody valency and size, and tumor architecture on antibody binding in multicell spheroids. *Nucl. Med. Biol.* **18**, 753–764 (1991).
20. R. Sutherland, F. Buchegger, M. Schreyer, A. Vacca and J. P. Mach, Penetration and binding of radiolabeled anti-carcinoembryonic antigen monoclonal antibodies and their antigen binding fragments in human colon multicellular tumor spheroids. *Cancer Res.* **47**, 1627–1633 (1987).
21. R. W. Howell, D. V. Rao, D-Y. Hou, V. R. Narra and K. S. R. Sastry, The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. *Radiat. Res.* **128**, 282–292 (1991).
22. S. M. Goddu, D. V. Rao and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation and subcellular distribution of radionuclides. *J. Nucl. Med.* **35**, 521–530 (1994).
23. J. F. Ward, C. L. Limoli, P. M. Calabro-Jones and J. Aguilera, An examination of the repair saturation hypothesis for describing shouldered survival curves. *Radiat. Res.* **127**, 90–96 (1991).
24. ICRU, *Stopping Powers for Electrons and Positrons*. Report 37, International Commission on Radiation Units and Measurements, Bethesda, MD, 1984.
25. R. Bruzzone, T. W. White and D. A. Goodenough, The cellular internet: On-line with connexins. *Bioessays* **18**, 709–718 (1996).
26. B. C. Koner, B. D. Banerjee and A. Ray, Organochlorine pesticide-induced oxidative stress and immune suppression in rats. *Ind. J. Exp. Biol.* **36**, 395–398 (1998).
27. B. Descampiaux, J. M. Leroux, C. Peucelle and F. Erb, Assay of free-radical toxicity and antioxidant effect on the Hep 3B cell line: A test survey using lindane. *Cell Biol. Toxicol.* **12**, 19–28 (1996).
28. R. Rosa, E. Rodriguez-Farre and C. Sanfeliu, Cytotoxicity of hexachlorocyclohexane isomers and cyclodienes in primary cultures of cerebellar granule cells. *J. Pharmacol. Exp. Ther.* **278**, 163–169 (1996).
29. K. A. Simon Giavarotti, L. Rodrigues, T. Rodrigues, V. B. Junqueira and L. A. Videla, Liver microsomal parameters related to oxidative stress and antioxidant systems in hyperthyroid rats subjected to acute lindane treatment. *Free Radic. Res.* **29**, 35–42 (1998).
30. P. Perocco, A. Colacci, C. Del Ciello and S. Grilli, Cytotoxic and cell transforming effects of the insecticide, lindane (γ-hexachlorocyclohexane) on BALB/c 3T3 cells. *Res. Commun. Mol. Pathol. Pharmacol.* **89**, 329–339 (1995).

RADIATION RESEARCH 155, 335–344 (2001)
0033-7587/01 $5.00
© 2001 by Radiation Research Society.
All rights of reproduction in any form reserved.

...DUCED ... MAY BE PROTECTED
BY COPYRIGHT LAW (TITLE 17, U.S. CODE)

Free Radical-Initiated and Gap Junction-Mediated Bystander Effect due to Nonuniform Distribution of Incorporated Radioactivity in a Three-Dimensional Tissue Culture Model

Anupam Bishayee,[a] Helene Z. Hill,[a] Dana Stein,[b] Dandamudi V. Rao[a,1] and Roger W. Howell[a,2]

[a] *Division of Radiation Research, Department of Radiology, and* [b] *Department of Pediatrics, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey 07103*

Bishayee, A., Hill, H. Z., Stein, D., Rao, D. V. and Howell, R. W. Free Radical-Initiated and Gap Junction-Mediated Bystander Effect due to Nonuniform Distribution of Incorporated Radioactivity in a Three-Dimensional Tissue Culture Model. *Radiat. Res.* **155, 335–344 (2001).**

To investigate the biological effects of nonuniform distribution of radioactivity in mammalian cells, we have developed a novel three-dimensional tissue culture model. Chinese hamster V79 cells were labeled with tritiated thymidine and mixed with unlabeled cells, and multicellular clusters (~1.6 mm in diameter) were formed by gentle centrifugation. The short-range β particles emitted by ^{3}H impart only self-irradiation of labeled cells without significant cross-irradiation of unlabeled bystander cells. The clusters were assembled in the absence or presence of 10% dimethyl sulfoxide (DMSO) and/or 100 μ*M* lindane. DMSO is a hydroxyl radical scavenger, whereas lindane is an inhibitor of gap junctional intercellular communication. The clusters were maintained at 10.5°C for 72 h to allow ^{3}H decays to accumulate and then dismantled, and the cells were plated for colony formation. When 100% of the cells were labeled, the surviving fraction was exponentially dependent on the mean level of radioactivity per labeled cell. A two-component exponential response was observed when either 50 or 10% of the cells were labeled. Though both DMSO and lindane significantly protected the unlabeled or bystander cells when 50 or 10% of the cells were labeled, the effect of lindane was greater than that of DMSO. In both cases, the combined treatment (DMSO + lindane) elicited maximum protection of the bystander cells. These results suggest that the bystander effects caused by nonuniform distributions of radioactivity are affected by the fraction of cells that are labeled. Furthermore, at least a part of these bystander effects are initiated by free radicals and are likely to be mediated by gap junctional intercellular communication. © 2001 by Radiation Research Society

INTRODUCTION

There is substantial interest in the role of bystander effects in the biological response of mammalian cells to ionizing radiation. It has long been believed that the principal genetic effects of ionizing radiation in mammalian cells are the direct result of DNA damage in irradiated cells that has not been repaired adequately. Therefore, when cells are exposed to external beams of radiation, only those cells that receive "hits" from the emitted radiations would be damaged. No effects would be observed in cells that are not "hit." These cells are referred to as bystanders. Studies from a number of laboratories suggest that these bystander cells do indeed incur damage as a consequence of being in the neighborhood of irradiated cells (*1*). Nagasawa and Little (*2*) first showed that Chinese hamster ovary (CHO) cells exposed to very low fluences of α particles exhibited a much higher incidence of cells with sister chromatid exchanges (SCEs) than expected based on the number of cells that were traversed by α particles. These authors found similar results when induction of *HPRT* mutations was used as the end point, thereby confirming that genetic damage does occur in bystander cells that are not irradiated (*3*). Deshpande *et al.* (*4*) reported increases of more than eightfold in the percentage of primary human fibroblast cells expressing an increased level of SCE over the actual number of nuclei traversed by an α particle. Hickman *et al.* (*5*) documented that α particles induced accumulation of the Tp53 tumor suppressor protein in rat lung epithelial cells in a higher percentage of cells than expected based on the number that would have received a direct nuclear traversal. Azzam *et al.* (*6*) made similar observations with altered expression of TP53, CDKN1A (also known as $p21^{Waf1}$), CDC2 (also known as $p34^{cdc2}$), cyclin B1 and RAD51 in human diploid fibroblast cells. Prise *et al.* (*7*) reported a higher frequency of apoptotic and micronucleated human fibroblast cells in cultures irradiated with a charged $^3He^{2+}$ particle microbeam. Mothersill and Seymour (*8*) demonstrated that addition of medium from epithelial cells irradiated with γ rays led to increased cell death and apoptosis of unirradiated cells.

[1] Present address: 2637 S. Saks Pt., Inverness, FL 34450.
[2] Author to whom correspondence should be addressed at Division of Radiation Research, Department of Radiology, MSB F-451, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103.

EXHIBIT
17
tabbies

Although consistent support for the existence of bystander effects is available in the literature, studies that probe the mechanisms that lead to damage in bystander cells are limited. Bystander effects have been attributed to the production of extracellular factors that lead to the generation of reactive oxygen species (ROS) (*9–11*). More recently, Iyer and Lehnert (*12*) have postulated that transforming growth factor β1 is the mediator of α-particle-induced bystander responses. Gap junctional intercellular communication (GJIC) has also been implicated as one of the mechanisms (*6, 13*). It has also been suggested that other mechanisms such as extranuclear-originating signal pathways, secreted diffusible factors, and apoptosis-inducing factors may be involved in the responses of bystander cells (*14, 15*). These findings suggest that different mechanisms may be operational for bystander effects depending on the cell type, the type of radiation, and other experimental conditions, including the end points studied.

The issue of bystander effects is relevant to the biological effects of nonuniform distribution of radioactivity; however, there is a paucity of data. This is of major importance to risk estimation in diagnostic nuclear medicine and radiation protection (e.g. inhalation of radon/radon progeny) as well as clinical outcome in therapeutic nuclear medicine. One of the major obstacles to predicting the biological response of tissues with nonuniform distribution of radioactivity is the absence of suitable experimental models that allow precise control of the degree of nonuniformity. To overcome this problem, we have recently developed a novel three-dimensional tissue culture model (*13*). Using this model, we have shown evidence of pronounced bystander effects in the form of decreased cell survival when ^3H is localized in the DNA of Chinese hamster lung fibroblast (V79) cells and is distributed nonuniformly in multicellular clusters (*13*). In the present communication, the same multicellular cluster model is used to investigate the impact of different magnitudes of nonuniform distribution of radioactivity on bystander effects. The underlying mechanisms of bystander effects that arise from nonuniform distribution of radioactivity are also studied.

MATERIALS AND METHODS

Radiochemical and its Quantification

Tritiated thymidine ([^3H]dThd) was obtained from NEN Life Science Products (Boston, MA) as a sterile aqueous solution at a concentration of 37 MBq/ml with a specific activity of 3000 GBq/mmol. The activity of ^3H was measured with a Beckman LS3800 automatic liquid scintillation counter (Fullerton, CA). The detection efficiency for the β particles emitted by ^3H was 0.65.

Cell Culture

V79 cells, kindly provided by Dr. A. I. Kassis (Harvard Medical School, Boston, MA), were used in the present study, with clonogenic survival serving as the biological end point. V79 cells are known to exhibit some degree of GJIC (*16–18*). The different minimum essential media (MEMA, MEMB and wash MEMA) and culturing conditions have been described in detail previously (*13*). The plating efficiency was about 64%.

Assembly of Multicellular Clusters

The protocols were as described earlier (*13*). Briefly, V79 cells were conditioned in 1 ml MEMB in 17 × 100-mm Falcon polypropylene culture tubes placed on a rocker-roller for 3–4 h in an incubator at 37°C, 5% CO_2, 95% air (2 or 4 × 10^6 cells/ml). Thereafter, 1 ml MEM containing various activities of [^3H]dThd was added and the tubes were returned to the rocker-roller. After 12 h, the cells were washed three times with wash MEMA, resuspended in 2 ml of MEMA, and passed several times through a 21 gauge needle. Additional tubes containing cells not labeled with ^3H were processed identically. The radiolabeled cells were then mixed with unlabeled cells to get 100, 50 or 10% radiolabeled cells, pelleted and transferred directly to a sterile 400-μl polypropylene microcentrifuge tube (Helena Plastics, San Rafael, CA). The tubes were centrifuged at 1000 rpm for 5 min at 4°C to form clusters with diameter ~1.6 mm (4 × 10^6 cells).

Treatment with DMSO and/or Lindane

To study the mechanisms underlying bystander effects, the multicellular clusters were assembled in the presence of the free radical scavenger DMSO and/or an inhibitor of GJIC, lindane (Sigma Chemical Co., St. Louis, MO). To achieve radioprotection, a DMSO concentration of 10% (1.28 *M*) was required as per our previous studies (*19*). Lindane was dissolved in DMSO (5 mg/ml) and subsequently diluted with MEMA to a final concentration of 100 μ*M* lindane–0.58% DMSO. These concentrations of lindane and DMSO were not cytotoxic (*13*).

Cell Survival

The microcentrifuge tubes containing the clusters were maintained at 10.5°C for 72 h to allow decay of ^3H in the absence of cell division. Under these conditions, V79 cells can withstand prolonged exposure to 10% DMSO and 100 μ*M* lindane without significant loss of plating efficiency (*13, 19*). The supernatant was then carefully removed and the tubes were vortexed to disperse the cell clusters. The cells were washed three times with 10 ml of wash MEMA, resuspended in 2 ml of wash MEMA, passed several times through a 21-gauge needle (resulting in a single cell suspension with a doublet frequency of only 2.4%), serially diluted, seeded in triplicate into 60 × 15-mm Falcon tissue culture dishes, and incubated at 37°C with 95% air and 5% CO_2. Aliquots were taken from each tube before serial dilution as above, and the mean radioactivity per cell was determined. After 7 days, the surviving fraction compared to control cells was determined.

Kinetics of Radioactivity in Cells

To ascertain the absorbed dose received by the cells, the kinetics of uptake and clearance of the radioactivity from the cells was followed. Multicellular clusters were prepared with 100% of the cells labeled as described above. At various times after the clusters were placed at 10.5°C, a cluster was dismantled and the level of radioactivity per cell was determined. Additionally, cells from clusters that had been maintained at 10.5°C for 72 h were washed with wash MEMA, and 1 × 10^6, 5 × 10^5, 2.5 × 10^5 and 2.5 × 10^4 cells were finally plated in duplicate into 75-cm^2 culture flasks. On each of the following 4 days, duplicate flasks with the same number of cells plated (in descending order) were removed from the incubator and the activity per cell was determined.

Monitoring of Radioactivity in Labeled and Unlabeled Cells

Studies were carried out to trace radioactivity in the labeled and unlabeled cells. The radiolabeled cells were dyed with 0.05 μ*M* carboxyfluorescein diacetate succinimidyl ester (CFDA SE) in phosphate-buffered saline (PBS) at 37°C for 15 min using a Vybrant® cell tracer kit

(Molecular Probes, Eugene, OR). CFDA SE passively diffuses into cells, where its acetate groups are cleaved by intercellular esterases to yield the highly fluorescent carboxyfluorescein succinimidyl ester that reacts with intracellular amines forming well-retained fluorescent conjugates. The radiolabeled and dyed cells (2×10^6) were mixed with an equal number of unlabeled, undyed cells. The pooled cells were used to form clusters containing a total of 4×10^6 cells (50% of the cells were radiolabeled and dyed) and the clusters were maintained at 10.5°C. After 72 h, the cells from two clusters were pooled, washed with PBS, resuspended in 5 μM EDTA in PBS to a concentration of 10^7 cells/ml, and passed through a 21-gauge needle five times to produce a single cell suspension. The cells were subjected to fluorescence-activated cell sorting (FACS) using a FACSCalibur flow cytometer (Becton Dickinson, San Francisco, CA). An air-cooled 488-nm argon-ion laser was used to excite the dye. The excitation and emission peaks of the fluorescent dye are 492 and 517 nm, respectively. Fluorescence in the FL-1 channel was collected along with forward-angle and 90° light scatter. The cells were sorted for dye-negative cells. A gate was applied around the cell population to evaluate cellular events. Single-parameter histograms based on 10,000 events were analyzed using CellQUEST software (Becton Dickinson). After sorting, the dye-negative cells (unlabeled and undyed) were collected, pooled, washed and finally resuspended in 2 ml of 5 μM EDTA in PBS. Aliquots were used to determine the mean activity per cell. An additional 0.5 ml of the suspension was subjected to FACS for the second time to check the purity of sorted cells (i.e. absence of dye-positive cells).

Assessment of GJIC by Flow Cytometry

The presence of functional GJIC between V79 cells in multicellular clusters maintained at 10.5°C in the absence and presence of lindane was monitored by flow cytometry. The method of Goldberg *et al.* (*20*) was used with modifications. Cells (4 or 2×10^6) were loaded with calcein AM (Molecular Probes). This fluorescent dye becomes membrane impermeant when it enters the cell. However, it can traverse functional gap junctions (*21*). The loading was achieved by incubating cells for 25 min at 37°C in the presence of 2 ml of 20 μM dye in PBS. The cells were then washed with PBS, resuspended in prewarmed MEMA, incubated at 37°C for 30 min, and centrifuged, and the supernatant was decanted. Undyed cells were treated similarly. These cells were used to form multicellular clusters containing 100 or 50% dyed cells. The clusters with 50% dyed cells were prepared in the presence or absence of 100 μM lindane. The clusters were then maintained at 10.5°C for 72 h. The cells from clusters were washed with PBS and resuspended in 1 ml of 5 μM EDTA in PBS. In the lindane-treated cells, all steps after the dyeing procedure, including washes and resuspensions, were carried out in the presence of lindane. GJIC was interpreted as the ability of the dye to pass from pre-dyed cells to undyed cells. This was determined by measuring the fluorescence of cells with the FACSCaliber flow cytometer with a 530-nm band pass filter using the technique of Tomasetto *et al.* (22). Fluorescent signals were processed over a four-decade logarithmic range. The clusters containing 100% dyed and undyed cells served as positive and negative controls. A no-dye-transfer control for the 50% dyed cell clusters was prepared by mixing equal volumes of the final suspensions of the 100% dyed and undyed cells, and the resulting mixture was immediately analyzed with the flow cytometer.

RESULTS

100% Labeling: Response of Multicellular Clusters and Cell Suspensions

Figure 1 illustrates the surviving fraction of cells maintained in multicellular clusters for 72 h as a function of ^3H activity (mBq) per labeled cell. Also shown is the survival curve obtained when cells were prepared identically except for maintenance as a single-cell suspension for 72 h at 10.5°C [see ref. (*19*) for experimental details]. The data were fitted by least squares to the relationship

$$SF = (1 - b)e^{-A/A_1} + b\, e^{-A/A_2}, \quad (1)$$

where SF is the surviving fraction, A is the activity per labeled cell, and b, A_1 and A_2 are the fitted parameters. The parameters A_1 and A_2 are analogous to D_0 values for the first and second components, respectively. With $b = 0$ for monoexponential response, the fitted values of A_1 for cluster and suspension are 0.80 ± 0.02 and 0.76 ± 0.04 mBq/cell, respectively. The response of the cells to incorporated [^3H]dThd is essentially the same whether the cells are arranged in the form of a cluster or are maintained as a single-cell suspension. Therefore, this suggests that the cells within the cluster do not receive any significant exposure to radiation from their neighbors (i.e. no cross-irradiation).

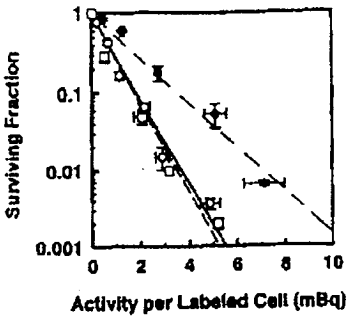


FIG. 1. Survival of V79 cells as a function of activity per labeled cell wherein 100% of the cell population was labeled with [^3H]dThd. Cells were maintained in MEMA as multicellular clusters in the absence (○) and presence (●) of 10% DMSO, or as suspensions in the absence of DMSO (□). Data points represent the average of eight (○), three (●) and two (□) experiments, respectively. Standard errors are shown accordingly.

100% Labeling: Modification of Response by DMSO and Lindane

The surviving fractions of cells in multicellular clusters assembled and maintained in the presence or absence of 10% DMSO are also shown in Fig. 1. A least-squares fit of the data for each treatment condition to Eq. (1) with $b = 0$ gives A_1 values of 1.5 ± 0.08 and 0.80 ± 0.02 mBq/cell for 10% and 0% DMSO, respectively (Table 1). The dose modification factor (DMF), which indicates the degree of protection provided to the labeled cells by 10% DMSO, is expressed by the ratio of A_1 values in the presence and absence of DMSO as follows (*19, 23*):

$$\text{DMF}([^3H]\text{dThd, 10\% DMSO}) = \frac{A_1\ (\text{with DMSO})}{A_1\ (\text{without DMSO})}. \quad (2)$$

As shown in Table 1, a DMF value of 1.9 ± 0.12 is obtained, which indicates that 10% DMSO is able to protect V79 cells against lethal damage in clusters when all cells are labeled with [^3H]dThd. As reported earlier (*13*), 100 μM lindane did not have any effect on the survival of cells

TABLE 1

Fitted Parameters for Survival Curves and Bystander Modification Factor for Multicellular Clusters

Treatment	Percentage of cells labeled	Number of experiments	b	A_1[a] (mBq/labeled cell)	A_2[a] (mBq/labeled cell)	Bystander modification factor[b]
[³H]dThd	100	8	0	0.80 ± 0.02[c]	—	—
[³H]dThd + 10% DMSO	100	2	0	1.5 ± 0.08	—	1.9 ± 0.12[d]
[³H]dThd	50	4	0.61 ± 0.09	0.29 ± 0.16	4.8 ± 1.1	—
[³H]dThd + 10% DMSO	50	5	0.52 ± 0.08	1.5 ± 0.28	11.1 ± 2.1	2.3 ± 0.68
[³H]dThd + 100 μ*M* lindane	50	3	0.68 ± 0.03	0.72 ± 0.15	18.2 ± 2.0	3.8 ± 0.94
[³H]dThd + 10% DMSO + 100 μ*M* lindane	50	3	0.61 ± 0.03	1.1 ± 0.20	29.5 ± 2.8	6.1 ± 1.5
[³H]dThd	10	2	0.87 ± 0.02	0.96 ± 0.40	49.4 ± 1.9	—
[³H]dThd + 10% DMSO	10	2	0.84 ± 0.02	1.4 ± 0.65	67.0 ± 4.3	1.4 ± 0.10
[³H]dThd + 100 μ*M* lindane	10	2	0.82 ± 0.02	1.4 ± 0.77	91.0 ± 6.7	1.8 ± 0.15
[³H]dThd + 10% DMSO + 100 μ*M* lindane	10	2	0.86 ± 0.03	3.5 ± 1.8	112.4 ± 7.5	2.3 ± 0.17

[a] A_1 and A_2 are analogous to the D_0's of the first (labeled cells) and second (bystander cells) components of the fitted survival curve (Eq. 1).
[b] A_2 (with treatment)/A_2 (without treatment).
[c] Mean ± SE.
[d] For 100% labeling, this quantity represents the traditional dose modification factor (DMF).

from a multicellular cluster when all cells were labeled with [³H]dThd. This suggests that the bystander effect does not significantly contribute to the killing of radiolabeled cells.

50% Labeling: Modification of Response by DMSO and/or Lindane

Figure 2A shows the cell surviving fraction as a function of the level of radioactivity per labeled cell for multicellular clusters in which 50% of the cells were labeled with [³H]dThd. The clusters were maintained in the presence of (1) MEMA, (2) 10% DMSO in MEMA, (3) 100 μ*M* lindane in MEMA, or (4) 10% DMSO + 100 μ*M* lindane in MEMA. As shown in Fig. 2A, a two-component exponential survival curve emerges when the data from each experimental condition are fitted to Eq. (1). As expected, the transition from the first to second component occurs near 50% survival. Table 1 summarizes the fitted parameters for the different treatment conditions. The fitted parameters for cases (1) and (3) are slightly different from those obtained previously (*13*) because the new values include results of additional experiments not reported earlier. In the absence of any treatment, as the activity per labeled cell increases, the surviving fraction drops sharply to about 50% and then continues to drop, albeit with a shallower slope. The first component of the two-component survival curve represents killing of the radiolabeled cells, whereas the second component represents killing of unlabeled bystander cells (*13*). Addition of 10% DMSO afforded significant protection against lethal damage to unlabeled bystander cells. In fact, the value of A_2 changes from 4.8 ± 1.1 to 11.1 ± 2.1 mBq/labeled cell (Table 1). If one considers this change a consequence of modification of the bystander effects imparted by the labeled cells to unlabeled cells, then the bystander modification factor (BMF) is defined as[3]

$$\text{BMF} = \frac{A_2 \text{ (with treatment)}}{A_2 \text{ (without treatment)}}. \qquad (3)$$

The bystander modification factor for 10% DMSO in multicellular clusters containing 50% cells labeled with [³H]dThd is 2.3 ± 0.68 (Table 1). A greater degree of protection of the bystander cells was achieved with 100 μ*M* lindane (bystander modification factor = 3.8 ± 0.94). This value is within statistical uncertainties of the previously reported value of 3.5 ± 1.0 (*13*). The most dramatic protection of the bystander cells was manifested by a combined treatment of 10% DMSO and 100 μ*M* lindane, which yields

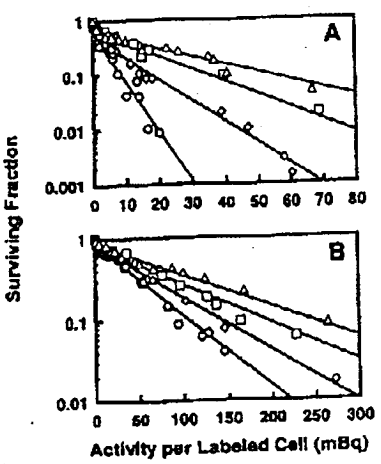


FIG. 2. Survival of V79 cells as a function of activity per labeled cell in which 50% (panel A) or 10% (panel B) of the cells were labeled with [³H]dThd and used to form multicellular clusters. The clusters were maintained in MEMA (○), 10% DMSO in MEMA (◇), 100 μ*M* lindane in MEMA (□), or 10% DMSO + 100 μ*M* lindane in MEMA (△). Data from two to five independent experiments are presented for each treatment. Standard errors for each data point are of the order of the dimensions of the symbols.

[3] In our previous communication, we designated the ratio of the A_2 values as a bystander blocking factor. This name was selected because lindane is a gap junction inhibitor in V79 cells. The change in name to bystander modification factor acknowledges the fact that other agents such as DMSO can modify the response through other mechanisms.

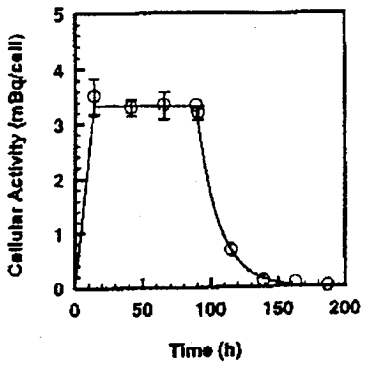


FIG. 3. Intracellular ^3H activity in V79 cells as a function of time. The period 0–12 h represents the uptake of the radiochemical. The period 12–84 h represents the 72-h period in which the cells were maintained at 10.5°C. The period beyond 84 h corresponds to the 1-week period of colony formation, when the cellular activity has an effective half-time of ~12 h. The area under the curve is proportional to the cumulative decays in the V79 cell nucleus. Approximately 76% of the intracellular decays occur when the cells were maintained at 10.5°C.

a sharp drop in the response curve to about 50% survival and only limited cell killing at higher activities per labeled cell (Fig. 2A). Under this experimental condition, a bystander modification factor of 6.1 ± 1.5 was obtained.

10% Labeling: Modification of Response by DMSO and/or Lindane

The multicellular clusters were also prepared with a mixture of 10% radiolabeled cells and 90% unlabeled cells. As in the case for 50% labeling, Fig. 2B shows a similar two-component exponential response for each treatment condition. As expected, the transition from the first to second component for 10% labeling occurs near 90% survival. The parameters resulting from least-squares fits to Eq. (1) are given in Table 1. The bystander modification factors corresponding to 10% DMSO, 100 μM lindane, and 10% DMSO + 100 μM lindane are 1.4 ± 0.10, 1.8 ± 0.15, and 2.3 ± 0.17, respectively (Table 1). These values for the case of 10% labeling were of lesser magnitude than those for the 50% labeling. However, like the data for 50% labeling, the data for 10% labeling show that the killing of unlabeled bystander cells does not saturate even though the number of labeled cells that come in contact with unlabeled cells is five times less than in the case of the 50% labeling.

Biokinetics of [^3H]dThd in Cells

The uptake, maintenance and clearance of [^3H]dThd in V79 cells as they proceed through different stages of the experiment are depicted in Fig. 3. The area under the curve is proportional to the cumulated activity (decays) of ^3H in V79 cell nuclei. The period of 0–12 h represents the uptake of the radiochemical at 37°C. Previous studies have shown that the uptake is linear in time during this period (*23*). The period of 12–84 h represents the 72-h period in which the cells were maintained at 10.5°C in the cluster configuration.

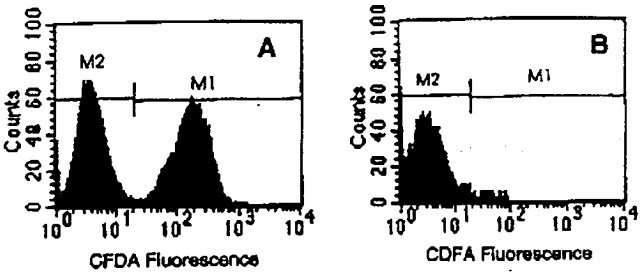


FIG. 4. Evidence of no migration of radioactivity from labeled to unlabeled cells in multicellular cluster containing 50% labeled cells. V79 cells were radiolabeled with [^3H]dThd, dyed with CFDA, and mixed with unlabeled and undyed cells, and cell clusters were prepared and maintained at 10.5°C for 72 h. Panel A: After 72 h, flow cytometry analysis shows that 49.6% of the cells remained dyed (M1). Panel B: The undyed cells (M2) were separated from the dyed cells (M1) by FACS. No significant radioactivity was found in the undyed cells.

As expected, the cellular activity did not change during this period. Finally, the curved region corresponds to the 1-week period of colony formation at 37°C, where the cellular activity has an effective half-time of ~12 h, which is in agreement with previous data (*23*). Integration under the curve and normalizing to 1 mBq give the mean cumulated activity (decays) in a labeled cell, $\tilde{A}$ = 343 Bq s per mBq in the cell. Finally, it should be noted that about 76% of the intracellular decays occurred while the cells were maintained in the cluster configuration at 10.5°C (*23*).

No Migration of Radioactivity in Multicellular Clusters

The multicellular clusters consisting of 50% radiolabeled and dyed cells and 50% unlabeled and undyed cells were maintained at 10.5°C for 72 h, dispersed, and analyzed by flow cytometry to verify percentage of cells dyed. The analysis of CFDA-positive and CFDA-negative cells is shown in Fig. 4A. The data confirm that 49.6% of the cells were dye-positive (M1 gate) and hence labeled with [^3H]dThd. The dye-negative cells (M2 gate) were then sorted and again analyzed by flow cytometry (Fig. 4B). Figure 4B shows that the undyed cells (M2 gate) were separated to a purity of ~97%. Aliquots of these cells were counted for radioactivity, and it was determined that they contained an average of 0.013 mBq/cell. The M1-gated (dye-positive) cells shown in Fig. 4A contained 1.5 mBq/cell. Since the dye-negative cells have a 3% contamination of radiolabeled and dyed cells, this population should have an average of at least 0.045 mBq/cell. This is well above the 0.013 mBq/cell observed. Therefore, these data provide strong evidence that there is no migration of [^3H]dThd from radiolabeled cells to surrounding unlabeled cells under the experimental conditions used.

Absorbed Dose to Labeled and Unlabeled Cells

The short-range β particles emitted by ^3H have a spectrum of energies from 0–18.6 keV (*24*), with ranges in water from 0–7 μm. The mean energy of the electron is only

5.7 keV, and it has a range of 1 μm in water. The mean diameter of a V79 cell is 10 μm, and the mean diameter of its nucleus is 8 μm (*25*). Using the model of Goddu *et al.* (*26*) and the full ^{3}H β-particle spectrum, the mean self-absorbed dose to the nucleus of a labeled cell per unit cumulated activity in the nucleus of the labeled cell is S_{self}(labeled←labeled) = 2.61 × 10^{-3} Gy/Bq s. The mean self-absorbed dose to the nucleus, D_{self}(labeled), is $\tilde{A}$ · S_{self}(labeled←labeled) = 0.895 Gy per mBq in the cell. Therefore, the mean lethal dose for 100% labeling is D_{37} = 0.80 mBq (0.895 Gy/mBq) = 0.72 Gy. In contrast, the mean cross-dose to a neighboring unlabeled cell per unit cumulated activity in a single labeled cell is S_{cross}(unlabeled←labeled) = 3.03 × 10^{-6} Gy/Bq s. For 50% labeling, each unlabeled cell has 12 neighbors, 6 of which are labeled (*26*). Therefore, S^{total}_{cross}(unlabeled←labeled) = 1.82 × 10^{-5} Gy/Bq s and D^{total}_{cross}(unlabeled←labeled) = 0.00624 Gy mBq^{-1} in the labeled cell. Therefore, the dose to the labeled cell is over 140 times that to the unlabeled cell.

Functional GJIC in Multicellular Clusters

The fluorescent dye calcein AM was used to ascertain the presence of functional GJIC. As shown in Fig. 5A, the background fluorescence associated with undyed cells resulted in a single low-intensity peak with a geometric mean of 2.9. When 100% of the cells were dyed, a single broad and intense peak was observed with a geometric mean of 290 (Fig. 5B). When 50% of the cells were loaded with the dye, two peaks emerged with geometric means of 13 and 203, respectively (Fig. 5C). The peak corresponding to initially undyed cells has shifted markedly to the right, indicating that dye has been transferred from dyed to undyed cells. Hence functional GJIC is present in the V79 multicellular clusters maintained at 10.5°C. Addition of lindane inhibited dye transfer, as shown by the similarity of its histogram (Fig. 5D, geometric means of 9 and 225) to that of the 50% control culture, where no dye transfer was possible (Fig. 5E, geometric means of 6 and 260). These data support the capacity of lindane to attenuate GJIC under the present experimental conditions.

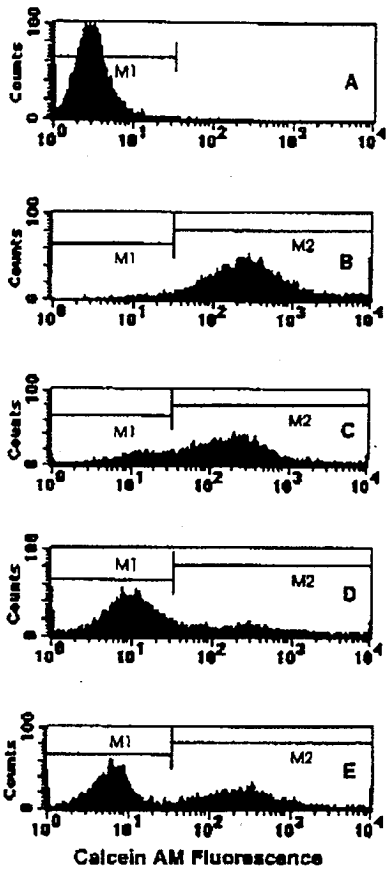


FIG. 5. Single-parameter histogram from flow cytometry of V79 cells from a multicellular cluster containing (panel A) 0%, (panel B) 100% or (panel C) 50% dyed cells. (panel D) 50% dyed cells in the presence of 100 μ*M* lindane, or (panel E) an equal mixture of 0% and 100% dyed cells followed by immediate analysis. Transfer of the fluorescent dye calcein AM from dyed to undyed cells in the case of 50% dyed cells is seen in the right lateral shift in the peak position corresponding to the cells that were initially undyed (panel C). Addition of lindane dramatically reduces the degree of dye transfer (panel D) as seen in the similarity to the 50% control histogram, where no dye transfer was possible (panel E).

DISCUSSION

The present study has used a three-dimensional tissue culture model (*13*) to quantify the lethal effect of nonuniform distributions of [^{3}H]dThd. This model affords a high degree of control over the percentage of radiolabeled cells in the cluster. The short range of the ^{3}H β particles prevents significant irradiation of unlabeled cells by radioactivity in the labeled cells (*13*). This is supported by the data in Fig. 1 that show that the mean lethal ^{3}H activity per cell required to achieve 37% survival is essentially the same for 100% labeling regardless of whether the cells are maintained as clusters (0.80 ± 0.02 mBq/cell) or in suspension, where no cross-dose is possible (0.76 ± 0.04 mBq/cell). It is also supported by theoretical calculations for 50% labeling (see Results section) that yield mean absorbed doses to the labeled and unlabeled cells of 0.895 and 0.00624 Gy mBq^{-1} per labeled cell, respectively. Since the activity per labeled cell required to effect 1% survival is about 20 mBq per labeled cell (Fig. 2A), the dose to the unlabeled cells in this case would be about 0.12 Gy. Assuming an equal effectiveness per gray between self- and cross-irradiation (worst-case scenario), this would produce an SF of 0.84 in the unlabeled cells which comprise 50% of the population. If all of the labeled cells were killed, and the killing of unlabeled cells was due to irradiation of their nuclei, this would yield an expected SF for the entire population of about 0.42. Similar results emerge for the case of 10% labeling. The difference between 0.42 and the observed SF of 0.01 suggests that mechanisms other than cross-irradia-

tion play the principal role in the observed killing of unlabeled bystander cells.

Three different radiolabeling conditions were investigated in which 100, 50 or 10% of the cells within the multicellular clusters were labeled, each resulting in markedly different survival curves. The data for 100% labeling in Fig. 1 yielded an exponential survival curve with a mean lethal cellular activity of 0.80 mBq/cell. In contrast, the data for 50 and 10% labeling required fits to a two-component exponential function (Fig. 2A, B). These fits and the resulting parameters (Table 1) indicate that about 50 and 10% of the cells are killed at relatively low activities per labeled cell because only the labeled cells are killed. However, the second components (Fig. 2) indicate that unlabeled cells are killed even though they do not receive significant irradiation. This strongly suggests that bystander effects are responsible for killing of unlabeled cells. Furthermore, the linearity of the second components suggests that the bystander signal increases exponentially with the dose to the labeled (irradiated) cells. This finding appears to differ from the data of Wu *et al.* (*27*), who found a saturation in the dose response for mutation induction in A_L cells irradiated through the cytoplasm with an α-particle microbeam. They observed a similar saturation in response when cell survival was used as the end point (data available only down to about 70% survival). They postulated that the saturation after eight α-particle traversals may have been due to the fact that the traversals were split between only two areas in the cytoplasm (*27*), suggesting that a more uniform irradiation of the cytoplasm may have eliminated the saturation. While this may be the case, the present data do not involve irradiation of the cytoplasm of the same cell; rather they involve death of unlabeled bystander cells adjacent to cells whose nucleus is irradiated by β particles emitted by [^{3}H]dThd. Therefore, given the very different irradiation conditions (cytoplasm of the same cell compared to the nucleus of a neighboring cell), it is possible that different mechanisms are operational, which could lead to different dose–response relationships. Furthermore, considering other differences between the experimental protocols (e.g. radiation type, cell type, dose of radiation to target cells, temperature, cell geometry), it is not surprising that differences were observed.

A more recent report from the same laboratory examined the mutagenic response in the surviving bystander cell population after irradiation of 0, 5, 10 or 20% of the cell nuclei with 20 α particles from the microbeam (*28*). Under these irradiation conditions, which were somewhat similar to those in the present study, they found a linear increase in the mutant fraction of bystander cells as the percentage of cell nuclei in the population that were hit with the 20 α particles was increased. A plateau was reached when 10% of the nuclei were hit such that there was no significant difference in mutation fraction when 20% of the cells in the population were hit. As indicated by the authors, this was perhaps expected in their two-dimensional monolayer model because the number of unirradiated cells in direct contact with an irradiated cell is not much different in the two cases. The data in the present work also suggest that percentage of cells labeled is an important determinant for bystander effects. However, in the present work, higher percentages of labeling appear to impart bystander effects more efficiently in the three-dimensional cluster model. The fitted values for A_2 (Table 1) differ by about 10-fold for 10% and 50% labeling, despite only a 5-fold difference in the percentage of cells labeled. Therefore, the efficiency of the transfer of bystander effects is reduced by a factor of two for 10% labeling compared to 50% labeling. It is possible that coupling of an average of about 1.2 radioactive cells to each bystander cell (10% labeling) results in transmission of damage signals to a single region of the bystander cell compared to a more uniform transmission when 6 radioactive cells are coupled to each bystander cell (50% labeling). This could lead to less efficient killing of the coupled bystander cell. Although we are not aware of any experimental data to support this hypothesis, this argument is analogous to that of Wu *et al.* (*27*) for cytoplasmic irradiation.

Gap junctional intercellular communication has been implicated as an important mediator of radiation-induced bystander effects (*6*). Gap junctions are intercellular membrane channels that directly link the interiors of neighboring cells. These channels have diameters of 1.5–2 nm and permit the direct passage of small (<2,000 Da) molecules between the cytoplasm of neighboring cells (*29*). V79 cells were reported to have some GJIC at physiological temperature (*16–18, 30*). It has also been shown by scrape-loading and dye transfer that V79 cells retained GJIC even when maintained for 72 h at 10.5°C as a confluent monolayer (*13*). While this served as evidence of GJIC in monolayers at 10.5°C, it was necessary to demonstrate the presence of functional coupling of V79 cells in multicellular clusters maintained at 10.5°C. This was studied in the present work by monitoring the transfer of the fluorescent dye calcein AM, which can traverse gap junctions, by flow cytometry. Figure 5 shows that functional gap junctions are indeed formed between the neighboring cells within the cluster, as shown by the transfer of calcein AM from dyed to undyed cells. Moreover, the current study also shows that GJIC between V79 cells in the multicellular cluster can be inhibited to some degree by lindane. This is in agreement with earlier findings (*17, 31*). It is perhaps not surprising that functional coupling occurs at 10.5°C. Ward *et al.* (*32*) have shown that V79 cells are able to repair DNA single-strand breaks at 10°C, albeit at a reduced rate. Double-strand breaks were not repaired at this temperature. Therefore, while 10.5°C does not represent normal body temperature, many important physiological processes such as repair and GJIC remain operational.

To elucidate the potential mechanisms responsible for bystander effects observed with 50 and 10% labeling, DMSO and/or lindane was added to the culture medium

before the multicellular clusters were formed. Our results show that 10% DMSO offered a fair degree of protection of bystander cells in both labeling situations (Table 1). A better protective effect was afforded by 100 μM lindane. However, concurrent treatment with DMSO and lindane brought about maximum protection of the bystander cells. For 50% labeling, the effect of a combined treatment was more prominent than for 10%, with bystander modification factor values of 6.1 ± 1.5 and 2.3 ± 0.17, respectively. This difference may be explained in light of the interactions between the labeled and unlabeled cells. The metabolic generation of ROS due to oxidative stress after exposure to ionizing radiation has recently been hypothesized as a mediator of bystander responses in unirradiated cells (*10, 33*). Exposure to high concentrations of ROS results in a wide spectrum of DNA damage, cell cycle arrest, senescence, and eventually cell death (*1, 34–36*). In the present study, significant protection (DMF = 1.9 ± 0.12) was afforded by DMSO against killing of cells in multicellular clusters in which 100% of the cells were labeled. The intracellular generation of ·OH by ^{3}H decays may be the principal cause of cell death. DMSO is a potent scavenger of ·OH (*37*), and it is therefore expected to attenuate the effect of [^{3}H]dThd provided that an adequate concentration of DMSO is used (*19*). However, ·OH are short-lived and can diffuse only about 4 nm (*38*). Thus, while these ·OH may account for lethal damage to labeled cells in the case of 100% labeling, no transmissible lethality to unlabeled bystander cells should occur with this source of ·OH. However, it is possible that there could be more persistent production of ·OH from another source such as superoxide ($O_2^{\cdot-}$) (*10, 39*). It is possible that free radicals, particularly ·OH, produced through these mechanisms could lead to membrane lipid peroxidation and consequent formation of a number of free radicals capable of producing DNA damage and cell death (*40–42*). While DMSO could block some of the above events in labeled cells and thereby reduce the concentration of free radicals in bystander cells, it is possible that some of the long-lived radicals that are not scavenged by DMSO may escape through gap junctions, reach the neighboring cells, and subsequently inflict lethal damage on these cells. The ability of lindane to block gap junctions may prevent the radicals from reacting with the DNA of bystander cells. However, lindane may not entirely abolish GJIC and provide complete protection against damage to the bystander cells by ROS originating in the labeled cells. This is likely in view of the fact that lindane affects GJIC by altering the permeability of gap junction channels and the number of gap junctions (*16, 43*). Further support for this is provided by the data in Fig. 5D, which shows that GJIC-mediated dye transfer was not completely blocked by lindane. Based on the above arguments, the presence of both DMSO and lindane might be expected to have an impact on both the ·OH-initiated events in the labeled cells and their propagation through gap junctions to the bystander cells. This may explain why the bystander modification factor is greater for the combined treatment with DMSO and lindane compared to treatment with either agent alone (Table 1).

Our results with lindane indicating the involvement of GJIC in bystander effects caused by nonuniform distributions of incorporated radioactivity are consistent with those of Azzam *et al.* (*6*), who reported a similar reduction of α-particle-induced bystander effects in human diploid fibroblast cell population by lindane. Recently, Zhou *et al.* (*28*) have provided evidence that irradiation of human–hamster A_L cells induces a bystander mutagenic response in unirradiated neighboring cells that can be inhibited by lindane but not by DMSO. They concluded that a signal transduction pathway other than ·OH-mediated oxidative stress might play a role in mediating the bystander responses for α particles. Although the present study with β particles implicates ·OH as the primary oxidant species responsible for the initiation of damage to bystander cells, it is also possible that other signaling mechanisms triggered by ROS may be involved as proposed by Iyer and Lehnert (*12*). In this context, it should be mentioned that oxidative stress has been correlated with the induction of signal transduction that is linked to a variety of deleterious effects of radiation (*34, 44–46*).

In conclusion, the present study provides new data on the biological effects of nonuniform distributions of incorporated radioactivity using a novel approach to specifically control the degree of nonuniformity. This study also establishes the response of V79 multicellular clusters to incorporated radioactivity and furnishes substantial evidence that bystander effects play a significant role in determining the biological effect of incorporated radioactivity. Furthermore, these data suggest that at least a part of the observed bystander effects are initiated by free radicals and mediated through gap junctions. These findings may ultimately enhance our capacity to predict the biological response of tumor and normal tissue in nuclear medicine and from environmental exposure to radioactivity.

ACKNOWLEDGMENTS

The authors greatly appreciate the insightful comments and suggestions provided by Drs. Edouard I. Azzam and Sonia M. de Toledo, Department of Radiology, UMDNJ–New Jersey Medical School. We are also thankful to Thomas N. Denny for providing access to the FACS. This work was supported in part by USPHS Grant Nos. R01 CA83838 (RWH) and shared instrumentation grant 1 S10 RR14753-01 (TND).

Received: July 17, 2000; accepted: October 6, 2000

REFERENCES

1. R. Iyer and B. E. Lehnert. Effects of ionizing radiation in targeted and nontargeted cells. *Arch. Biochem. Biophys.* **376**, 14–25 (2000).
2. H. Nagasawa and J. B. Little. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* **52**, 6394–6396 (1992).
3. H. Nagasawa and J. B. Little. Unexpected sensitivity to the induction

of mutations by very low doses of alpha-particle irradiation: Evidence for a bystander effect. *Radiat. Res.* **152**, 552–557 (1999).

4. A. Deshpande, E. H. Goodwin, S. M. Bailey, B. L. Marrone and B. E. Lehnert, Alpha-particle-induced sister chromatid exchange in normal human lung fibroblasts: Evidence for an extranuclear target. *Radiat. Res.* **145**, 260–267 (1996).
5. A. W. Hickman, R. J. Jaramillo, J. F. Lechner and N. F. Johnson, Alpha-particle-induced p53 protein expression in a rat lung epithelial cell strain. *Cancer Res.* **54**, 5797–5800 (1994).
6. E. I. Azzam, S. M. de Toledo, T. Gooding and J. B. Little, Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiat. Res.* **150**, 497–504 (1998).
7. K. M. Prise, O. V. Belyakov, M. Folkard and B. D. Michael, Studies on bystander effects in human fibroblasts using a charged particle microbeam. *Int. J. Radiat. Biol.* **74**, 793–798 (1998).
8. C. Mothersill and C. Seymour, Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. *Int. J. Radiat. Biol.* **71**, 421–427 (1997).
9. B. E. Lehnert and E. H. Goodwin, Extracellular factor(s) following exposure to alpha particles can cause sister chromatid exchanges in normal human cells. *Cancer Res.* **57**, 2164–2171 (1997).
10. P. K. Narayanan, E. H. Goodwin and B. E. Lehnert, Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. *Cancer Res.* **57**, 3963–3971 (1997).
11. P. K. Narayanan, K. E. A. LaRue, E. H. Goodwin and B. E. Lehnert, Alpha particles induce the production of interleukin-8 by human cells. *Radiat. Res.* **152**, 57–63 (1999).
12. R. Iyer and B. E. Lehnert, Factors underlying the cell growth-related bystander responses to alpha particles. *Cancer Res.* **60**, 1290–1298 (2000).
13. A. Bishayee, D. V. Rao and R. W. Howell, Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. *Radiat. Res.* **152**, 88–97 (1999).
14. C. Mothersill and C. B. Seymour, Cell–cell contact during gamma irradiation is not required to induce a bystander effect in normal kidney keratinocytes: Evidence for release during irradiation of a signal controlling survival into the medium. *Radiat. Res.* **149**, 252–262 (1998).
15. G. Kroemer, The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nat. Med.* **3**, 614–620 (1997).
16. S. B. Yancey, J. E. Edens, J. E. Trosko, C. C. Chang and J. P. Revel, Decreased incidence of gap junctions between Chinese hamster V-79 cells upon exposure to the tumor promoter 12-*o*-tetradecanoyl phorbol-13-acetate. *Exp. Cell Res.* **139**, 329–340 (1982).
17. M. H. El-Fouly, J. E. Trosko and C. C. Chang, Scrape-loading and dye transfer: A rapid and simple technique to study gap junctional intercellular communication. *Exp. Cell. Res.* **168**, 422–430 (1987).
18. H. Banrud, S. O. Mikalsen, K. Berg and J. Moan, Effects of ultraviolet radiation on intercellular communication in V79 Chinese hamster fibroblasts. *Carcinogenesis* **15**, 233–239 (1994).
19. A. Bishayee, D. V. Rao, L. G. Bouchet, W. E. Bolch and R. W. Howell, Radioprotection by DMSO against cell death caused by intracellularly localized iodine-125, iodine-131 and polonium-210. *Radiat. Res.* **153**, 416–427 (2000).
20. G. S. Goldberg, J. F. Beckberger and C. C. G. Naus, A pre-loading method of evaluating gap junctional communication by fluorescent dye transfer. *BioTechniques* **18**, 490–497 (1995).
21. W. Zhang, W. T. Couldwell, M. F. Simard, H. Song, J. H. C. Lin and M. Nedergaard, Direct gap junction communication between malignant glioma cells and astrocytes. *Cancer Res.* **59**, 1994–2003 (1999).
22. C. Tomasetto, M. J. Nepeu, J. Daley, P. K. Horan and R. Sager, Specificity of gap junction communication among human mammary cells and connexin transfectants in culture. *J. Cell Biol.* **122**, 157–167 (1993).
23. R. W. Howell, S. M. Goddu, A. Bishayee and D. V. Rao, Radioprotection against lethal damage caused by chronic irradiation with radionuclides *in vitro*. *Radiat. Res.* **150**, 391–399 (1998).
24. E. Browne and R. B. Firestone, *Table of Radioactive Isotopes*. Wiley, New York, 1986.
25. R. W. Howell, D. V. Rao, D-Y. Hou, V. R. Narra and K. S. R. Sastry, The question of relative biological effectiveness and quality factor for Auger emitters incorporated into proliferating mammalian cells. *Radiat. Res.* **128**, 282–292 (1991).
26. S. M. Goddu, D. V. Rao and R. W. Howell, Multicellular dosimetry for micrometastases: Dependence of self-dose versus cross-dose to cell nuclei on type and energy of radiation and subcellular distribution of radionuclides. *J. Nucl. Med.* **35**, 521–530 (1994).
27. L. J. Wu, G. R. Randers-Pehrson, A. Xu, C. A. Waldren, C. R. Geard, Z. L. Yu and T. K. Hei, Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. *Proc. Natl. Acad. Sci. USA* **96**, 4959–4964 (1999).
28. H. Zhou, G. Randers-Pehrson, C. A. Waldren, D. Vannais, E. J. Hall and T. K. Hei, Induction of a bystander mutagenic effect of alpha particles in mammalian cells. *Proc. Natl. Acad. Sci. USA* **97**, 2099–2104 (2000).
29. J. E. Trosko and R. J. Ruch, Cell–cell communication in carcinogenesis. *Front. Biosci.* **3**, 208–236 (1998).
30. M. J. Zeilmaker and H. Yamasuki, Inhibition of junctional intercellular communication as a possible short-term test to detect tumor-promoting agents: Results with nine chemicals tested by dye transfer assay in Chinese hamster V79 cells. *Cancer Res.* **46**, 6180–6186 (1986).
31. G. Tsushimoto, C. C. Chang, J. E. Trosko and F. Matsumura, Cytotoxicity, mutagenic, and cell–cell communication inhibitory properties of DDT, lindane, and chlordane on hamster cells *in vitro*. *Arch. Environ. Contam. Toxicol.* **12**, 721–730 (1983).
32. J. F. Ward, C. L. Limoli, P. M. Calubro-Jones and J. Aguilera, An examination of the repair saturation hypothesis for describing shouldered survival curves. *Radiat. Res.* **127**, 90–96 (1991).
33. S. M. Clutton, K. M. S. Townsend, C. Walker, J. D. Ansell and E. G. Wright, Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures. *Carcinogenesis* **17**, 1633–1639 (1996).
34. Q. Chen and B. N. Ames, Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc. Natl. Acad. Sci. USA* **91**, 4130–4134 (1994).
35. G. McLennan, L. W. Oberley and A. Autor, The role of oxygen-derived free radicals in radiation-induced damage and death of non-dividing eukaryotic cells. *Radiat. Res.* **84**, 122–132 (1980).
36. J. E. Spencer, A. Jenner, O. I. Aruoma, C. E. Cross, R. Wu and B. Halliwell, Oxidative DNA damage in human respiratory tract epithelial cells: Time course in relation to DNA strand breakage. *Biochem. Biophys. Res. Commun.* **224**, 17–22 (1996).
37. M. R. Raju, M. E. Schillachi, S. G. Carpenter, D. T. Goodhead and J. F. Ward, Radiobiology of ultrasoft X rays. V. Modification of cell inactivation by dimethyl sulfoxide. *Radiat. Res.* **145**, 563–567 (1996).
38. R. Roots and S. Okada, Protection of DNA molecules of cultured mammalian cells from radiation-induced single-strand scissions by various alcohols and SH compounds. *Int. J. Radiat. Biol.* **21**, 329–342 (1972).
39. P. A. Riley, Free radicals in biology: Oxidative stress and the effects of ionizing radiation. *Int. J. Radiat. Biol.* **65**, 27–33 (1994).
40. B. Peter, M. Wartena, H. H. Kampinga and A. W. T. Konings, Role of lipid peroxidation and DNA damage in paraquat toxicity and the interaction of paraquat with ionizing radiation. *Biochem. Pharmacol* **43**, 705–715 (1992).
41. C. L. Limoli, A. Hartmann, L. Shephard, C. Yang, D. A. Boothman, J. Bartholomew and W. F. Morgan, Apoptosis, reproductive failure, and oxidative stress in Chinese hamster ovary cells with compromised genomic integrity. *Cancer Res.* **58**, 3712–3718 (1998).
42. Y. Higuchi and S. Matsukawa, Glutathione depletion induces giant DNA and high-molecular-weight DNA fragmentation associated with

apoptosis through lipid peroxidation and protein kinase C activation in C6 glioma cells. *Arch. Biochem. Biophys.* **363**, 33–42 (1999).

43. X. Guan, W. J. Bonney and R. J. Ruch, Changes in gap junction permeability, gap junction number, and connexin43 expression in lindane-treated rat liver epithelial cells. *Toxicol. Appl. Pharmacol.* **130**, 79–86 (1995).

44. J. E. Trosko and T. Inoue, Oxidative stress, signal transduction, and intercellular communication in radiation carcinogenesis. *Stem Cells* **15** (Suppl. 2), 59–67 (1997).

45. R. H. Burdon, Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic. Biol. Med.* **18**, 775–795 (1995).

46. J. Remacle, M. Raes, O. Toussaint, P. Renard and G. Rao, Low levels of reactive oxygen species as modulators of cell function. *Mutat. Res.* **316**, 103–122 (1995).

NCBI PubMed National Library of Medicine NLM

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM

Search PubMed for bystander 3H-thymidine Go Clear

Limits Preview/Index History Clipboard Details

About Entrez

Display Summary Show: 20 Sort Send to Text

Items 1-8 of 8 One pag

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: de Boer OJ, van der Wal AC, Houtkamp MA, Ossewaarde JM, Teeling P, Becker AE. Related Articles, Link

Unstable atherosclerotic plaques contain T-cells that respond to Chlamydia pneumoniae.
Cardiovasc Res. 2000 Dec;48(3):402-8.
PMID: 11090835 [PubMed - indexed for MEDLINE]

2: Waldman WJ, Knight DA, Adams PW. Related Articles, Link

Cytolytic activity against allogeneic human endothelia: resistance of cytomegalovirus-infected cells and virally activated lysis of uninfected cells.
Transplantation. 1998 Jul 15;66(1):67-77.
PMID: 9679824 [PubMed - indexed for MEDLINE]

3: Zhu NL, Wu L, Liu PX, Gordon EM, Anderson WF, Starnes VA, Hall FL. Related Articles, Link

Downregulation of cyclin G1 expression by retrovirus-mediated antisense gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation.
Circulation. 1997 Jul 15;96(2):628-35.
PMID: 9244236 [PubMed - indexed for MEDLINE]

4: Hirschowitz EA, Ohwada A, Pascal WR, Russi TJ, Crystal RG. Related Articles, Link

In vivo adenovirus-mediated gene transfer of the Escherichia coli cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine.
Hum Gene Ther. 1995 Aug;6(8):1055-63.
PMID: 7578418 [PubMed - indexed for MEDLINE]

5: Rayner DC, Petrycky-Cox LD, Diocee M, Rector E. Related Articles, Link

Major histocompatibility complex class II-restricted cytotoxicity by self-myelin basic protein-reactive T-cell hybridomas: evidence for a tumour necrosis factor-independent nucleolytic mechanism.
Immunology. 1993 Feb;78(2):273-8.
PMID: 7682537 [PubMed - indexed for MEDLINE]

6: Mullen CA, Kilstrup M, Blaese RM. Related Articles, Link

Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system.
Proc Natl Acad Sci U S A. 1992 Jan 1;89(1):33-7.
PMID: 1729703 [PubMed - indexed for MEDLINE]

7: Ashwell JD, Cunningham RE, Noguchi PD, Hernandez D. Related Articles, Link

Cell growth cycle block of T cell hybridomas upon activation with antigen.

EXHIBIT tabbies 18

J Exp Med. 1987 Jan 1;165(1):173-94.
PMID: 3491868 [PubMed - indexed for MEDLINE]

8: Fidler IJ, Kleinerman ES. Related Articles, Link

Lymphokine-activated human blood monocytes destroy tumor cells but not normal cells under cocultivation conditions.
J Clin Oncol. 1984 Aug;2(8):937-43.
PMID: 6379124 [PubMed - indexed for MEDLINE]

Display Summary Show: 20 Sort Send to Text

Items 1-8 of 8 One pag

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Freedom of Information Act | Disclaimer

Sep 4 2003 10:00

NCBI PubMed National Library of Medicine

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM 3c

Search PubMed for tritiated thymidine and bystander Go Clear

Limits Preview/Index History Clipboard Details

About Entrez

Display Summary Show: 20 Sort Send to Text

Items 1-4 of 4 One pag

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: Bishayee A, Hill HZ, Stein D, Rao DV, Howell RW. Related Articles, Link

Free radical-initiated and gap junction-mediated bystander effect due to nonuniform distribution of incorporated radioactivity in a three-dimensional tissue culture model.
Radiat Res. 2001 Feb;155(2):335-44.
PMID: 11175669 [PubMed - indexed for MEDLINE]

2: Zhou D, Lu Y, Steiner MS, Dalton JT. Related Articles, Link

Cytochrome P-450 2C9 sensitizes human prostate tumor cells to cyclophosphamide via a bystander effect.
Antimicrob Agents Chemother. 2000 Oct;44(10):2659-63.
PMID: 10991840 [PubMed - indexed for MEDLINE]

3: Haberkorn U, Khazaie K, Morr I, Altmann A, Muller M, van Kaick G. Related Articles, Link

Ganciclovir uptake in human mammary carcinoma cells expressing herpes simplex virus thymidine kinase.
Nucl Med Biol. 1998 May;25(4):367-73.
PMID: 9639298 [PubMed - indexed for MEDLINE]

4: Ruddle NH. Related Articles, Link

Delayed hypersensitivity to soluble antigens in mice. II. Analysis in vitro.
Int Arch Allergy Appl Immunol. 1979;58(1):44-52.
PMID: 422279 [PubMed - indexed for MEDLINE]

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Freedom of Information Act | Disclaimer

Sep 4 2003 16:00

NCBI PubMed National Library of Medicine

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM 2

Search PubMed for bystander 3H Go Clear

Limits Preview/Index History Clipboard Details

About Entrez

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

Display Summary Show: 20 Sort Send to Text

Items 1-18 of 18 One pag

1: Dotti G, Savoldo B, Takahashi S, Goltsova T, Brown M, Rill D, Rooney C, Brenner M. Related Articles, Link

Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells. A model for immunotherapy.
Exp Hematol. 2001 Aug;29(8):952-61.
PMID: 11495701 [PubMed - indexed for MEDLINE]

2: de Boer OJ, van der Wal AC, Houtkamp MA, Ossewaarde JM, Teeling P, Becker AE. Related Articles, Link

Unstable atherosclerotic plaques contain T-cells that respond to Chlamydia pneumoniae.
Cardiovasc Res. 2000 Dec;48(3):402-8.
PMID: 11090835 [PubMed - indexed for MEDLINE]

3: Evrard A, Cuq P, Ciccolini J, Vian L, Cano JP. Related Articles, Link

Increased cytotoxicity and bystander effect of 5-fluorouracil and 5-deoxy-5-fluorouridine in human colorectal cancer cells transfected with thymidine phosphorylase.
Br J Cancer. 1999 Aug;80(11):1726-33.
PMID: 10468288 [PubMed - indexed for MEDLINE]

4: Degreve B, De Clercq E, Balzarini J. Related Articles, Link

Bystander effect of purine nucleoside analogues in HSV-1 tk suicide gene therapy is superior to that of pyrimidine nucleoside analogues.
Gene Ther. 1999 Feb;6(2):162-70.
PMID: 10435100 [PubMed - indexed for MEDLINE]

5: McIntosh KR, Drachman DB. Related Articles, Link

Induction of apoptosis in activated T cell blasts by suppressive macrophages a possible immunotherapeutic approach for treatment of autoimmune disease.
Cell Immunol. 1999 Apr 10;193(1):24-35.
PMID: 10202110 [PubMed - indexed for MEDLINE]

6: Waldman WJ, Knight DA, Adams PW. Related Articles, Link

Cytolytic activity against allogeneic human endothelia: resistance of cytomegalovirus-infected cells and virally activated lysis of uninfected cells.
Transplantation. 1998 Jul 15;66(1):67-77.
PMID: 9679824 [PubMed - indexed for MEDLINE]

7: LeMay DR, Kittaka M, Gordon EM, Gray B, Stins MF, McComb JG, Jovanovic S, Tabrizi P, Weiss MH, Bartus R, Anderson WF, Zlokovic BV. Related Articles, Link

Intravenous RMP-7 increases delivery of ganciclovir into rat brain tumors and enhances the effects of herpes simplex virus thymidine kinase gene therapy.
Hum Gene Ther. 1998 May 1;9(7):989-95.
PMID: 9607410 [PubMed - indexed for MEDLINE]

8: Zhu NL, Wu L, Liu PX, Gordon EM, Anderson WF, Starnes VA, Hall FL. Related Articles, Link

Downregulation of cyclin G1 expression by retrovirus-mediated antisense gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation.
Circulation. 1997 Jul 15;96(2):628-35.
PMID: 9244236 [PubMed - indexed for MEDLINE]

9: Ishii-Morita H, Agbaria R, Mullen CA, Hirano H, Koeplin DA, Ram Z, Oldfield EH, Johns DG, Blaese RM. Related Articles, Link

Mechanism of 'bystander effect' killing in the herpes simplex thymidine kinase gene therapy model of cancer treatment.
Gene Ther. 1997 Mar;4(3):244-51.
PMID: 9135738 [PubMed - indexed for MEDLINE]

10: Rowley S, Lindauer M, Gebert JF, Haberkorn U, Oberdorfer F, Moebius U, Herfarth C, Schackert HK. Related Articles, Link

Cytosine deaminase gene as a potential tool for the genetic therapy of colorectal cancer.
J Surg Oncol. 1996 Jan;61(1):42-8.
PMID: 8544459 [PubMed - indexed for MEDLINE]

11: Haberkorn U, Oberdorfer F, Gebert J, Morr I, Haack K, Weber K, Lindauer M, van Kaick G, Schackert HK. Related Articles, Link

Monitoring gene therapy with cytosine deaminase: in vitro studies using tritiated-5-fluorocytosine.
J Nucl Med. 1996 Jan;37(1):87-94.
PMID: 8544010 [PubMed - indexed for MEDLINE]

12: Hirschowitz EA, Ohwada A, Pascal WR, Russi TJ, Crystal RG. Related Articles, Link

In vivo adenovirus-mediated gene transfer of the Escherichia coli cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine.
Hum Gene Ther. 1995 Aug;6(8):1055-63.
PMID: 7578418 [PubMed - indexed for MEDLINE]

13: Bi WL, Parysek LM, Warnick R, Stambrook PJ. Related Articles, Link

In vitro evidence that metabolic cooperation is responsible for the bystande effect observed with HSV tk retroviral gene therapy.
Hum Gene Ther. 1993 Dec;4(6):725-31.
PMID: 8186287 [PubMed - indexed for MEDLINE]

14: Rayner DC, Petrycky-Cox LD, Diocee M, Rector E. Related Articles, Link

Major histocompatibility complex class II-restricted cytotoxicity by self-myelin basic protein-reactive T-cell hybridomas: evidence for a tumour necrosis factor-independent nucleolytic mechanism.
Immunology. 1993 Feb;78(2):273-8.
PMID: 7682537 [PubMed - indexed for MEDLINE]

☐ **15:** Mullen CA, Kilstrup M, Blaese RM. Related Articles, Link

Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system.
Proc Natl Acad Sci U S A. 1992 Jan 1;89(1):33-7.
PMID: 1729703 [PubMed - indexed for MEDLINE]

☐ **16:** Gromkowski SH, Hepler KM, Janeway CA Jr. Related Articles, Link

Low doses of interleukin 2 induce bystander cell lysis by antigen-specific CD4+ inflammatory T cell clones in short-term assay.
Eur J Immunol. 1988 Sep;18(9):1385-9.
PMID: 2901965 [PubMed - indexed for MEDLINE]

☐ **17:** Ashwell JD, Cunningham RE, Noguchi PD, Hernandez D. Related Articles, Link

Cell growth cycle block of T cell hybridomas upon activation with antigen.
J Exp Med. 1987 Jan 1;165(1):173-94.
PMID: 3491868 [PubMed - indexed for MEDLINE]

☐ **18:** Fidler IJ, Kleinerman ES. Related Articles, Link

Lymphokine-activated human blood monocytes destroy tumor cells but not normal cells under cocultivation conditions.
J Clin Oncol. 1984 Aug;2(8):937-43.
PMID: 6379124 [PubMed - indexed for MEDLINE]

Display Summary Show: 20 Sort Send to Text

Items 1-18 of 18 One pag

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Freedom of Information Act | Disclaimer

Sep 4 2003 10:00

3/29/01

The following has occurred this week:

3/23 Friday Anupam thawed aliquots of V79 and plated them into 2 T175's.

3/26 Monday Marek observes that both 3/23 flasks are contaminated. A 3rd bottle containing 1522 cells was OK. Marek observed that Anupam was probably using one of the contaminated T175's to set up an experiment. He also believes that Anupam split the cells and put them back into the incubator in a flask marked 3/23. Anupam sets up an experiment on rollers in the top of the double 37° incubator as seen by Marek.

3/27 Tuesday Anupam collects the cells from the rollers and sets up 7 Helena tubes which are placed in the 10.5° incubator as observed by Marek.

3/28 Wednesday Marek and I both observed a T175 in the trash marked 3/23 that was clearly contaminated. Once Anupam was gone, we took samples from the 7 Helena tubes – marked in green, 1 through 7-- in the 10.5° incubator that we presumed had been plated from the contaminated flask of 3/23. He sampled nos. 1 and 7 by withdrawing a small volume from each and placing in 10 ml each of growth medium in P100's. A 3rd P100 contained only medium (control).

3/29 Thursday We observed both P100's under inverted phase. With careful focusing it is possible to see rods in both of them.

In the top of the double incubator in which we found the contaminated V79 T175's, there is a tray containing P60's which would have come from experiments done last week – probably from the FACS. There are two rollers, both empty. I looked at all the dishes and observed the following:

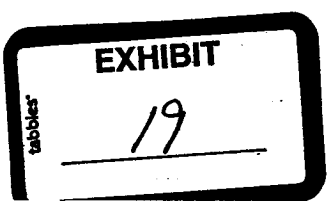
P60's in the front of the orange tray – marked with green marker:

Label	contaminated/total
1.2S	1/3
3.2S	1/3
4	1/3
6.3S	0/3
5.2S	0/3

P60's in the back of the orange tray – also marked with green marker

1.2	3/3
2.2	3/3
3.2	3/3
4.2	3/3
5.2	3/3
7.3	3/3
6.3	3/3

There are no colonies to be seen on any of the dishes in this set. Colonies can't be seen in the front set but they are probably from a more recent run than the second set.



There are 2 T175's in the incubator today. One is the same 1522 flask as seen the day before and is OK i.e. not contaminated, did not look at cells. The other is a contaminated flask of V79 marked with the date 3/28 probably indicating that it was thawed yesterday.

Around 4 PM Anupam seemed to be in a great rush and asked Marek to give him some V79 which he did. This was observed by Ed.

3/30/01 Friday ~ 8:30 AM (The day clusters from Helena tubes should be prepared for FACS, sorted and plated).

Small incubator: 1 T175 of V79 from Marek from yesterday – no date, 100% confluent, no contamination.
1T175 of AG1522: cells are floating, none attached. Flask is not dated. Probably plated yesterday.

Double Incubator:
Top:
2 empty rollers
1T175 of V79 – date is 3/28, cells are not attached, there are floaters but no growth – somewhat cloudy.
The P60's are all gone

Bottom: empty

Trash:
P60's from the incubator – from yesterday – green marker
1 T175 with AG 1522: has some very sick looking cells

10.5° incubator:
orange rack with 7 Helena tubes marked in green #s1-7

Incubator in F468: 3 P100's; Control: medium is clear, #1 and #7 are very contaminated.

12:54 PM 7 Helena tubes are still in the 10.5° incubator
1:34 same
1:39 Anupam is working in the laminar air flow hood and just told Marek that he is doing an experiment with clusters.
ca 3:00 PM all 7 Helena tubes are still there. Anupam goes to Denny's lab to do the FACS separation (but the cells for FACS are still in the 10.5° incubator in Helena tubes in clusters).

At the time that Roger leaves, Anupam returns from FACS, says that things went better than the last time. Roger says that this is a 50:50 ^{3}H cluster experiment. Marek asks if it had been incubated at 10.5°, Anupam says 'yes'.

5:00 PM 10.5° incubator now has only 6 Helena tubes, #s 1-6. #7 is gone (this is the same tube that we sampled yesterday and found to be contaminated).

Conclude: Flow was done with newly harvested V79 cells that Anupam received from Marek yesterday. These will be plated and should come out OK.

6:00 PM: 6 Helena tubes are still in the 10.5° incubator. A full set of P60's are incubating in the small incubator.

Took pictures with digital camera – no date-stamp – didn't know how – files are P1010004-P1010009.

6:40 PM: We depart. There are still 6 Helena's in the 10.5° incubator.

3/31/01 Saturday

10.5° incubator: orange rack with 6 Helena tubes marked 1-6 in green still there.

Small incubator: orange tray: Front: stacks of 3 P60's each marked with green marker 1-2,2-2,3-2,4-2,5-2,6-2,7-2 all also say (-)

Back: stacks of 3 P60's each marked with green marker 1-2,2-2,3-2,4-2,5-3,6-3,7-4 all also say (+)

Lower shelf: 2 T175's of V79 no dates, green marker, cells OK, no signs of contamination
1 T175 of AG1522 no date, floaters, very few cells attached, no signs of contamination

Double incubator:

Top: 2 empty rollers
Bottom: 1P60: PBS
1P60: M

Radioactive trash: 4 Helena tubes marked in purple 4,5,6,7 (no 1,2,3), no other Helenas. We hid the radioactive trash so that it can be examined later. If the experiment had been done properly, it should contain the 7 green-marked Helena tubes from the clusters that had been incubated for 3 days at 10.5°. It does not contain such tubes (as we know they are still in the 10.5° incubator at this time).

Large red lined trash can: much trash but: 1 Helena tube marked 7 in green – aspirated remaining fluid to count radioactivity. Assume this tube to be the missing #7 from the 10.5° incubator.

Took more pictures. Now they are date-stamped.

Marek took samples from the Helena tubes to count for radioactivity and to test for bacterial contamination. The latter are incubated in a cluster plate along with a medium control in the F468 incubator. The demonstrated radioactivity is not quantitative.

4/1/01 Sunday

As reported by Marek: 6 Helena tubes are still in the 10.5° incubator – photo taken with digital camera – date/stamped

4/2/01 Monday

6 Helena tubes are still in the 10.5° incubator, there are no attached cells in the T175 flask containing 1522 cells. Marek, Roger & Anupam had a conference. Roger asked Anupam if he had plenty of 1522 cells to which he replied 'yes' which was not true. See notes from 3/31. After this time, he obtained a fresh T75 of 1522's from Sonia.

The 5 samples in the cluster plate in the F468 incubator are contaminated with bacteria. The control is not.

Both Marek and Anupam set up new experiments this evening. Anupam used the 2 rollers in the upper incubator, Marek used the lower ones.

Digital camera now attached to the microscope. Not easy to use anymore.

4/3/01 Tuesday

2 rolling rollers in upper incubator (Anupam) 2 in lower (Marek)

Helena tubes are still in the 10.5° incubator. Photo taken with my camera – date/stamped.

Both Marek and Anupam process their cells. When Marek put his in the 10.5° incubator, Anupam had not removed the old clusters. He now has two sets of clusters. Marek asks if he has two experiments running at the same time and he replies 'yes' which we know is a lie because the first set are the same that were there since last Tuesday. Photo taken of the three sets of clusters – date/stamped.

9:30 PM Marek reports that the + dishes that were plated on Friday are contaminated. So far, the – dishes are OK. Tom Denny told me that contamination is a big problem for the FACS so it may be that Anupam used the good cells that he got from Marek but they got contaminated anyway.

p60 (see above) marked PBS is contaminated. The one marked M seems to be OK.

4/4/01 Wednesday

Small incubator: Bottom shelf: 1 T75 of AG1522 from Sonia, 1 T175 of same – cells all dead; 1 T175 of V79 of Anupam's from Marek

2nd shelf fr bottom: P60's in the back (marked +) are all contaminated. The P60's in front (marked -) are OK

Upper shelves: Marek's experiments

Double incubator: Upper: 2 empty rollers, Bottom: same

10.5° incubator: 2nd shelf fr top: 2 orange holders both with green numbers. The one in front is from last week with 6 Helena tubes, the one in front is the new one from yesterday with 7 Helena tubes.

3rd shelf fr top: 1 white rack with 10 Helena tubes with blue markings from Marek.

Incubator in F468: control for cluster plate is still clear, as is the P100 control.

4/5/01 Thursday

Morning: 10.5° incubator: 3 racks of Helena tubes: the 2 of Anupam, 1 of Marek

Small incubator: T175 w 1522, T75 w same (from Sonia), P60's as before, no V79's

Large trash can: 4 T175's – all contaminated, 3 are V79 and 1 is 1522.

Double incubator: 2 empty rollers on top, nothing in the bottom

Marek observed in late afternoon that the Helena tubes from last week that were in the 10.5° incubator are now gone. I confirm at about 9PM. I look in the radioactive waste for the 6 tubes and do not find them. I also look in all the other trash baskets without finding them.

4/6/01 Friday 9:30 AM

10.5° incubator: 2 sets of clusters, one is Marek's, the other (7 tubes) is Anupam's.

Small incubator: P60's are all gone – the (–) ones were counted and discarded in the large red trash bag. Colonies look OK.
The (+) ones are by the sink – rinsed, no colonies, all were very contaminated.

Roger looks very angry.

Later that day, I see the notebook containing the protocol for the experiment. The date at the top of the first page is 3/26/01 which would be the day that the cells were first harvested to begin the experiment. The first page has the standard protocol that involves rolling the first night, harvesting, making 50:50 clusters the next day and incubating them at 10.5° for 3 days. I did not get a chance to see when the dye was to be added. The second page indicates that, on 3/31, the clusters are broken up and the cells are taken to the FACS machine for separation of dyed-radioactive cells from the bystander-non-radioactive cells. I also observe that there are colony counts on the second page and that Roger has signed this page. I observe the notebook containing this protocol on Anupam's desk after Roger has left for the day.

Roger asks Marek to do a mutagenesis study along with Anupam using the cells that Anupam has collected from clusters today. Marek offers to help Anupam count the cells in the 14 samples using the Coulter Counter. Anupam says that he will do it. Marek is very surprised at how quickly Anupam comes up with the counts. In fact, he figures the time was so short that Anupam did not have time to count them. He looks at the Coulter Counter and notes that it is set to count 0.5 ml. However, Anupam always counts 0.05 ml. Had he counted 0.5 ml, the numbers he got would be 10X those he would have expected and 10X those that he recorded. We conclude that Anupam did not count the samples but made up the numbers.

Anupam is still in the lab when Marek leaves.

4/7/01 Saturday

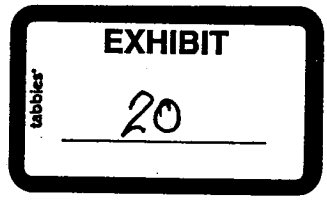
I went to verify the dates and the protocol for the experiment started 3/26, but the notebook is nowhere to be found.

Incubator inventory:

Double incubator: top – 2 empty rollers; bottom – nothing
Small incubator: top – 2 sets of p60's: 8x3 (-); 8x3 (+)
3rd, 4th & 5th shelves: p60's and p100's
6th shelf: 1 T175 of V79: OK
1T75 of 1522 fr Sonia – very contaminated; 1T175 of 1522: mold growing in the middle
10.5°: empty

Figures

Figure #	File name	Date	
1	p1010004	3/30	L: contaminated T175 of V79 probably thawed and plated 3/28. R: uncontaminated T175 of AG1522 (cf notes of 3/29, p2)
2	p1010006	3/30	L: either sample 1 or 7 sampled on 3/28 – shows contamination. R: Control medium – no contamination
3	p1010008	3/30	K is control – uncontaminated, 1 and 7 are contaminated. Same dishes as in Figure 2.
4	p1010009	3/30	After 5 PM: 6 Helena tubes in the 10.5° incubator – should not be there – should have been processed for FACS. #7 is gone – was last seen at 3:00 PM.
5	p1010010 (3310)	3/30	10.5° incubator: Same as #4 but have figured out how to date stamp.
6	p3310011	3/31	10.5° incubator: Same as #'s 4 and 5 but with black cardboard behind for contrast. See notes, p3.
7	p3310012	3/31	10.5° incubator: Same as #6.
8	p4010005	4/1	10.5° incubator: Helena tubes are still there.
9	p4010006	4/1	10.5° incubator: Helena tubes are still there. Marek took out tube #2 and put it back in the wrong place.
10	p4010007	4/1	Small incubator: The p60 culture dishes plated from the sort resulting from the FACS: - in front, + in back.
11	p4010008	4/1	Same as 10.
12	Photo		4/3 AM Tube #2 is back where it belongs.
13	Photo		4/3 PM Now 2 sets of Helena tubes are on the upper shelf on the left – belonging to Anupam and 1 set on the lower shelf on the right – belonging to Marek. The set from the 3/26 experiment is in front on the upper shelf. The one in the rear is from the new experiment started yesterday, 4/2, has 7 tubes and rolled overnight, along with Marek's experiment.
14	Photo	4/5	The old experiment (3/26) is gone (had a radioactivity label on the left end). The new experiment (4/2) has 7 Helena tubes. Marek's experiment is still there on the lower shelf.



15 Photo 4/5 Same as 14.

16 Photo 4/7 3 P100's sampled on 3/28: Top (K = control) is still uncontaminated, 1 and 7 are cloudy as before.

17 Photo 4/7 'Cluster' plate (sorry for the confusing nomenclature). K is on the R front and is uncontaminated. The other 5 are contaminated.

Figure 1

P1010004

3/30/01

EXHIBIT

tabbies

21

Figure 2

P1010006 3/30/01

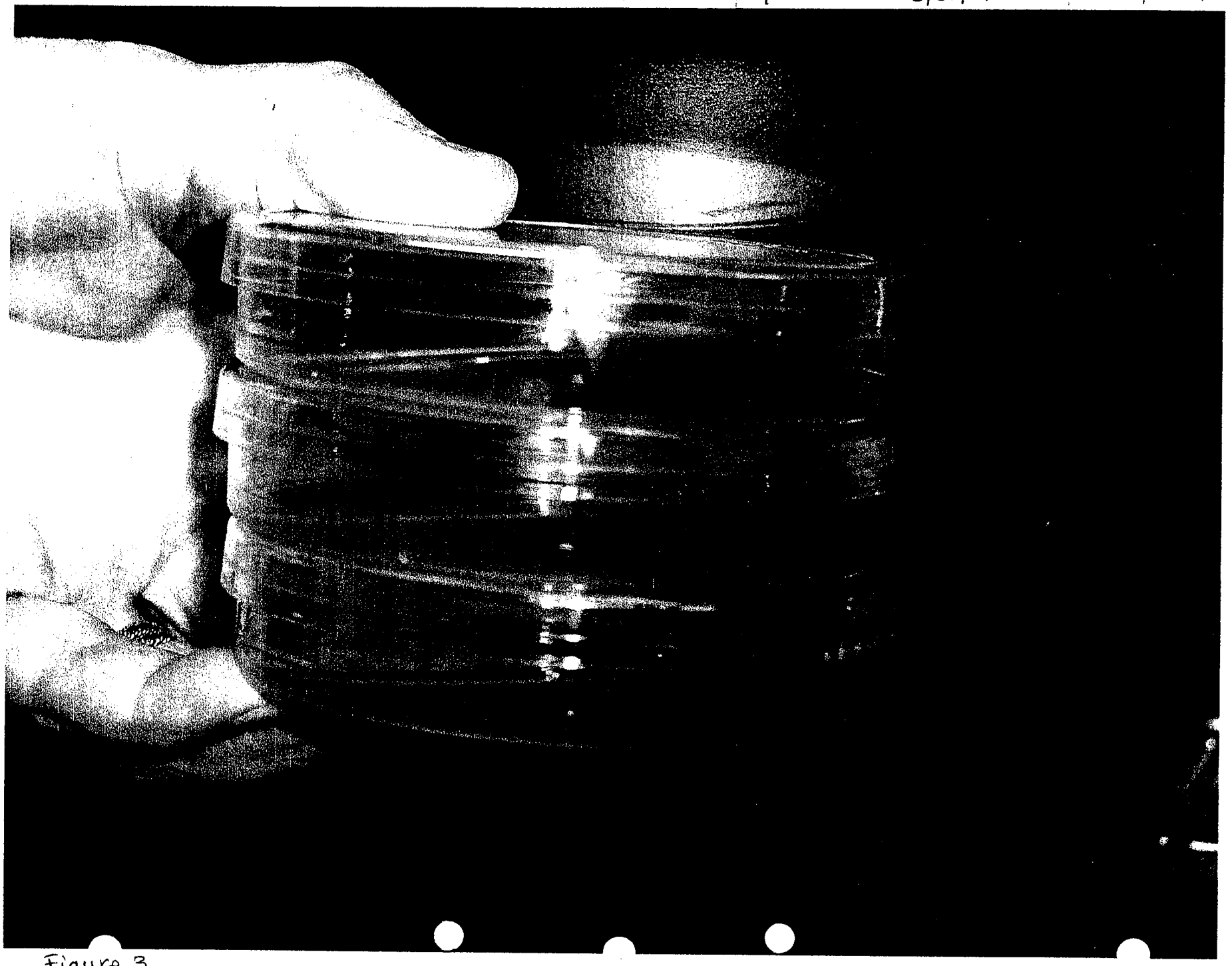


Figure 3

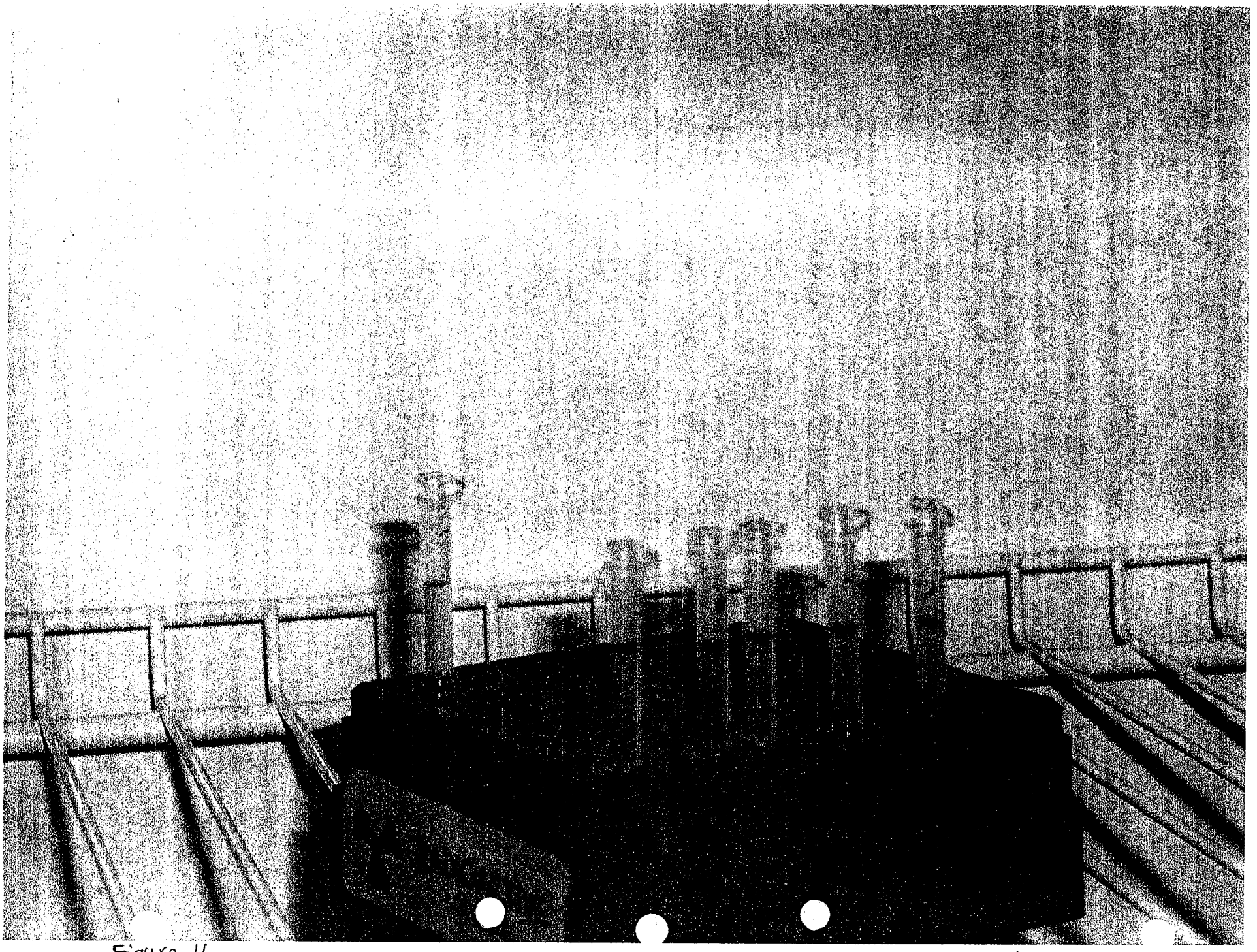


Figure 4

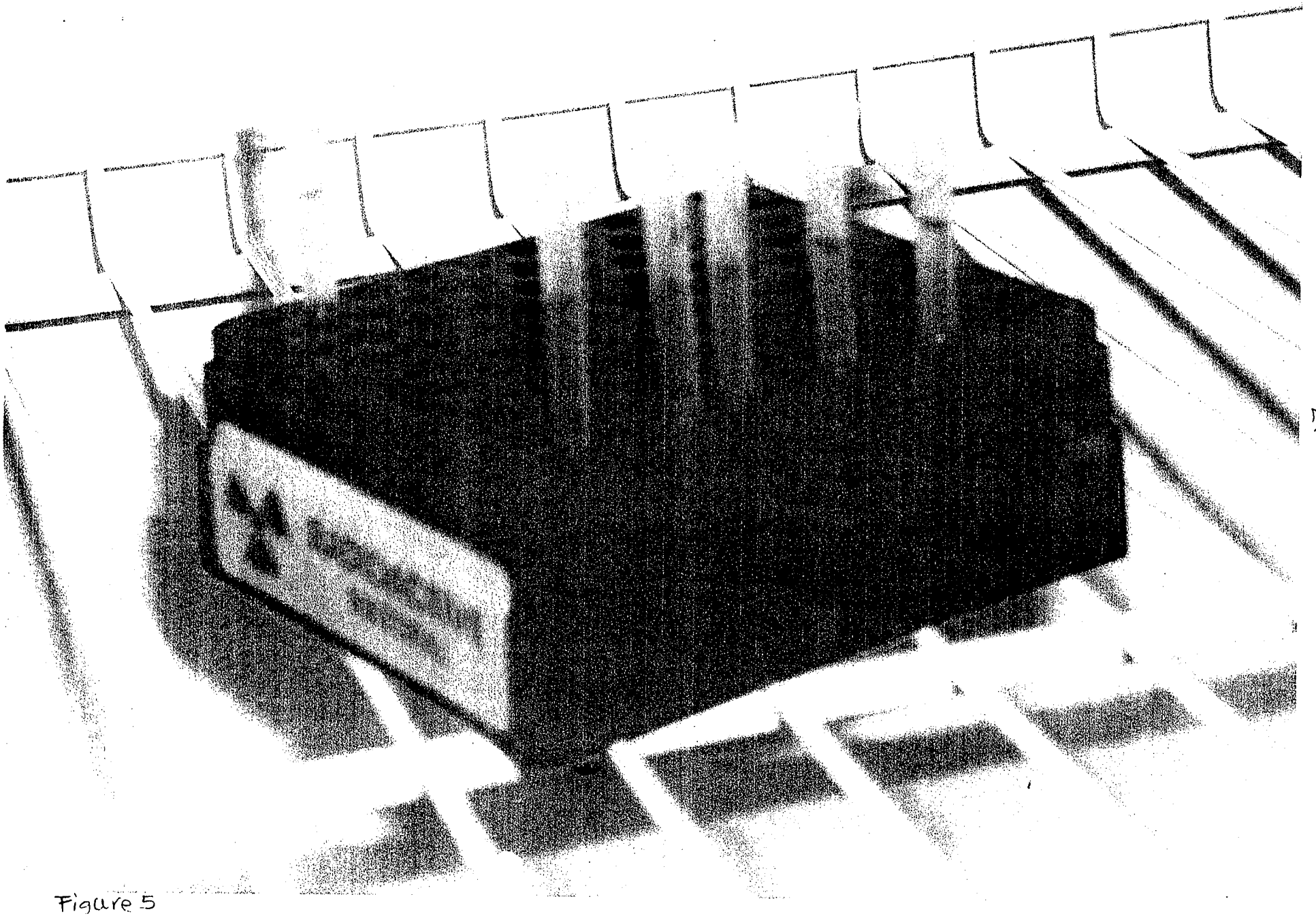


Figure 5

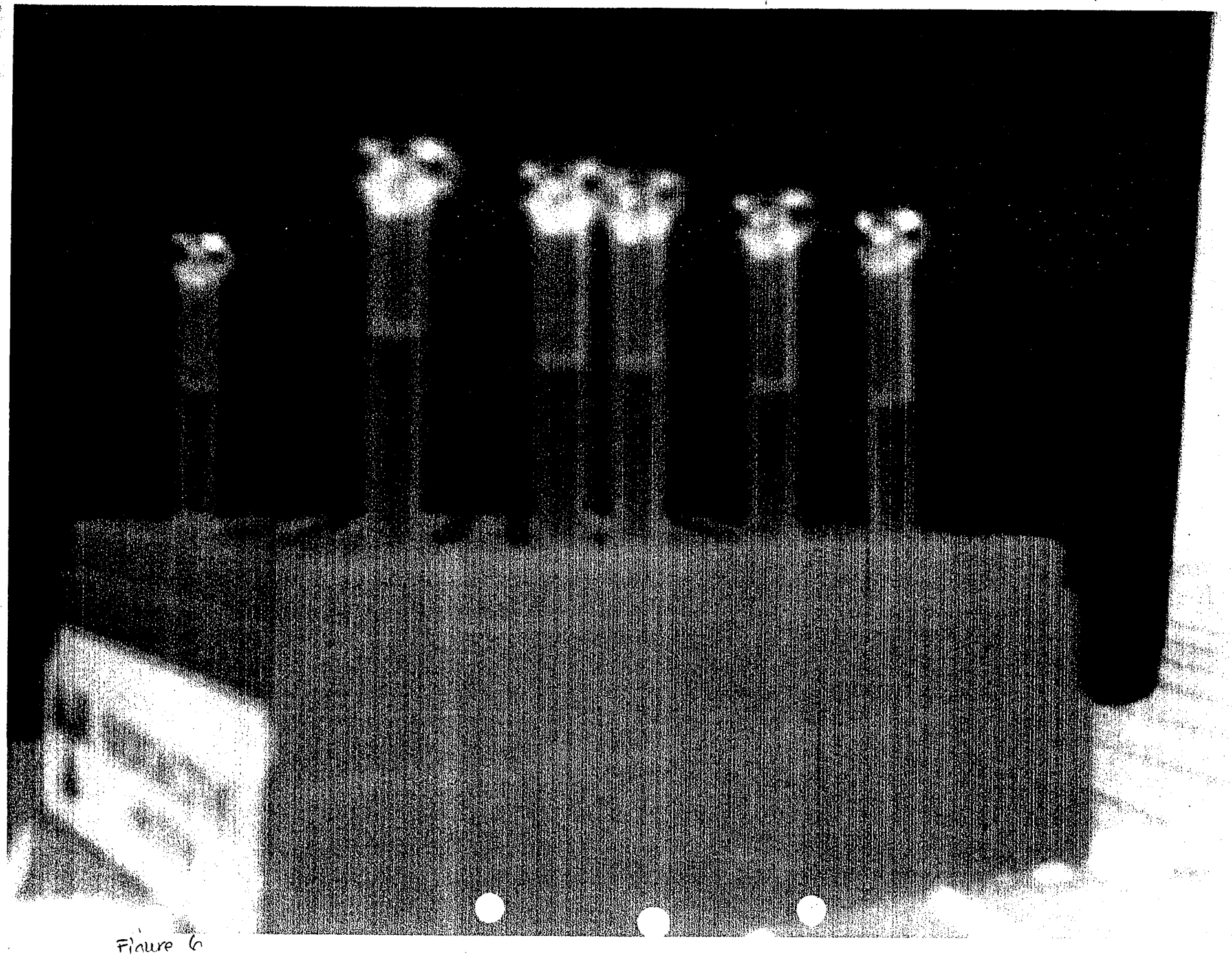


Figure 6

Figure 4

P351 CW12 5/31/01

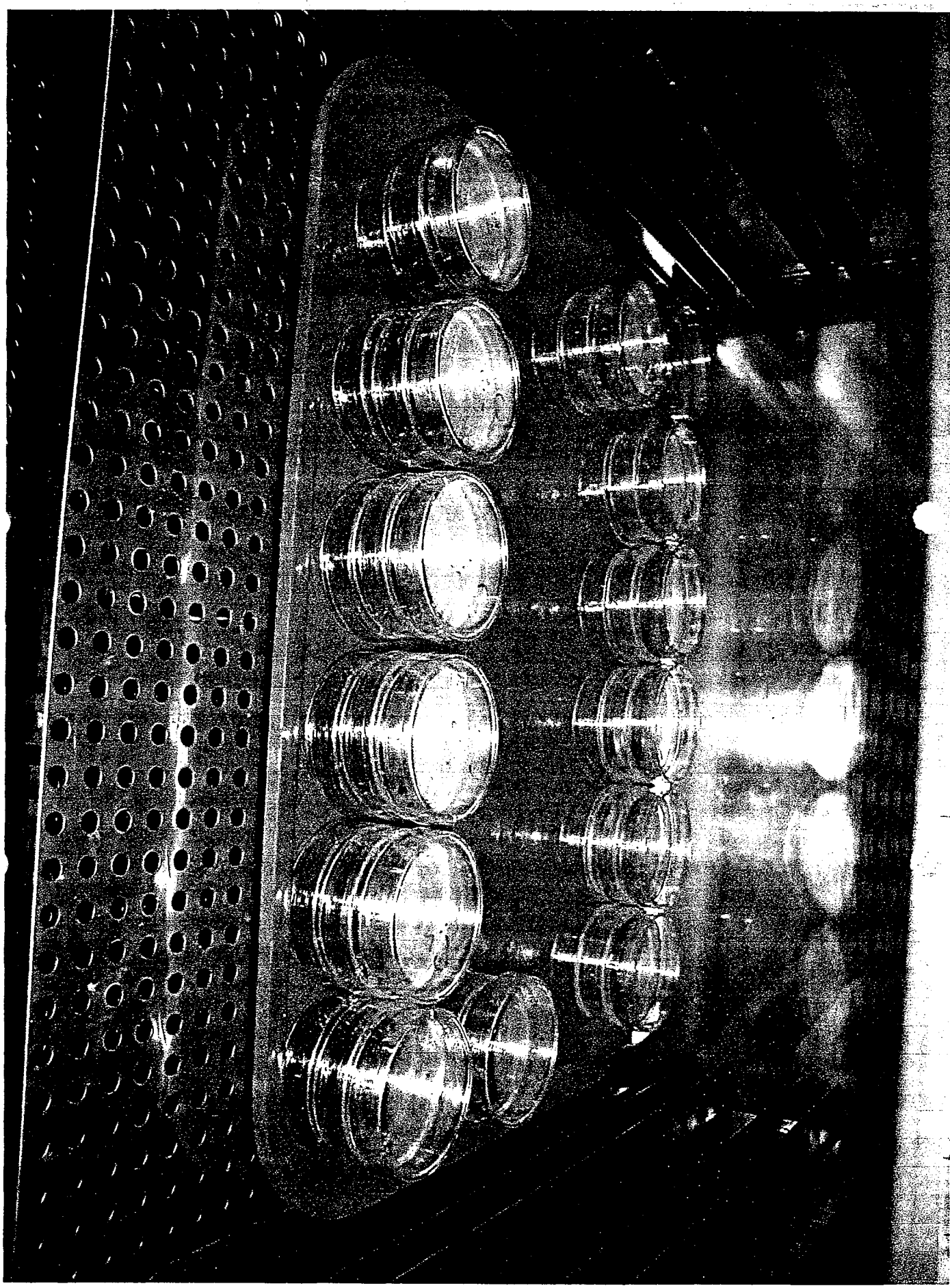
Figure 8

4010005 4/11/01

Figure 9

Fig 10

Fig 11



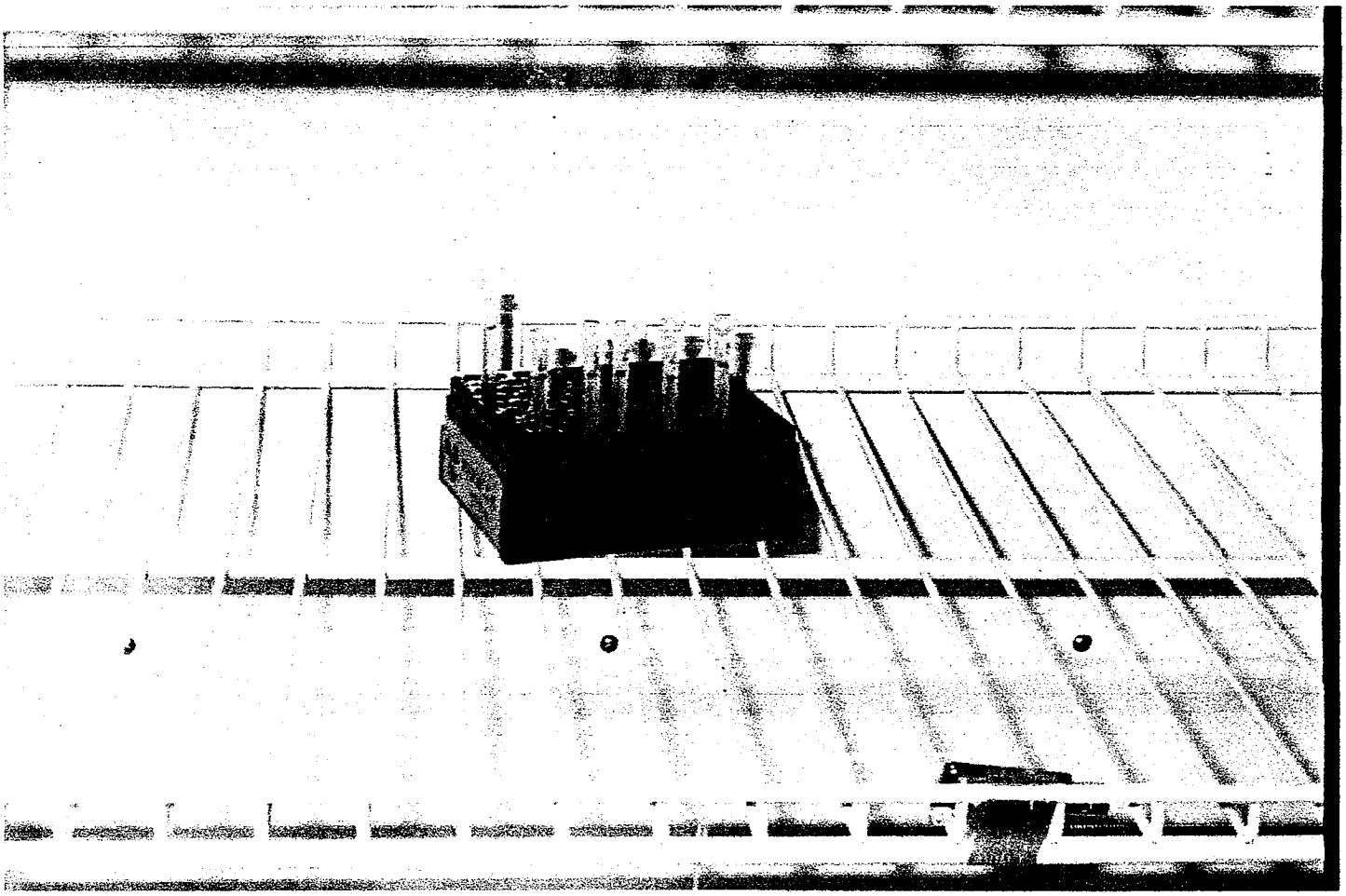


Fig 12 AM 4/3/01 photo10301 scan of photo

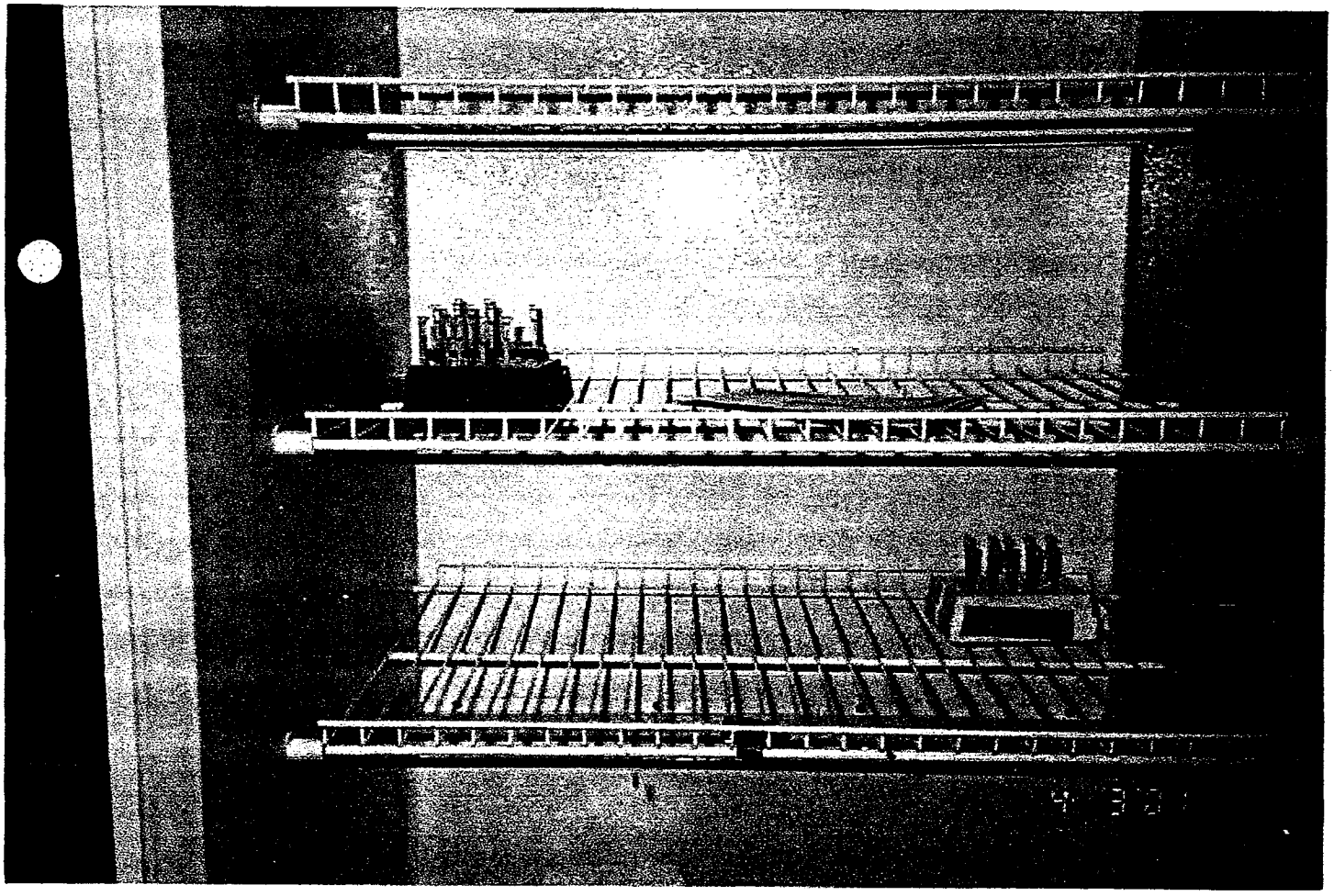


Fig 13 PM 4/3/01 scan of photo

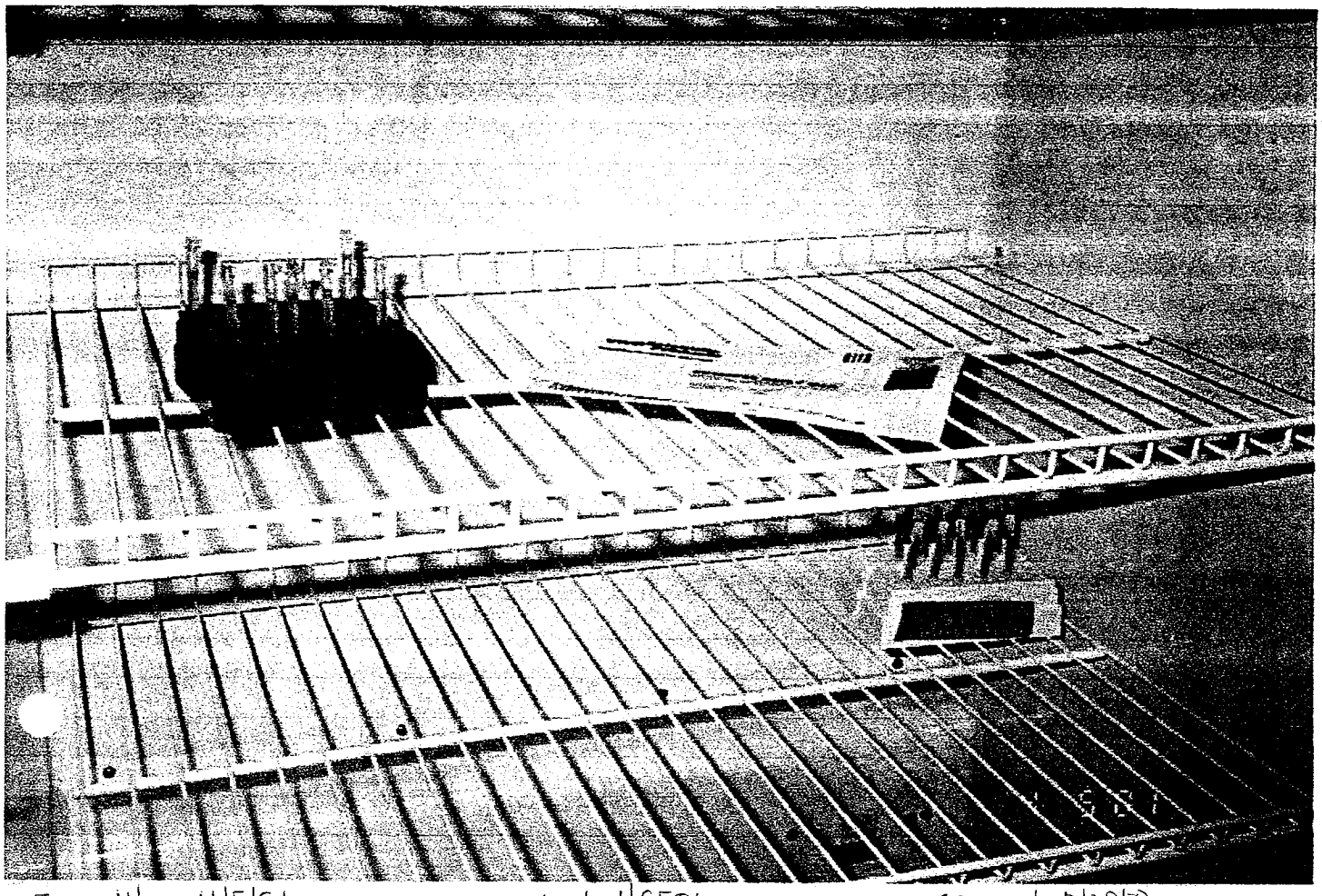


Fig 14 4/5/01 photo 40501 scan of photo

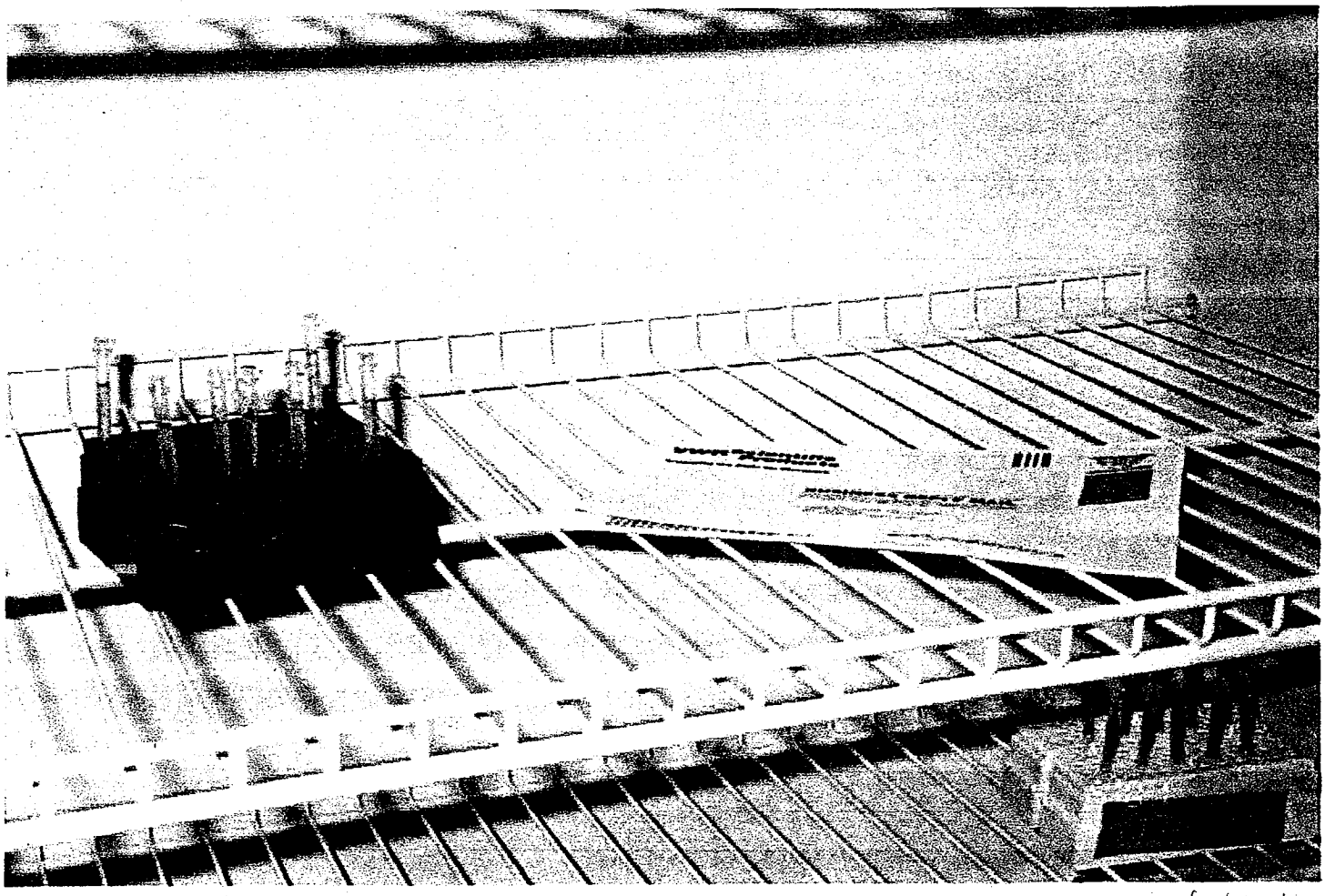


Fig 15 4/5/01 scan of photo

Fig 16 4/7/01 photo 40701 scan of photo

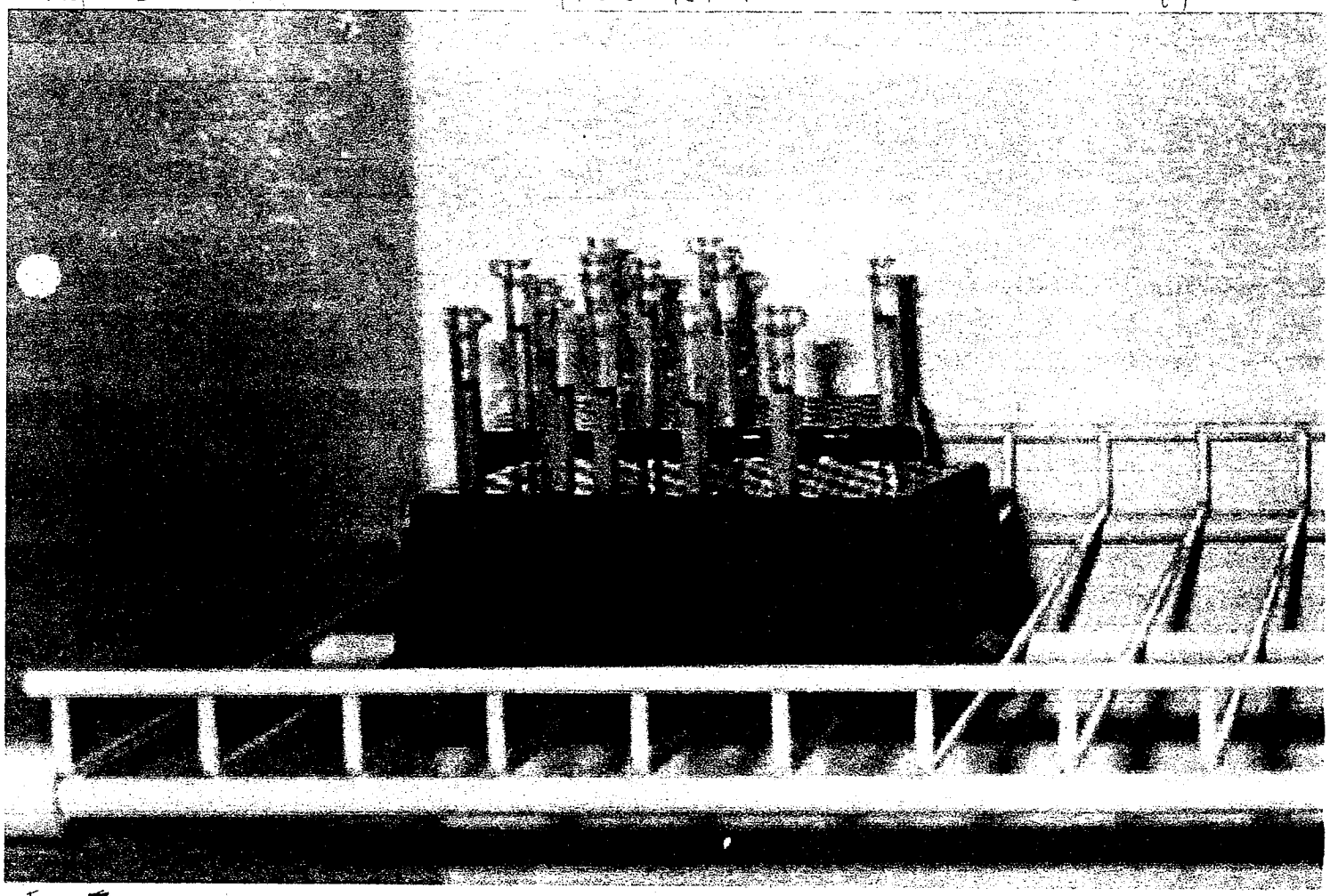


Fig 17 Enlargement of 4/3/01 scan of photo

Inventory and Disposition Record

LICENSEE: R. HOWELL | ISOTOPE FORM: H3 | THYMIDINE [METHYL-3H] | ACTIVITY IN mCi: 5.00000 mCi

DATE: 2/15/01
CAL DATE:
MANUFACTURER: PERKINELME
CATALOG #: NET027Z
LOT #: 3106412

PHYSICAL FORM: liquid/solid
STORED AT: 4C/-20C/room temperature
STORED IN ROOM: 451A

Date Used	Activity Used (uCi)	Disposal Separate by isotope!!	Balance (uCi)
02/15/01	640	80 %Liquid 20 %Solid %LSV	4360
02/19/01	90	80 %Liquid 20 %Solid %LSV	4250
03/6/01	400	90 %Liquid 10 %Solid %LSV	3850
03/8/01	640	80 %Liquid %Solid %LSV	3210
03/09/01	60	60 %Liquid 40 %Solid %LSV	3150
03/13/01	100	90 %Liquid 10 %Solid %LSV	3050
03/20/01	360	324 %Liquid 36 %Solid %LSV	2690
03/19/01	175	60 %Liquid 40 %Solid %LSV	
03/27/01	360	324 %Liquid 36 %Solid %LSV	
03/26/01	36	60 %Liquid 40 %Solid %LSV	
04/02/01	300	60 %Liquid 40 %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
		%Liquid %Solid %LSV	
Example:			
1/1/98	150	50%Liquid 30%Solid 20%LSV	100

← This shows that 36 µCi of ^{3}Hd Thd were taken to the Experiment on March 26. On page 28, 31.98 uCi were used in the experiment so this is consistent with the requirement

INSTRUCTIONS:

1. Post Inventory and Disposition Form on or near storage area
2. Update Inventory form after EACH and EVERY USE, this is a requirement of NRC
3. Radionuclides with a half-life <120 days are Decayed-On-Site
4. Dispose of all Radioactive Wastes through the Office of Radiation Safety Services (ORSS)
   Therefore: Deface all materials associated with these isotopes PRIOR to disposal into the LLRW
5. Maintain this record for a minimum of three (3) years

Questions, or concerns please call 2-5305, 2-5306, or by pager 899-8438

EXHIBIT
tabbies
22

V79 COLONY FORMING ASSAY FOLLOWING FACS

Experiment Name: Cell separation by FACS and SF (^{3}HTdR cluster, 50% labeling, five ^{3}HTdR conc.)

Exp. # 3; **Investigator:** A. Bishayee **Date:** 03/26/01

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 80-90% confluent 175 cm^2 flasks, subcultured 4-5 days before) with PBS, trypsinize cells, each resuspend in 7 ml MEMB, pool, pass five times through 5 or 10 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~2,000,000 cells/ml in MEMB [Actual count : 5,200,000 cells/ml)
3. Transfer 1 ml of cell suspension into two sets of tubes (7 tubes per set; Falcon plastic test tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 03/26/01; 4-0 pm
5. Prepare MEMB containing radioactivity in hood

   36 µl ^{3}HTdR (Stock : 1 µCi/µl on 2/15/01) + 3 ml MEMB
6. After 3-4 h, remove first set of tubes from roller and add MEMB with or without radioactivity according to Table below. i.e. 2nd set gets no radioactivity **Date/Time:** 03/26/01; 7-1 pm

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR 12uCi/ml (ml)	CFDA in PBS (1 uM) (ml)
1	0	1.0	1.0	0	2
2	0	1.0	1.0	0	2
3	1	1.0	0.835	0.165	2
4	2	1.0	0.665	0.335	2
5	3	1.0	0.5	0.5	2
6	4	1.0	0.335	0.665	2
7	6	1.0	0	1	2

Radioactivity used

12 X 0.165 = 1.98 µCi

12 X 0.335 = 4.02

12 X 0.5 = 6.0

12 X 0.665 = 7.98

12X 1.0 = 12.0

Total radioactivity for this exp: 31.98 µCi

7. Add 1 ml of MEMB tube and return test tubes to roller for 14 h. **Date/Time:** 03/26/01; 7- pm
8. Next day, while test tubes are in roller label tubes (13 X 100 mm VWR glass test tube)

EXHIBIT
tabbies
23

9. After ~14 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (*precooled centrifuge*).

Date/Time: 03/27/01; 9-30 a

10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled tubes.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex
18. Add 8 ml of PBS in each tube, vortex and transfer the content to 15-ml plastic centrifuge tube
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes,vortex
21. Add 2 ml of 1 uM CFDA in prewarmed PBS as per the Table and PBS in the remaining tubes.
22. Incubate all tubes at 37°C for ~~15~~ 30 min.
23. Centrifug tubes for 10 min at 2000 rpm, 4°C
24. Decant supernatant, click tubes, vortex, add 2 ml prewarmed MEMA
25. Incubate all tubes at 37°C for 30 min.
26. Centrifuge and decant the supernatant, suspend in 5 ml MEMA
27. Follow steps 11-24 for second set of tubes
28. Transfer the content of one tube from one set to the corresponding tube of another set
29. Centrifuge, decant the supernatant
30. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
31. Again add 200 ul MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
32. Centrifuge tubes for 5 min at 1000 rpm, 4°C
30. Transfer tubes at 10°C for 72 h.

Date/Time: 03/~~28~~ 27/01; 1-00 PM

33. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to eight 15 ml tubes containing 10 ml PBS by using pasteur pipet

Date/Time: 03/30/01; 12-45 pm

34. Again add 200 ul PBS in microcentrifuge tubes, resuspend and transfer the cell suspensions in 15 ml tubes

35. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
36. Decant supernatant, click tubes, vortex, pooled cells from corresponding tubes, centrifuge, decant the supernatant, resuspend in 2 ml PBS with, syringe and transfer aliquots for cell count (100 ul) and radioactivity count (50 ul)
37. Centrifuge, decant, resuspend in 1 ml PBS for each tube and transfer ~1ml in Falcon 12x75 mm polystyrene 6 ml tube, wrap the tubes with aluminium foil, put in ice and transfer for FACS study.
38. During sorting, collect both dye-positive and dye-negative cells in VWR 12x75 mm glass tube (pre-cooled in ice) containing 1 ml PBS with 100 U penicillin and 100 µg streptomycin (add 20 µl Pen-Strep from the commercial stock in 1 ml PBS to get the desired concentrations).
39. Transfer cells in PBS in 15-ml plastic centrifuge tube, add 7 ml of PBS, and centrifuge
40. Decant, vortex, resuspend in 1 ml of PBS, and transfer 100 µl for cell count
41. Transfer 300 µl in Falcon 12x75 mm polystyrene 6 ml tube for FACS analysis to check the purity of the sorted cells.
42. Dilute remaining cells (three 10-fold dilution by transferring 0.5 ml cells to 4.5 ml MEMA)
43. Plate required number of cells (200, 2000 or 20,000) in Falcon 60 mm tissue culture dish (in 4 ml total volume of MEMA).
44. Count colonies following a week.

4/6/2001 7:00am initiate colony ~~RMA~~ staining and counting

* ALL Dye positive colonies contaminated.
ALL Dye negative colonies <u>not</u> contaminated

1.2 - 167, 178
2.2 - 193, 204, 190
3.2 - 166, 152, 169
4.2 - 150, 132, 148
5.2 - 129, 135, 136
6.2 - 100, 127, 119
7.2 - 81, 75, 100

30µl medium

R: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 27 MAR 2001 10:04
PLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
1 AQC:N QCF:N RCM:N
NNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
A CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
F LIFE(DAYS):N

POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
**- 1	1	59.00	26.04	1.00	1.45	76.0	
**- 2	1	9.00	66.67	1.00	3.02	75.0	
**- 3	1	12.00	57.74	1.00	4.59	77.0	
**- 4	1	4.00	100.0	1.00	6.17	76.0	
**- 5	1	8.00	70.71	1.00	7.73	76.0	
- 6	1	9.00	66.67	1.00	9.37	79.0	
**- 7	1	30037.14	1.95	0.35	10.27	77.0	
**- 8	1	*12.00	57.74	1.00	11.90	73.0	
**- 9	1	**64890.00	1.76	0.20	12.65	85.0	
**-10	1	56870.00	1.88	0.20	13.46	78.0	
**-11	1	54540.00	1.91	0.20	14.27	77.0	
**-12	1	56435.00	1.88	0.20	15.02	78.0	
**-13	1	80786.66	1.82	0.15	15.72	76.0	
**-14	1	84560.00	1.78	0.15	16.43	78.0	
**-15	1	87933.33	1.74	0.15	17.13	77.0	
**-16	1	101253.33	1.62	0.15	17.83	77.0	
**-17	1	113006.66	1.54	0.15	18.53	76.0	
**-18	1	121893.33	1.48	0.15	19.23	78.0	
**- 1	1	172990.00	1.52	0.10	20.00	74.0	
**- 2	1	166430.00	1.55	0.10	20.71	74.0	
**- 3	1	166500.00	1.27	0.15	21.42	76.0	

* Sample was not added
** Sample was added twice

Experiment: H-3/50%/FACS3
Date: 3/26/2001

Tube #	Medium count (CPM) 1st	2nd	3rd	CPM Average	CPM - corrected for control	DPM CPM/(y e)	At µCi/ml on counting	Ao µCi/ml at addition [At/e-0.693t/T]	Ao kBq/ml at addition
1	*59*	*9*	*12*	*17*	*0*	*0*	*0*	*0*	*0*
2	*4*	*8*	*9*		*0*	*0*	*0*	*0*	*0*
3	30037	32445	32445	31642	31626	48655	0.7305	0.7306	27.0329
4	56870	54540	56435	55948	55932	86048	1.2920	1.2921	47.8091
5	80786	84560	87933	84426	84410	129861	1.9499	1.9500	72.1516
6	101253	113006	121893	112051	112034	172360	2.5880	2.5882	95.7643
7	172990	166430	166500	168640	168623	259420	3.8952	3.8956	144.1358
8				*#DIV/0!*	*0*	*0*	*0*	*0*	*0*
9					*0*	*0*	*0*	*0*	*0*
10				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

23-5

ENTER=FAX

Sand alley

:R: 6 ID:H3 HOWELL PRESET TIME: 1.00 MON 02 APR 2001 09:41
IPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
1 AQC:N QCF:N RCM:N
UNNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
LIFE(DAYS):N

	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
	**- 1	1	9.00	66.67	1.00	1.43	70.0	
1c	**- 2	1	9.00	66.67	1.00	3.05	70.0	
	**- 3	1	5.00	89.44	1.00	4.68	69.0	
	**- 4	1	12.00	57.74	1.00	6.30	71.0	
2c	**- 5	1	10.00	63.25	1.00	7.93	71.0	
	**- 6	1	10.00	63.25	1.00	9.49	69.0	
	**- 7	1	2255.00	4.21	1.00	11.12	73.0	
3c	**- 8	1	2500.00	4.00	1.00	12.69	73.0	
	**- 9	1	2502.00	4.00	1.00	14.26	72.0	
	**-10	1	4333.00	3.04	1.00	15.82	77.0	
4c	**-11	1	4212.00	3.08	1.00	17.40	74.0	
	**-12	1	3324.00	3.47	1.00	19.02	72.0	
	-13	1	3934.00	3.19	1.00	20.65	72.0	
5c	**-14	1	4703.00	2.92	1.00	22.22	77.0	
	**-15	1	3881.00	3.21	1.00	23.79	73.0	
	**-16	1	6757.00	2.43	1.00	25.42	76.0	
6c	**-17	1	8521.00	2.17	1.00	26.99	81.0	
	**-18	1	6181.00	2.54	1.00	28.56	77.0	
	**- 1	1	8844.00	2.13	1.00	30.20	78.0	
7c	**- 2	1	12981.25	1.96	0.80	31.62	84.0	
	**- 3	1	11244.44	1.99	0.90	33.08	81.0	
	**- 4	1	29762.86	1.96	0.35	34.03	-1.0 →	Standard

Experiment: H-3/50%/FACS3
Date: 03/26/01

Tube #	Suspension count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	A_t µCi/ml on counting	A_o µCi/ml after uptake	A_o kBq/ml after uptake
1	*9*	*9*	*5*	*9*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*12*	*10*	*10*			*0*	*0.00000*	*0*	*0.0000*
3	2255	2500	2502	2419	2410	3707	0.03340	0.03340	1.2358
4	4333	4212	3324	3956	3947	6073	0.05471	0.05471	2.0242
5	3934	4703	3881	4173	4164	6405	0.05771	0.05771	2.1351
6	6757	8521	6181	7153	7144	10991	0.09901	0.09901	3.6635
7	8844	12981	11244	11023	11014	16944	0.15265	0.15265	5.6481
8				*#DIV/0!*	*0*	*0*	*0.00000*	*0*	*0.0000*
9						*0*	*0.00000*	*0*	*0.0000*
10				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

CoulterSurvival

Experiment: H-3/50%/FACS3
Date/Time: 3/26/01

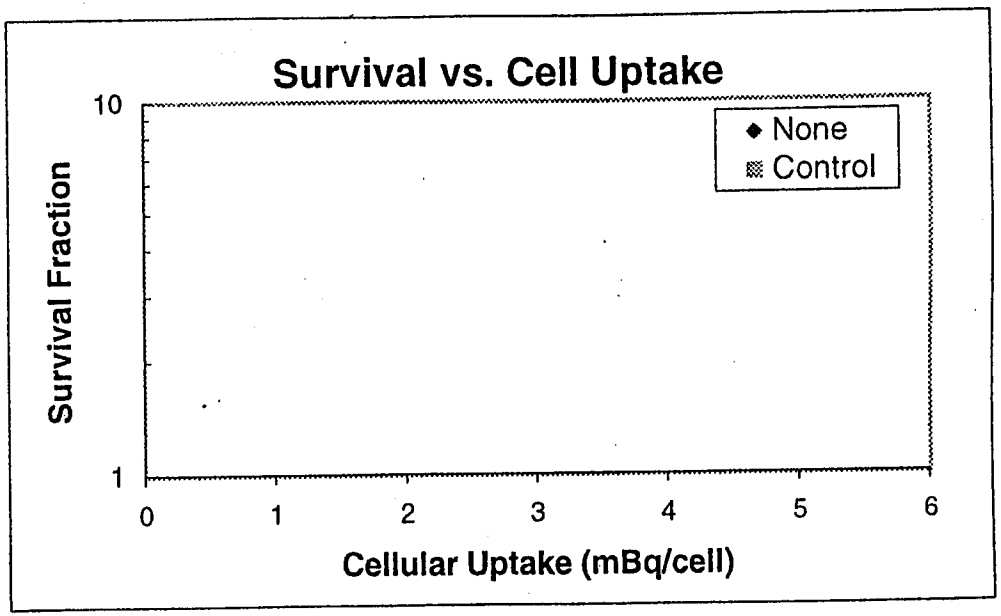
Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*612*	*632*	*643*	*629*	*2505333*				
2	*633*	*621*	*654*	*636*	*2533333*				
3	634	654	666	651	2594667				
4	635	619	644	633	2520000				
5	579	598	609	595	2370667				
6	599	641	642	627	2498667				
7	598	601	582	594	2364000				
8				*#DIV/0!*	*#DIV/0!*				
9				*#DIV/0!*	*#DIV/0!*				
10				#DIV/0!	#DIV/0!				
11				#DIV/0!	#DIV/0!				
12				#DIV/0!	#DIV/0!				
13				#DIV/0!	#DIV/0!				
14				#DIV/0!	#DIV/0!				

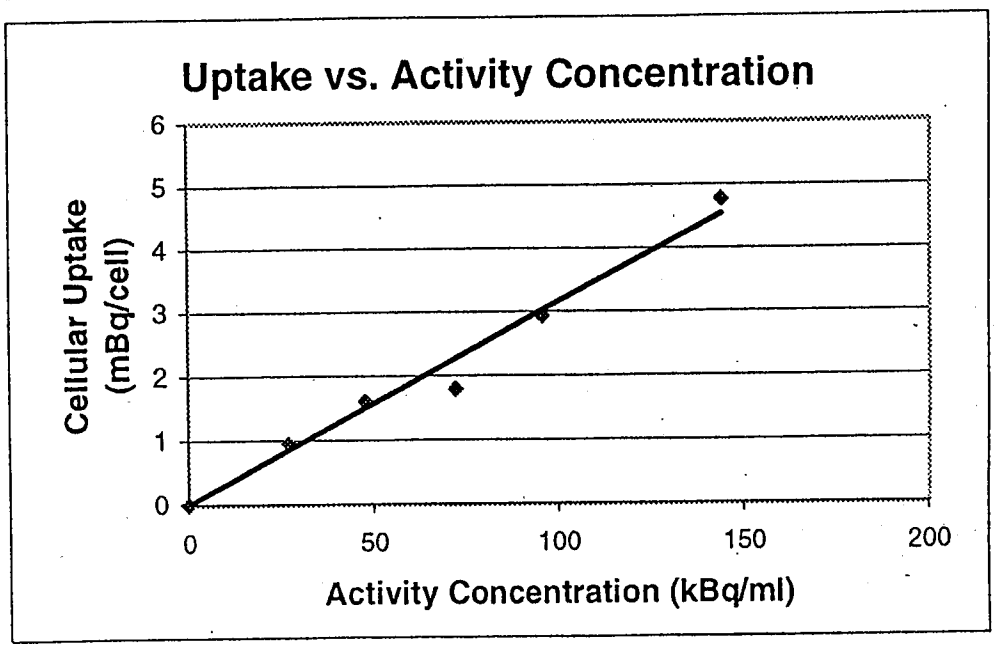
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrecte	SF Corrected
1	200	*251*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
2	200	*253*							
3	200	259				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
4	200	252				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
5	200	237				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6	200	250				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
7	200	236				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
8		*#DIV/0!*				*#DIV/0!*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
9		*#DIV/0!*							
10		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14		#DIV/0!				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Summary

Experiment:
Date/Time: 3/26/01

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*#DIV/0!*	*#DIV/0!*
2	*0.000*	*0.000*		
3	27.033	0.953	#DIV/0!	#DIV/0!
4	47.809	1.606	#DIV/0!	#DIV/0!
5	72.152	1.801	#DIV/0!	#DIV/0!
6	95.764	2.932	#DIV/0!	#DIV/0!
7	144.136	4.778	#DIV/0!	#DIV/0!
8	*0.000*	*#DIV/0!*	*#DIV/0!*	*#DIV/0!*
9	*0.000*	*#DIV/0!*		
10	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
11	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
12	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
13	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
14	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!





SORT

DATE: 3/30/01

TIME: 2:45 - 4:30

INVESTIGATOR:

	LEFT SORT	RIGHT SORT	ABORT	FREQUENCY
	+	−		
TUBE 1	201255	203487		
TUBE 2	563425	501016		
TUBE 3	500572	570786		
TUBE 4	528945	500562		
TUBE 5	522404	500873		
TUBE 6	571869	500629		
TUBE 7	555058	469014		
TUBE 8				
TUBE 9				
TUBE 10				

3/30/01

Cell count after sorting

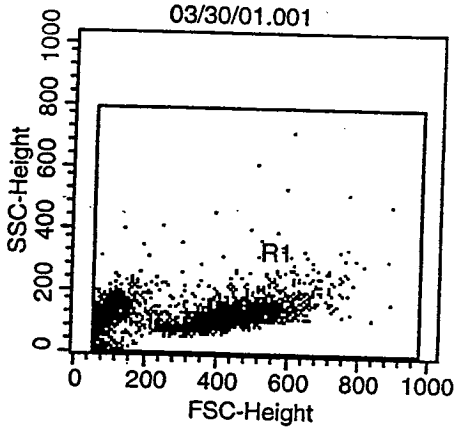
MS = 500 µl ; background = 21

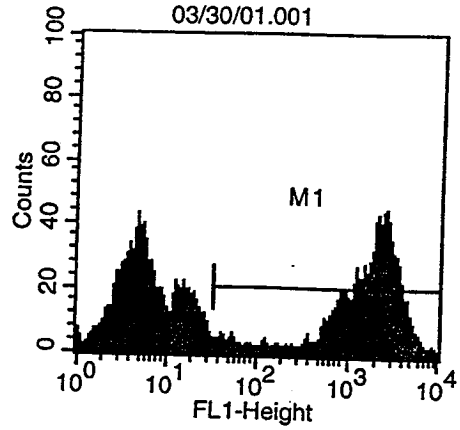
Dye(+)ve cells

Tube #	Coulter Counts	Avg.	Cell Conc. (#/ml)	# of cells plated (dil)	vol. pl (µl)
1	385, 395, 361	380	152133	200 (1:1000)	1315
2	1211, 1235, 1224	1223	489333	200 (1:1000)	410
3	1192, 1185, 1197	1191	476533	200 (1:1000)	420
4	1209, 1215, 1222	1215	486133	200 (1:1000)	411
5	1175, 1187, 1195	1185	474266	200 (1:1000)	420
				2000 (1:100)	420
6	1190, 1181, 1192	1187	475066	200 (1:1000)	420
				2000 (1:100)	420
7	1165, 1180, 1172	1172	468933	2000 (1:100)	425
				20,000 (1:10)	425

Dye(-)ve cells

1	365, 380, 377	374	149600	200 (1:1000)	1335
2	1127, 1135, 1145	1135	454266	200 (1:1000)	440
3	1207, 1229, 1217	1217	487066	200 (1:1000)	410
4	1177, 1189, 1162	1176	470400	200 (1:1000)	425
5	1150, 1169, 1141	1153	461333	200 (1:1000)	433
6	1135, 1169, 1147	1150	460133	200 (1:1000)	435
7	1081, 1065, 1047	1064	425733	200 (1:1000)	470



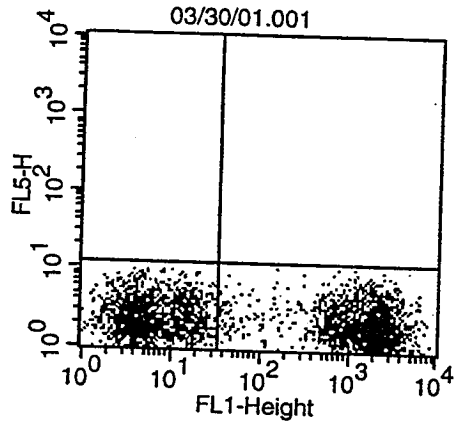


Histogram Statistics

File: 03/30/01.001
Sample ID: control 1
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9995	100.00	99.95	981.64	99.16	131.47	54.25	2458
M1	32, 9910	5130	51.33	51.30	1905.05	1407.60	64.14	1826.92	2458



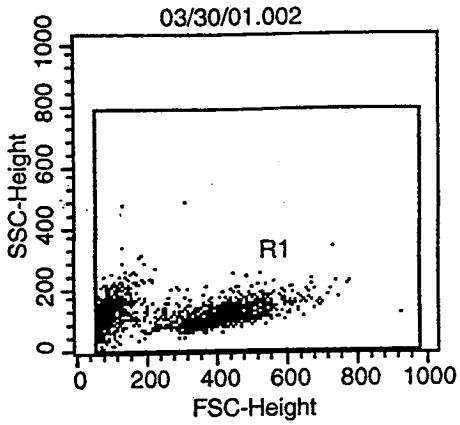
Quadrant Statistics

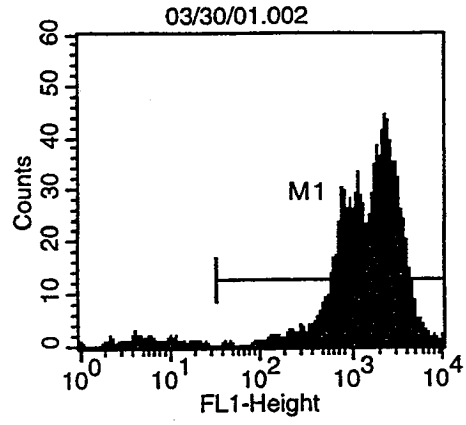
File: 03/30/01.001
Sample ID: control 1
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 33, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4881	48.83	48.81	8.02	6.08	2.77	2.44
LR	5114	51.17	51.14	1910.91	1424.28	2.45	2.16

After sorting



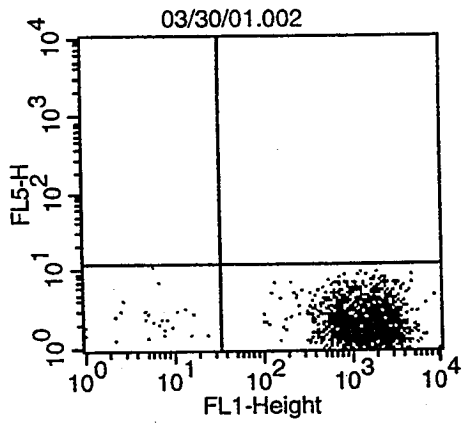


Histogram Statistics

File: 03/30/01.002
Sample ID: left +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5067
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5070

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5067	100.00	99.94	1738.06	1341.26	61.30	1610.76	2035
M1	32, 9910	4988	98.44	98.38	1765.47	1461.06	59.54	1640.00	2035



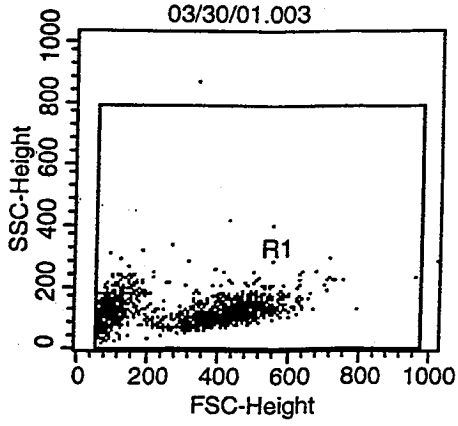
Quadrant Statistics

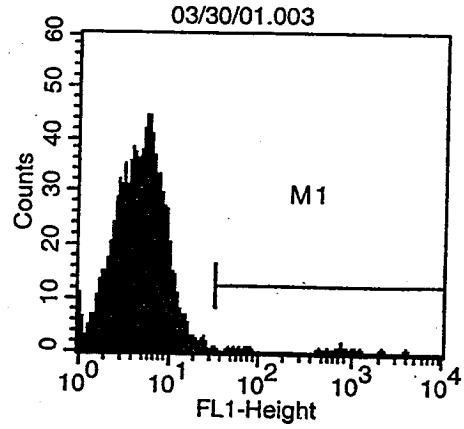
File: 03/30/01.002
Sample ID: left +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5067
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 33, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5070
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.02	0.02	1144.44	1144.44	12.30	12.30
LL	79	1.56	1.56	7.56	6.05	2.62	2.29
LR	4987	98.42	98.36	1765.59	1461.14	2.42	2.14

After sorting



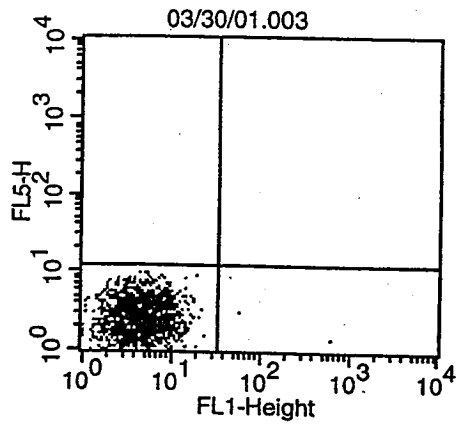


Histogram Statistics

File: 03/30/01.003
Sample ID: right -
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5068
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5070

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5068	100.00	99.96	7.68	4.54	876.54	4.53	5
M1	32, 9910	17	0.34	0.34	754.24	346.91	121.50	626.43	710

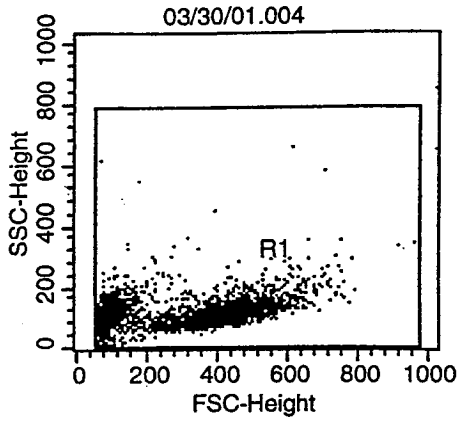


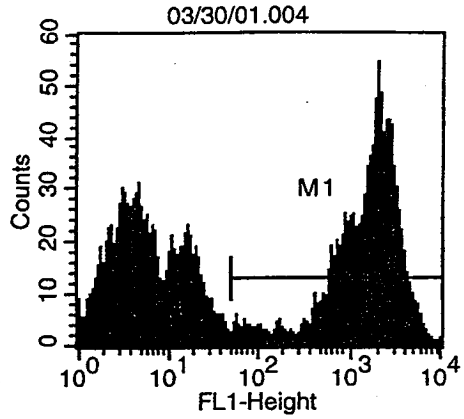
Quadrant Statistics

File: 03/30/01.003
Sample ID: right -
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5068
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 33, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5070
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5051	99.66	99.63	5.17	4.47	2.80	2.47
LR	17	0.34	0.34	754.24	346.91	2.53	2.21



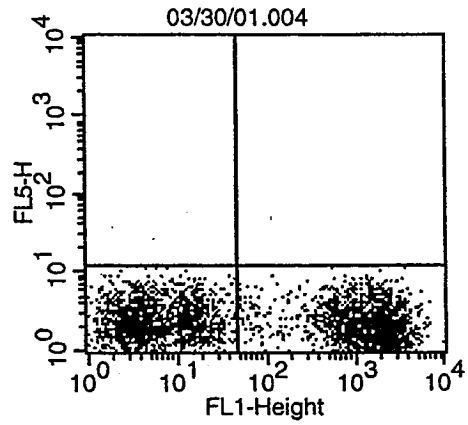


Histogram Statistics

File: 03/30/01.004
Sample ID: control 2
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9995	100.00	99.95	941.76	107.99	126.81	375.16	1762
M1	48, 9910	5344	53.47	53.44	1753.94	1353.65	63.73	1625.31	1762

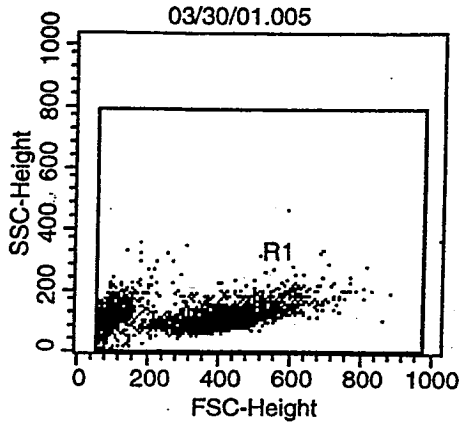


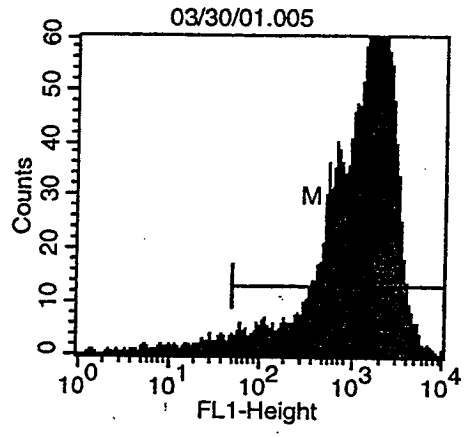
Quadrant Statistics

File: 03/30/01.004
Sample ID: control 2
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 48, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.01	4.22	4.22	13.22	13.22
UR	0	0.00	0.00	***	***	***	***
LL	4650	46.52	46.50	8.55	5.91	2.84	2.49
LR	5344	53.47	53.44	1753.94	1353.65	2.49	2.19



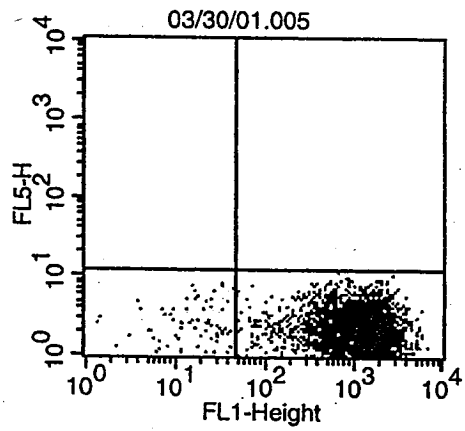


Histogram Statistics

File: 03/30/01.005
Sample ID: 100%
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9996
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9996	100.00	99.96	1397.07	1050.14	62.28	1321.58	1945
M1	48, 9910	9796	98.00	97.96	1425.13	1140.87	60.08	1345.57	1945

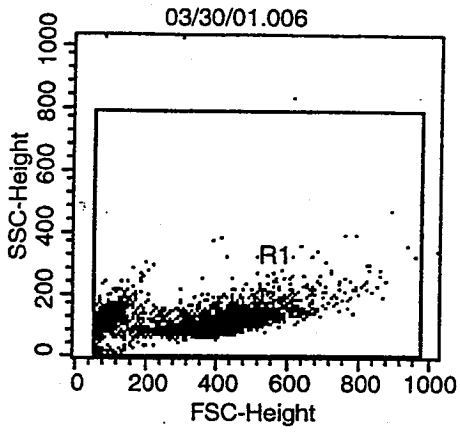


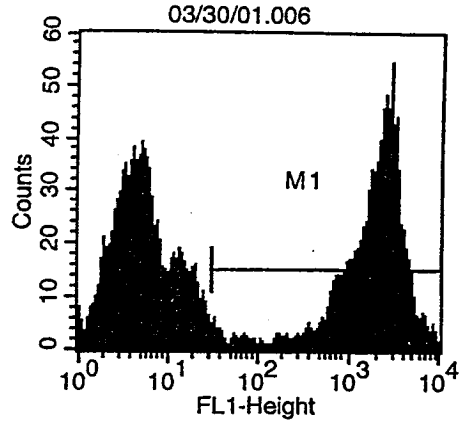
Quadrant Statistics

File: 03/30/01.005
Sample ID: 100%
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9996
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 48, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	1	0.01	0.01	3718.03	3718.03	12.41	12.41
LL	200	2.00	2.00	22.73	18.13	2.98	2.62
LR	9795	97.99	97.95	1424.89	1140.73	2.47	2.18



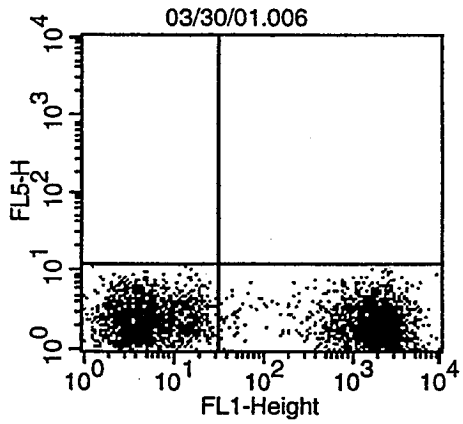


Histogram Statistics

File: 03/30/01.006
Sample ID: 3
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9988
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9988	100.00	99.88	998.49	83.98	133.77	22.98	2617
M1	30, 9910	4882	48.88	48.82	2035.89	1580.93	61.05	1919.57	2617

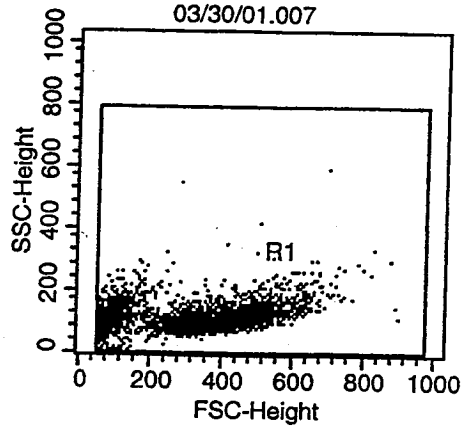


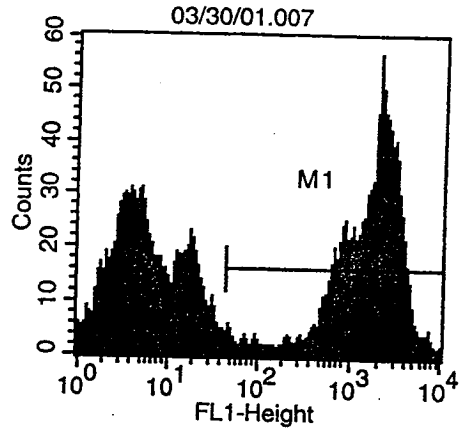
Quadrant Statistics

File: 03/30/01.006
Sample ID: 3
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9988
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 31, 12

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5126	51.32	51.26	6.69	5.11	2.78	2.45
LR	4862	48.68	48.62	2044.14	1606.84	2.45	2.16



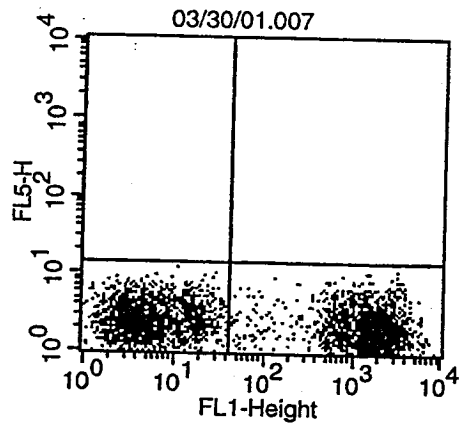


Histogram Statistics

File: 03/30/01.007
Sample ID: 4
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9993
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9993	100.00	99.93	963.26	100.42	128.89	199.89	1860
M1	42, 9910	5170	51.74	51.70	1854.25	1450.56	62.30	1762.36	1860



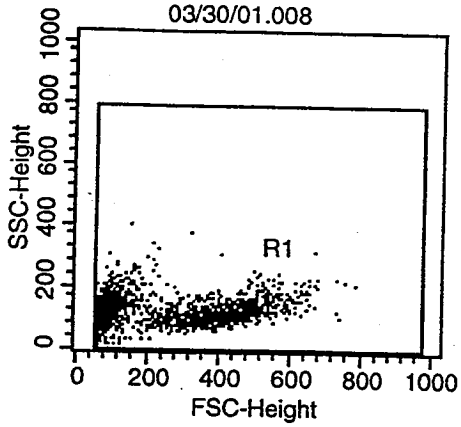
Quadrant Statistics

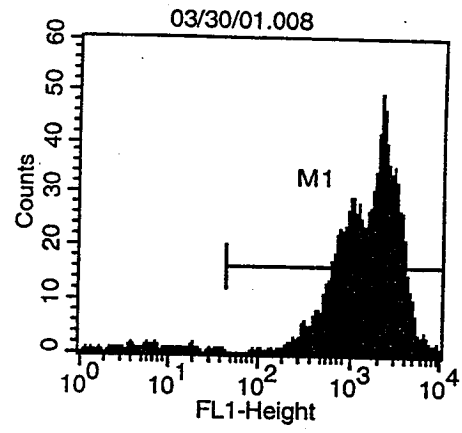
File: 03/30/01.007
Sample ID: 4
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9993
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 41, 14

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4823	48.26	48.23	8.17	5.74	2.83	2.49
LR	5170	51.74	51.70	1854.25	1450.56	2.46	2.16

After sorting



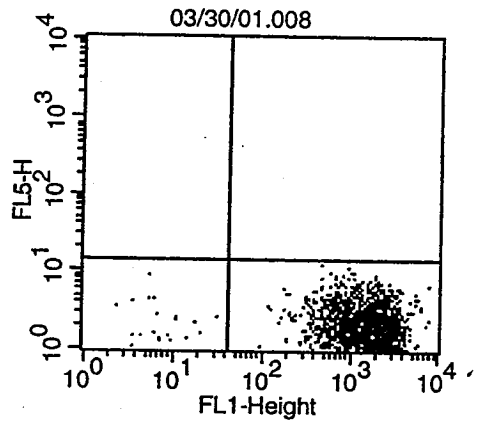


Histogram Statistics

File: 03/30/01.008
Sample ID: sort 4 +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5190
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5190

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5190	100.00	100.00	1715.64	1348.71	60.05	1596.34	1928
M1	42, 9910	5126	98.77	98.77	1736.93	1438.54	58.66	1625.31	1928



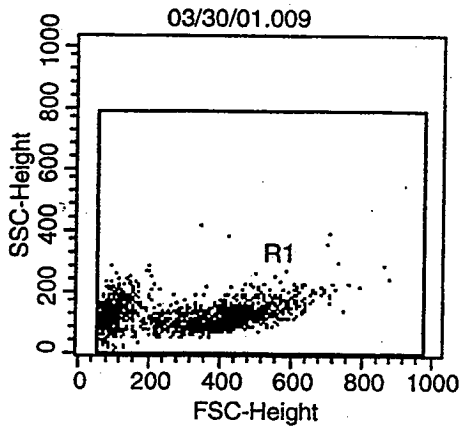
Quadrant Statistics

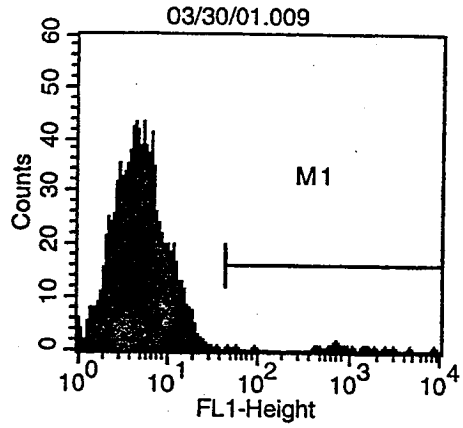
File: 03/30/01.008
Sample ID: sort 4 +
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5190
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 41, 14

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5190
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	64	1.23	1.23	10.31	7.71	2.66	2.37
LR	5126	98.77	98.77	1736.93	1438.54	2.48	2.18

After sorting



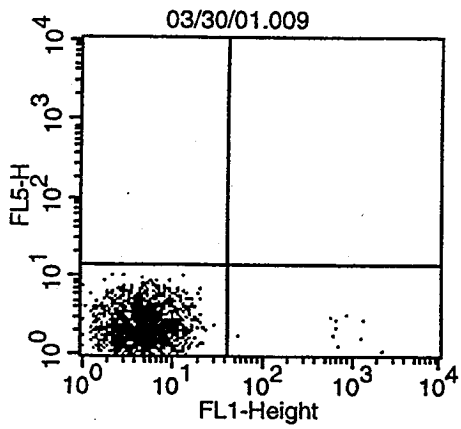


Histogram Statistics

File: 03/30/01.009
Sample ID: sort 4 -
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5277
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5280

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	5277	100.00	99.94	12.64	4.75	1157.19	4.53	4
M1	42, 9910	30	0.57	0.57	1265.48	706.89	118.72	720.15	637

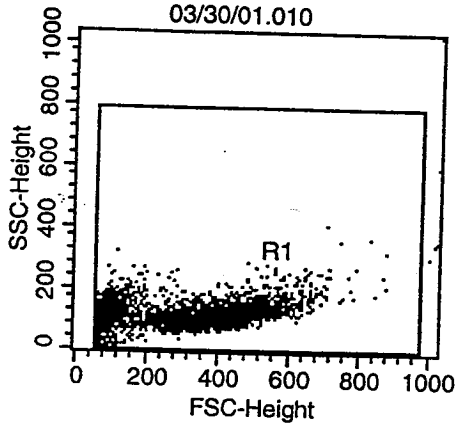


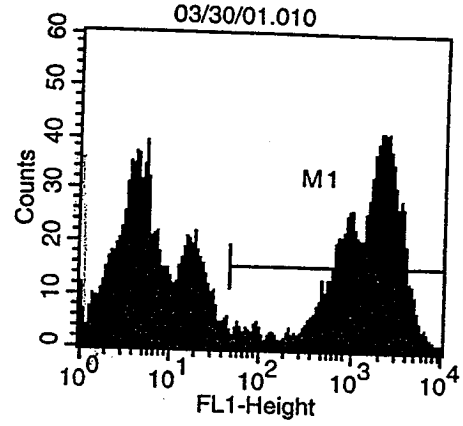
Quadrant Statistics

File: 03/30/01.009
Sample ID: sort 4 -
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 5277
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 41, 14

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 5280
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	5247	99.43	99.38	5.48	4.62	2.80	2.47
LR	30	0.57	0.57	1265.48	706.89	2.55	2.32



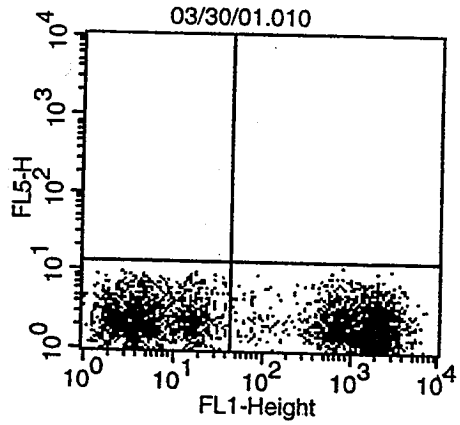


Histogram Statistics

File: 03/30/01.010
Sample ID: 5
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9990
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9990	100.00	99.90	871.01	92.55	131.47	88.57	1810
M1	45, 9910	5095	51.00	50.95	1699.82	1319.65	63.61	1582.04	1810

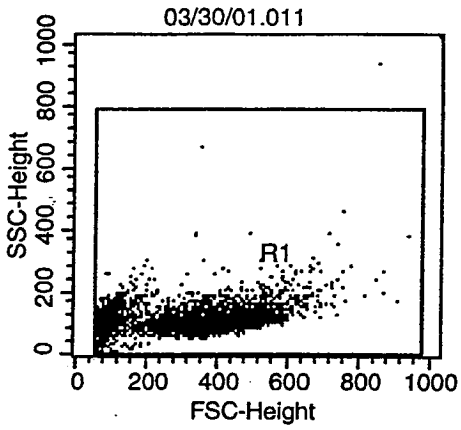


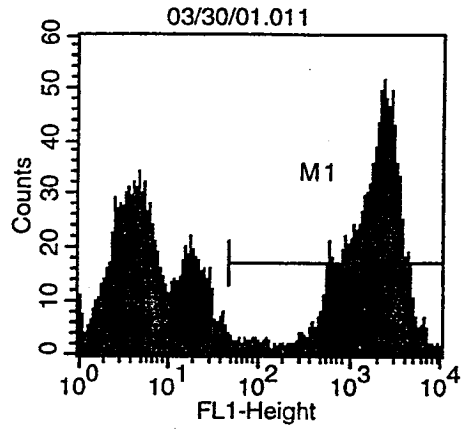
Quadrant Statistics

File: 03/30/01.010
Sample ID: 5
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9990
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 45, 13

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4895	49.00	48.95	8.33	5.82	2.84	2.50
LR	5095	51.00	50.95	1699.82	1319.65	2.49	2.19



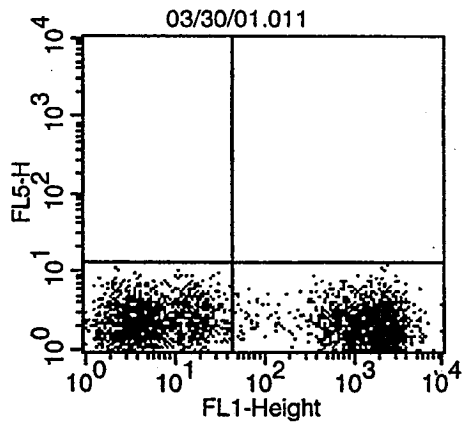


Histogram Statistics

File: 03/30/01.011
Sample ID: 6
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9995	100.00	99.95	991.07	109.18	123.99	441.09	2053
M1	45, 9910	5279	52.82	52.79	1868.94	1501.44	59.23	1778.28	2053

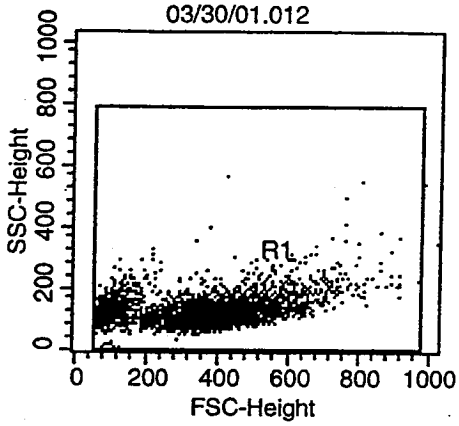


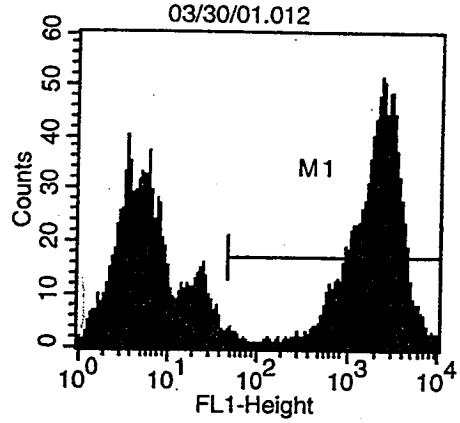
Quadrant Statistics

File: 03/30/01.011
Sample ID: 6
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9995
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 45, 13

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4716	47.18	47.16	8.39	5.81	2.80	2.46
LR	5279	52.82	52.79	1868.94	1501.44	2.45	2.16



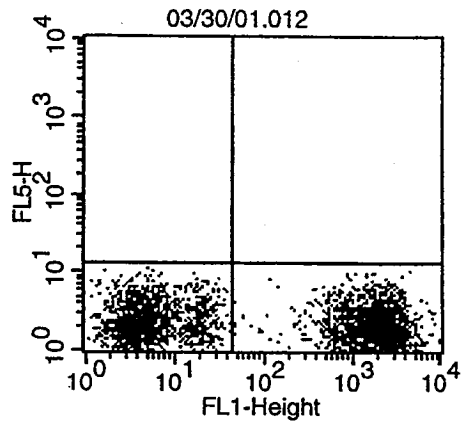


Histogram Statistics

File: 03/30/01.012
Sample ID: 7
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9992
X Parameter: FL1-H FL1-Height (Log)

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9992	100.00	99.92	1169.46	128.43	118.83	685.39	2090
M1	45, 9910	5417	54.21	54.17	2150.51	1793.80	56.18	2016.91	2090



Quadrant Statistics

File: 03/30/01.012
Sample ID: 7
Tube:
Acquisition Date: 30-Mar-01
Gated Events: 9992
X Parameter: FL1-H FL1-Height (Log)
Quad Location: 45, 13

Log Data Units: Linear Values
Patient ID:
Panel:
Gate: G1
Total Events: 10000
Y Parameter: FL5-H (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	***	***	***	***
UR	0	0.00	0.00	***	***	***	***
LL	4575	45.79	45.75	7.86	5.66	2.77	2.44
LR	5417	54.21	54.17	2150.51	1793.80	2.42	2.13

23-23

wipe test

PAGE: 1

ER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 03 APR 2001 14:14
AMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
#: 1 AQC:N QCF:N RCM:N
HANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
ATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
ALF LIFE(DAYS):N

AM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	9.00	66.67	1.00	1.47	73.0	
2	**- 2	1	14.00	53.45	1.00	3.11	79.0	
3	**- 3	1	13.00	55.47	1.00	4.73	73.0	
4	**- 4	1	12.00	57.74	1.00	6.36	73.0	
5	**- 5	1	6.00	81.65	1.00	7.92	74.0	
6	**- 6	1	8.00	70.71	1.00	9.58	80.0	
	**- 7	1	14.00	53.45	1.00	11.21	75.0	
	**- 8	1	9.00	66.67	1.00	12.83	77.0	
9	**- 9	1	7.00	75.59	1.00	14.46	76.0	
10	**-10	1	7.00	75.59	1.00	16.03	70.0	
11	**-11	1	11.00	60.30	1.00	17.61	71.0	Background

Set of counts

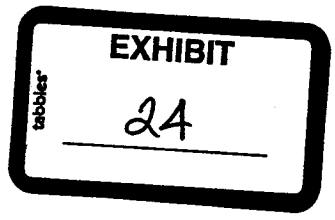
PAGE: 1

: 6 ID:H3 HOWELL PRESET TIME: 1.00 SUN 01 APR 2001 11:16
LE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
1 AQC:N QCF:N RCM:N
INEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
A:A CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000
ALF LIFE(DAYS):N

AM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
1	28- 1	1	8.00	70.71	1:00	1.42	74.0		– KONTR (Ekolume Medium)
2	28- 2	1	41.00	31.23	1:00	3.00	76.0		– S-1
3	28- 3	1	15.00	51.64	1.00	4.62	79.0		– S-2
4	28- 4	1	11128.42	1.95	0.95	6.18	82.0		– S-3
5	28- 5	1	8244.00	2.20	1.00	7.80	82.0		– S-4
6	28- 6	1	22222.00	1.90	0.50	8.86	78.0		– S-5
7	28- 7	1	555.00	8.49	1.00	10.48	82.0		– S-7
8	28- 8	1	30024.00	1.88	0.38	11.52	0.0		– Background Positive control H3 – DPM~98200

These are radioactivity counts of aliquots taken from the Helena tubes in the 10,5° incubator on Saturday, March 31, 2001.

Samples 28-2 and 28-3 are non-radioactive controls The others are all radioactive. There was no attempt here to be quantitative.



2nd set of counts

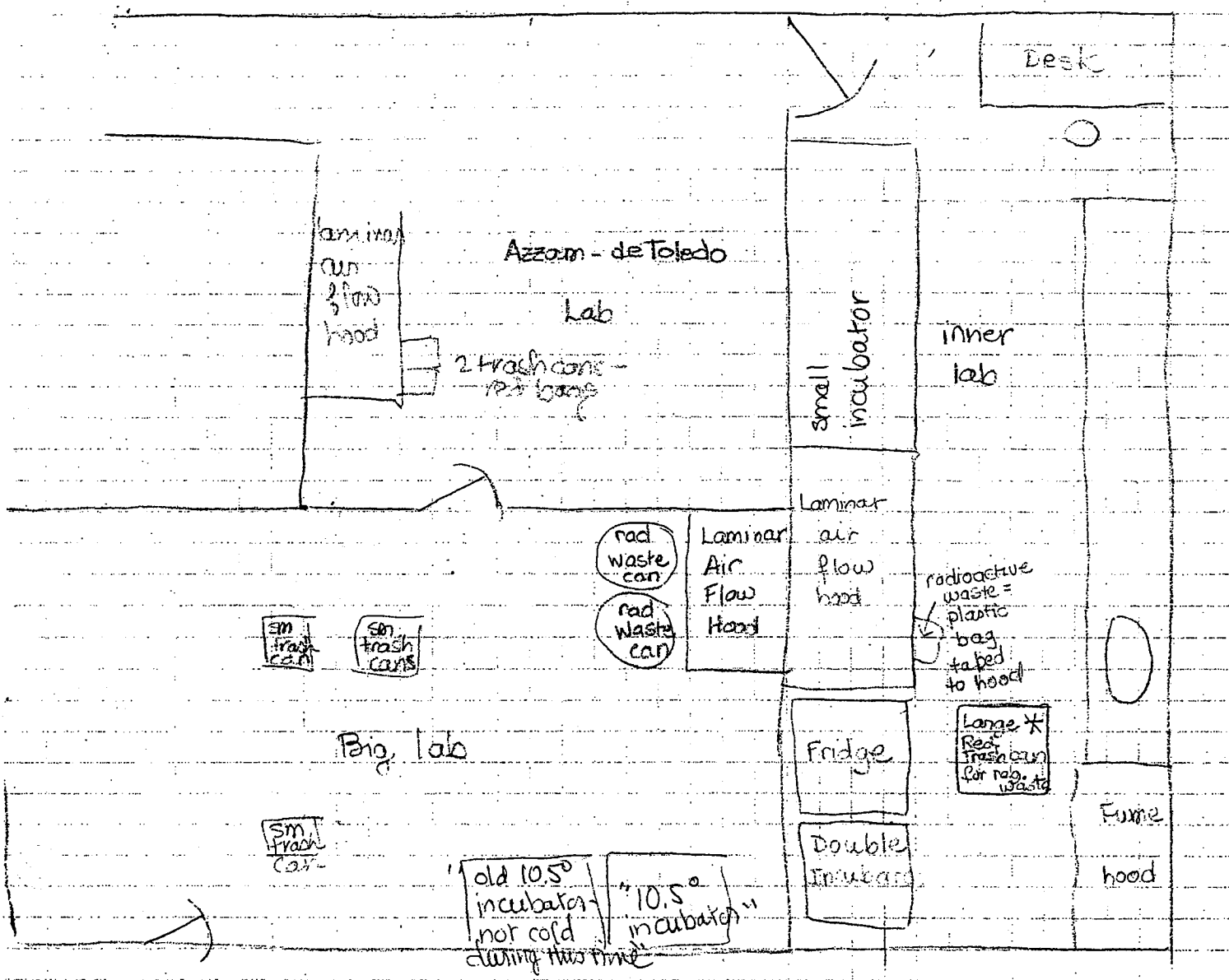
PAGE: 1

UP : 6 ID:H3 HOWELL PRESET TIME: 1.00 SUN 01 APR 2001 16:03
SA. LE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#		ERR
1	29- 1	1	13.00	55.47	1.00	1.42	73.0	- CONTR	
2	29- 2	1	56.00	26.73	1.00	2.99	75.0	- 1	
3	29- 3	1	15.00	51.64	1.00	4.56	80.0	- 2	
4	29- 4	1	10846.32	1.97	0.95	6.08	82.0	- 3	
5	29- 5	1	8367.00	2.19	1.00	7.65	82.0	- 4	
6	29- 6	1	21684.00	1.92	0.50	8.72	77.0	- 5	
7	29- 7	1	516.00	8.79	1.00	10.34	81.0	- 7	
8	29- 8	1	29709.33	1.89	0.38	11.38	1.0	- 15 DPM=98200	

These are a second set of radioactivity counts

Gave to Dr. R on Monday, 4/16/01



* Found tube #7 on Saturday, 3/31/01

I looked for tubes 1 ~~and~~ "thru" 6 in the inner lab in the plastic bag for rad waste and in the large red trash can for regular waste. I also looked in various small trash baskets around the lab and in the 2 large rad waste cans by the fumehood big

-HZ 11/7 11

" " added on 4/16 in Dr R's office - may not be exact

EXHIBIT
tabbies
26

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

CONFIDENTIAL

185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

April 12, 2001

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB E586g
Newark, New Jersey 07103

Dear Dr. Hill:

Pursuant to the UMDNJ policy on Misconduct in Science (copy enclosed), this letter is to inform you of an Initial Inquiry into an allegation of misconduct in science opened April 11, 2001 by the Newark Campus Committee on Research Integrity. You are the complainant and the respondent is Anupam Bishayee, Ph.D., Research Associate III in the department of Radiology

The allegation involves possible falsification and/or fabrication of data for NIH grant RO1 #CA83838.

You will be given the opportunity to be heard and will be expected to cooperate fully in this and any subsequent proceedings. Pursuant to University policy, confidentiality will be maintained to the extent possible and permitted by law. The policy states that appropriate action will be taken against those who attempt to retaliate against those reporting misconduct in good faith. The policy also states that appropriate action will be taken against individuals found to have made an unsubstantiated allegation which the complainant knew or had reason to know was false, or an allegation made with reckless disregard for or willful ignorance of facts that would disprove the allegation. A written report summarizing the conduct of the Initial Inquiry and the Committee's recommendations will be prepared at the conclusion of the proceeding. You will have the opportunity to comment on the report.

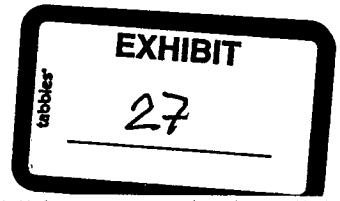
Please contact me if I can be of assistance in this matter.

Sincerely yours,

Elizabeth Raveché

Elizabeth Raveché, Ph.D.
Chair, Newark Campus Committee on Research Integrity
Professor, Pathology and Laboratory Medicine, UMDNJ-New Jersey Medical School

Enclosure: University Policy on Misconduct in Science



UMD
NEW JERSEY

University of Medicine & Dentistry of New Jersey

Vice President for Academic Affairs
Phone: (973) 972-4380
Fax: (973) 972-5320
E-mail: putterma@umdnj.edu

65 Bergen Street, Room 1441
University Heights
Newark, NJ 07107-3001

CONFIDENTIAL

April 16, 2001

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB F-542
Newark, NJ 07103

Dear Dr. Hill:

Please let this letter serve as confirmation of your meeting with the Newark Initial Inquiry Committee on **Tuesday, April 17, 2001 from 1:00P.M. to 3:00 P.M.** The meeting will be held in the Bergen Building, Suite 1441 conference room located at 65 Bergen Street in Newark. The purpose of this meeting is to discuss with you the allegation of misconduct in science which you made against Anupam Bishayee, Ph.D., Research Associate III in the Department of Radiology at UMDNJ-New Jersey Medical School.

Please call me at 973-972-4380 if you have any questions.

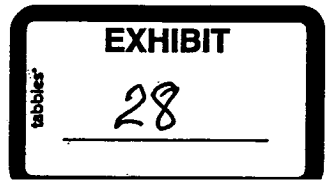
Thank you for your cooperation.

Sincerely,

Karen Putterman

Karen Putterman, M.D., M.P.H.
Vice President for Academic Affairs

c: Dr. Elizabeth Raveché, Chair, Newark Initial Inquiry Committee



UMDNJ
UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY

UNIVERSITY POLICY

SUBJECT: ACADEMIC AFFAIRS **TITLE:** MISCONDUCT IN SCIENCE

CODING: 00-01-20-60:00 **ADOPTED:** 07/15/89 **AMENDED:** 02/01/01

I. PURPOSE

To establish policy and procedures for the University's response to allegations and apparent occurrences of misconduct in science for which the University is the grantee, or which is conducted by or under the direction of any employee or agent of the University in connection with his or her institutional responsibilities. The objective of this policy is to ensure the prompt and appropriate investigation of alleged or apparent misconduct while protecting the rights of individuals, both those who report misconduct and those about whom allegations are made.

This policy is intended to implement the Federal Law 42 U.S.C. Section 289b and the regulations promulgated pursuant thereto. 42 CFR Part 50, Subpart A and 45 CFR Part 689.

II. APPLICABILITY

This policy applies to faculty members, housestaff, trainees, students (including postdoctoral fellows), volunteers, attending physicians, and staff members.

III. ACCOUNTABILITY

Under the direction of the President, the Senior Vice President for Academic Affairs shall ensure compliance with this policy. The Vice President for Academic Affairs shall implement this policy.

IV. DEFINITIONS

A. **"Misconduct in science"** - fabrication, falsification, plagiarism or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting or reporting research. Misconduct does not include those factors intrinsic to the process of science, such as honest error, conflicting data, or differences in interpretations or judgments about data or experimental design.

Other research practices or activities that deviate from those commonly accepted within the scientific community, and civil or criminal misconduct and regulatory violations occurring in the course of research (such as theft or other financial misfeasance, harassment, discrimination, and violations of regulations or University policies governing human subjects, animals, recombinant DNA or use of hazardous materials) shall be addressed by the University administratively or through laws and regulations.

B. **SVPAA** - shall mean the Senior Vice President for Academic Affairs.

C. **VPAA** - shall mean the Vice President for Academic Affairs.

D **Complainant** - the individual who made an allegation of misconduct in science.

E. **Respondent** - the individual against whom the allegation was made.

EXHIBIT 29 tabbies

F. **Allegation made in bad faith** - the intentional filing of an allegation which the complainant knew or had reason to know was false; or an allegation made with reckless disregard for or willful ignorance of facts that would disprove the allegation.

V. POLICY

A. The faculties and administration of UMDNJ have an important responsibility to maintain high ethical standards in scientific research that is conducted on University premises by University personnel. These standards, based upon well-established principles of scientific research, include validity, accuracy and honesty in proposing and performing research, in collecting, analyzing and reporting research results, and in reviewing the research of others. Failure to observe these principles that results in misconduct in science damages the University's image, the general public trust and the entire scientific community. In addition, University personnel who commit research misconduct breach their obligations to the University.

B. The University shall make diligent efforts to assure that:

1. those reporting alleged misconduct in good faith are protected from retaliation;

2. appropriate action will be taken against individuals who attempt to retaliate against those reporting misconduct in good faith;

3. appropriate action will be taken against individuals found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F);

4. the reputations of those unfairly accused are not damaged or are restored.

C. Campus Committees on Research Integrity

Three Campus Committees on Research Integrity shall be established, one each for Newark, Piscataway/New Brunswick and Camden/Stratford. These Committees shall be called together by the Chairperson or his/her designee on an as-needed basis to review allegations and reports of misconduct in science and apparent instances of misconduct, but in any event at least semiannually.

1. Membership

Membership of the Campus Committees shall consist of tenured faculty members representing the schools on that campus. Members shall represent a mixture of the basic and clinical sciences, and shall have strong research experience and other appropriate qualifications to judge the issues raised by allegations of misconduct in science. The members should be of appropriate status and stature with regard to their school's research enterprise in order to be effective in carrying out initial inquiries.

a. the Newark Committee shall have seven members, two faculty members from New Jersey Medical School (one of which shall be from the basic sciences and the other from the clinical sciences), and one faculty member each from the Graduate School of Biomedical Sciences-Newark Division, New Jersey Dental School, School of Health Related Professions, School of Nursing, and School of Public Health;

b. the Piscataway/New Brunswick Committee shall have six members, three faculty members from Robert Wood Johnson Medical School (representing both the basic and clinical sciences) and one faculty member each from the Graduate School of Biomedical Sciences-Piscataway Division, School of Health Related Professions, and School of Public Health; and

c. the Camden/Stratford Committee shall have five members, one faculty member each from Robert Wood Johnson Medical School-Camden, School of

Osteopathic Medicine, Graduate School of Biomedical Sciences-Stratford Division, School of Nursing and School of Health Related Professions.

2. Appointment

Members shall be appointed by the SVPAA upon the recommendations of the Deans following consultation with their faculties.

3. Term of Appointment

Members of the Campus Committees shall serve for terms of three years which may be renewed. In the event of an extended absence or resignation of a Campus Committee member, an alternate to serve out the term shall be appointed by the SVPAA in the same manner as original appointments.

4. Chair

Each Campus Committee shall elect a chairperson who should be at the rank of full professor. The Chairperson or designee shall call all meetings, whether regularly scheduled or in response to the receipt by any member of the Campus Committee of a report or allegation of misconduct in science.

5. Functions

The functions of the Campus Committees shall be to:

a. receive reports or allegations of misconduct in science from any source within or external to the University about University individuals working on that campus or whose primary academic appointment is at a school on that campus; however, when appropriate, any given allegation may be assigned by the Campus Committee for action to another Campus Committee;

b. conduct initial inquiries of allegations of misconduct in science, and send resulting reports to the SVPAA; and

c. supply the VPAA with the information needed to make the University's annual submission to the Office of Research Integrity (ORI) of the Office of the Secretary for Health pursuant to 42 CFR Part 50.

6. Expenses of the Campus Committees

Expenses related to the general functioning and training of the Campus Committees shall be borne by the schools on that campus.

D. Initial Inquiry

The initial inquiry shall involve information gathering and initial fact finding to determine whether an allegation of misconduct in science or apparent instance of misconduct warrants further investigation.

1. Preliminary Assessment of Allegation

The Campus Committee shall perform a preliminary assessment of an allegation or report to determine if an initial inquiry is warranted. This determination shall be limited to whether the allegation meets the definition of misconduct in science as set forth in Section IV.A, and whether there is or could be adequate information available to proceed with an initial inquiry. This determination shall take place within ten (10) working days of the Committee's receipt of the allegation or report. In the case of research disputes when an initial inquiry is not felt to be warranted, the Committee may recommend the services of the School's research ombudsperson. In the case of other types of alleged

professional misconduct or breach of research ethics not within the definition of misconduct in science, the Committee may refer the case to the appropriate School or University committee or administrator. When an initial inquiry is felt not to be warranted, the Committee's reasons shall be documented and the complainant shall be informed. The identification of the respondent shall be kept confidential from everyone without a need to know.

2. Initiation of Initial Inquiry

The Campus Committee (hereinafter the Initial Inquiry Committee) shall meet to begin the initial inquiry within ten (10) working days of its determination that the allegation warrants an initial inquiry.

3. Conflict of Interest/Bias

It is the responsibility of each member of the Initial Inquiry Committee to divulge potential conflicts of interest. In the event that any member of the Initial Inquiry Committee has any real or apparent personal or professional conflicts of interest or bias with respect to the respondent, complainant or case, that member shall be recused. Such conflicts include, but are not limited to, involvement with the research in question, competition with the respondent, and a previous or ongoing close professional or academic relationship with either respondent or complainant.

4. Notification of Initial Inquiry

Within fourteen (14) calendar days of the initiation of the initial inquiry, the respondent, the complainant, the Dean of the appropriate School or the Vice President of the pertinent patient care or administrative unit (in the case of a non-faculty respondent who is an employee of such unit), and the VPAA shall be notified in writing of the inquiry by the Chairperson of the Initial Inquiry Committee. Under certain circumstances set forth in Section V.H., the Office of Research Integrity (ORI) in the case of research conducted under a PHS grant or another pertinent funding agency must be immediately notified.

5. Rights and Obligations of the Respondent

The respondent shall be informed of the charges, of the opportunity to be heard, as well as the obligation to cooperate fully, and that unreasonable refusal to supply relevant material or other uncooperative behavior shall constitute violation of this policy. Legal counsel may not participate in the initial inquiry.

6. Sequestering of Data and Other Materials

At the time, the respondent is notified of the inquiry, the Initial Inquiry Committee shall, with the assistance of the Dean's or Vice President's office and/or of campus security and/or Information Services & Technology (IST) personnel if necessary, take custody of and sequester any original data, research records and other material and documents necessary to the conduct of the initial inquiry and potential future investigation. An inventory shall be made of each item removed. This inventory shall be signed by the Initial Inquiry Committee Chairperson, and a copy given to the respondent. Efforts should be made to permit the research to continue while the initial inquiry and other procedures go forward. If original materials are required for the proceedings, the Committee Chairperson or designee shall give the respondent reasonable access to original or unduplicatable materials during the proceedings. In addition, copies of original documents and records shall be made for the respondent. Materials sequestered shall be stored in a manner to ensure their preservation.

7. Consultants and *ad hoc* Members for Initial Inquiry Committee

For purposes of the initial inquiry, the Initial Inquiry Committee in its discretion may seek expert scientific advice and/or decide to add *ad hoc* members such as experts in a

particular field, student representatives or postdoctoral fellow representatives, especially if a student or postdoctoral fellow is the respondent.

8. Duration of Initial Inquiry

The Initial Inquiry Committee shall complete the inquiry and submit its final report in writing to the SVPAA and VPAA within sixty (60) calendar days from the date the initial inquiry began. If circumstances warrant a longer period, the records shall include documentation of the reasons for exceeding the 60-day period and the Initial Inquiry Committee may request an extension of time from the VPAA. If such an extension is granted, the respondent shall be so notified.

9. Decision of Initial Inquiry Committee

The Initial Inquiry Committee shall decide by majority opinion whether the initial inquiry reveals:

a. finding of no cause: i.e., insufficient credible evidence of misconduct in science to warrant further investigation. The reasons for this decision shall be documented in sufficient detail to permit later assessments of this decision if necessary. A written report summarizing the conduct of the initial inquiry and its conclusions shall be prepared for the SVPAA. The respondent, the complainant and the appropriate Dean or Vice President shall be notified in writing of the Committee's decision. The Initial Inquiry Committee may also make recommendations to the SVPAA regarding actions to restore the reputation of the respondent, and may consult with the respondent in this regard. The Initial Inquiry Committee may also make recommendations to the SVPAA concerning actions against a complainant found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

b. finding of cause: i.e., of the existence of credible evidence of misconduct in science sufficient to warrant further investigation. In this event, the Initial Inquiry Committee shall recommend to the SVPAA that an investigation be initiated to formally examine and evaluate all relevant facts to determine if misconduct has occurred.

A written report summarizing the conduct of the initial inquiry and its conclusions shall be prepared for the SVPAA with a copy to the respondent, the complainant and the appropriate Dean or Vice President. Comments of the respondent and of the complainant on the Committee's report, if any, must be filed with the SVPAA within five (5) working days of receipt of the report.

If the Initial Inquiry Committee finds there is a high probability that false or misleading information has been or may be disseminated to the scientific community and that such dissemination could cause significant harm, the Committee may recommend that the SVPAA, if he or she initiates an investigation, inform the following individuals of the existence and status of the investigation: (1) editors of scientific journals in which articles or other publications concerning the research under investigation have been published or are pending publication; and (2) program directors of scientific meetings at which the research under investigation is scheduled to be presented.

10. Decision and Actions of the SVPAA

The SVPAA has the sole discretion to accept, reject or modify the recommendations of the Initial Inquiry Committee. The SVPAA shall make a decision concerning the recommendations of the Initial Inquiry Committee within ten (10) working days of receipt of the Committee's report.

a. If the SVPAA accepts the recommendation of the Initial Inquiry Committee that there is insufficient credible evidence of misconduct in science to warrant further investigation, the case shall be closed.

The SVPAA shall notify in writing the respondent, the complainant, all individuals interviewed or otherwise informed of the allegation, and the appropriate Dean or Vice President of the disposition of the allegation. In the event that the ORI or another pertinent funding agency or sponsor was notified during the initial inquiry, under the circumstances enumerated in Section V.H., the same shall be informed by the SVPAA of the finding of no cause following the inquiry and that the University considers the case closed. When necessary and in consultation with the respondent, diligent efforts shall be undertaken fully to restore the reputation of the respondent.

If the SVPAA finds that the allegation was made in bad faith (see definition, Section IV.F), pursuant to a recommendation of the Initial Inquiry Committee, he/she shall determine whether and what administrative actions should be taken against the complainant pursuant to applicable University policies, procedures or contracts.

All research records, original data and other original materials sequestered by the Initial Inquiry Committee from the respondent or furnished by others shall be returned.

The Chairperson of the Initial Inquiry Committee shall gather the original records of the proceedings of the initial inquiry and copies of all documents and other materials furnished to the Committee. This file shall be sent to the SVPAA who shall seal it and retain it in a locked confidential cabinet for at least five (5) years and preferably indefinitely. The documents shall, upon request, be provided to authorized personnel representing the funding agency or sponsor. Otherwise, access to materials in the file shall be available only upon authorization of the SVPAA for exceptional cause.

b. If the SVPAA accepts the recommendation of the Initial Inquiry Committee that there is credible evidence of misconduct in science sufficient to warrant further investigation, the SVPAA shall initiate an investigation. All files accumulated by the Initial Inquiry Committee in this matter shall be transferred to the Office of the SVPAA.

The SVPAA shall provide notice of the investigation in writing to the respondent, the complainant, the appropriate Dean or Vice President, the Vice President of the Office of Legal Management, and the Director of the Office of Research Integrity (ORI) if the research in question was funded by PHS, or the director of another pertinent funding agency or sponsor if required (see paragraph below). Notification should include the name of the respondent, the nature of the charges, and the specific applications or grant numbers involved. The SVPAA may also decide to notify certain editors of journals or program directors of scientific meetings pursuant to a recommendation from the Initial Inquiry Committee.

Under certain circumstances set forth in Section V.H., the funding agency must be immediately notified.

c. If the SVPAA for good reason cannot accept the recommendation of the Initial Inquiry Committee, the SVPAA shall document his or her reasons and communicate these in writing to the Committee, the respondent, the complainant, and the appropriate Dean or Vice President. In accordance with the SVPAA's decision, either the procedure outlined in Section V.D.10.a or Section V.D.10.b shall be followed.

11. Expenses of the Initial Inquiry

Expenses of initial inquiries shall be borne by the Dean or Vice President in whose school or unit the respondent's research in question has been or is being conducted.

E. Investigation

The investigation shall be a formal examination and evaluation of all relevant facts to determine if misconduct in science has occurred. It shall include interviewing the complainant and the respondent as well as others who might have relevant information; reviewing original data, research records and other necessary material and documents; talking with experts; considering materials and/or comments submitted by the respondent and complainant; reviewing relevant literature, publications, correspondence, memos, etc.

1. Formation of Investigative Panel

An investigative panel shall be appointed by the SVPAA, consisting of three scientists with strong research experience and other appropriate qualifications to judge the issues raised in the investigation. These individuals may be internal to the University or external. University faculty serving on investigative panels must be tenured. Members of the Initial Inquiry Committee shall not be appointed to the Investigative Panel.

2. Conflict of Interest/Bias

In making appointments to the Investigative Panel, precautions shall be taken against real or apparent personal or professional conflicts of interest or bias with respect to the respondent, complainant or the case. For example, Panel members should not be involved with the research in question, should not be professional competitors with the respondent, and should not have a previous or ongoing close professional or academic relationship with either respondent or complainant.

3. Rights and Obligations of Respondent

The respondent shall be notified of the charges, the opportunity to be heard, and the obligation to cooperate fully with the investigation. Such notice shall inform the respondent that the investigation may determine (a) whether or not misconduct has occurred; and/or (b) if the actions or conduct investigated are/is otherwise unacceptable within the University for proposing, performing or reviewing research or reporting research results. The respondent shall also be informed that unreasonable refusal to supply relevant material or other uncooperative behavior constitutes violation of this policy.

4. Objections to Proposed Investigative Panel Members

The respondent and the complainant shall be informed of the proposed membership of the Investigative Panel. If the respondent or the complainant objects to the participation of any member of the Investigative Panel based upon personal or professional conflict of interest or bias with respect to the respondent, complainant or the case, this objection must be made within five (5) working days in writing to the SVPAA who shall decide whether to replace the challenged member. The decision of the SVPAA shall be final. Such challenges to the membership of the Investigative Panel must be resolved prior to the official appointment of the members by the SVPAA.

5. Appointment of and Charge to Investigative Panel

The SVPAA shall appoint the members of the Investigative Panel and shall administer the charge to the Panel. The official date of the initiation of the investigation shall be the date of the first meeting of the Investigative Panel. This shall be within thirty (30) calendar days of the completion of the initial inquiry (transmission of written report to SVPAA by Initial Inquiry Committee).

6. Chairperson of Investigative Panel

The Investigative Panel shall choose its chairperson at its first meeting.

7. Staff to Investigative Panel

The SVPAA and the Vice President for Legal Management shall assign staff to assist the Investigative Panel. Staff shall consider themselves and their activities for the Investigative Panel as strictly confidential.

8. Protection of Complainant from Retaliation

The SVPAA shall coordinate the University's efforts to protect the complainant from retaliation during and after the inquiry and investigation, working with all relevant University offices in these efforts.

9. Conduct of Investigation

a. Procedural Protection

Every effort shall be made to ensure a comprehensive, fair, expeditious and judicious investigation. Before the Investigative Panel, the respondent shall have the opportunity to examine all evidence forwarded to the Panel; to be represented by legal counsel; and to present evidence and to cross-examine witnesses, including the complainant, unless the evidence supporting the allegation of misconduct is independently verifiable. Anonymous third-party statements are not admissible as evidence.

b. Confidentiality

The respondent and the complainant shall be afforded confidential treatment to the maximum extent possible. Files shall be kept in a central location in a locked cabinet accessible only to the appropriate individuals taking part in the investigation.

c. Testimony before the Investigative Panel

Tape recordings shall be made of all testimony given. Documentation (including original data) substantiating the Investigative Panel's findings will be carefully secured, prepared and maintained. Transcriptions of the taped interviews shall be provided to the persons interviewed for comment or revision and included as part of the investigative file.

d. Sequestering of Additional Data and Material

The Investigative Panel may secure and sequester additional pertinent original research data, records, documents and other material from the respondent or others, using inventories and signed receipts for all material taken. If additional original materials are required for the proceedings, the Panel chairperson or designee shall give the respondent copies of original documents and records and reasonable access to original or unduplicatable materials during the proceedings. Materials sequestered shall be stored in a manner to ensure their preservation.

e. Consultants and Assistance for Investigative Panel

The Investigative Panel may seek additional expert scientific advice and/or the advice of students or postdoctoral fellows as appropriate to the status of the respondent.

f. Broadening/Change in Subject Matter of Investigation

If, during the investigation, information becomes available which the Panel considers substantially related to the original charge from the SVPAA, the Panel may broaden the scope of its charge and give written notice to the respondent of the new scope. If the Panel does not consider the new information substantially related to the original charge, the Panel may refer the new information to the Campus Committee as the basis of a new allegation.

10. Decision of Investigative Panel

In reaching a conclusion on whether there was misconduct in science, the burden of proof is on the University to support its case by a preponderance of the evidence.

The Investigative Panel's decisions shall be the majority opinions. There may be a minority report. The results of any vote taken shall be made known to the SVPAA in the written report of the Investigative Panel.

11. Duration of Investigation

The investigation shall be completed within ninety (90) calendar days of its initiation date to allow sufficient time for review of the Investigative Panel's report by the complainant, respondent and the SVPAA, and submission of the University's report, including the decision of the SVPAA, to the funding agency, within a total of 120 calendar days of the initiation of the investigation. If the investigation cannot be completed within these time limits, the University may request an extension of time from the ORI (in the case of research conducted under a PHS grant) or another pertinent funding agency or sponsor if required. If such an extension is granted, the respondent shall be so notified.

12. Report of the Investigative Panel

Upon conclusion of its investigation, the Investigative Panel shall prepare a written report. A copy of the report shall be given to the complainant, the respondent, the Chairperson of the Initial Inquiry Committee, the appropriate Dean or Vice President, and the SVPAA. Comments, if any, must be filed with the SVPAA within ten (10) working days of receipt of the Investigative Panel's report. These comments shall be considered by the SVPAA and made part of the report.

The report shall describe the policies and procedures under which the investigation was conducted and how and from whom information relevant to the investigation was obtained. It shall also include a recommendation as to whether a finding of misconduct should be made, the basis for that recommendation as well as a recommendation about the appropriate corrective measures to be taken if any.

The report may also include recommendations that a finding be made that the respondent has engaged in practices that are unacceptable within the University for proposing, performing or reviewing research, or reporting research results, but which do not constitute misconduct in science as defined in Section IV of this policy. The report may make recommendations about corrective actions, if any, to be taken under these circumstances.

The report may also include the Panel's concerns that violations of other University policies or of Federal or state regulations may have occurred, with recommendations to refer these concerns for administrative action.

In addition, the Panel may make recommendations concerning notification of law enforcement agencies, professional societies, licensing boards, journal editors, collaborators of the respondent or other concerned parties of the outcome of the investigation.

In the event of a recommendation that there be no finding of misconduct, the Investigative Panel, after consultation with the respondent, may make recommendations to the SVPAA regarding actions to restore the reputation of the respondent. The Investigative Panel may also make recommendations to the SVPAA concerning actions against a complainant found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

13. Expenses of the Investigation

The expenses of the investigation, including external consultants' fees if any, shall be borne by the pertinent Dean or Vice President.

14. Decision and Actions of the SVPAA

The SVPAA shall review the final report of the Investigative Panel and shall make a final decision in writing on behalf of the University. If the SVPAA for good reason cannot accept the recommendation of the Investigative Panel, the SVPAA shall document his or her reasons and communicate these in writing to the Panel, the respondent, the complainant and the pertinent Dean or Vice President.

The SVPAA may make one of the following decisions:

a. finding of no misconduct: When necessary, diligent efforts shall be undertaken in consultation with the respondent fully to restore the reputation of the respondent, and appropriate action shall be taken against complainants found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

b. finding of misconduct: The decision shall include the SVPAA's determination about the appropriate corrective actions. The SVPAA shall either accept the Investigative Panel's recommendation about corrective actions or impose alternatives. If the SVPAA decides to seek the termination of a faculty member with a full title, he or she shall direct the appropriate Dean immediately to initiate "Termination for Cause Proceedings" pursuant to the University Bylaws, Article VI, Title C. Evidence gathered during the investigation, recommendations of the Investigative Panel, and the decision of the SVPAA shall be forwarded to all individuals involved in the termination-for-cause proceedings for their consideration. Discipline imposed for misconduct in science shall be exempt from grievance and arbitration proceedings. The SVPAA may withdraw from publication all pending abstracts and papers that are considered to be of questionable scientific validity as a result of the finding, and may notify the editors of journals, books and other publications in which the respondent's previous papers and abstracts have appeared during the preceding five years.

c. finding that actions or conduct investigated are/is unacceptable within the University for proposing, performing or reviewing research or for reporting research results, but do/does not constitute misconduct in science as defined in Section IV of this policy: The decision shall include the SVPAA's determination about appropriate corrective actions.

15. Notification of Decision of SVPAA

The SVPAA shall provide a copy of his/her final decision to the respondent, the complainant, the Chairperson of the Initial Inquiry Committee, the Investigative Panel, the pertinent Dean or Vice President, and the Vice President for Legal Management.

The SVPAA shall forward to the ORI (in the case of research conducted under a PHS grant) or to another external funding agency or sponsor a copy of his/her final decision, along with the names of the Investigative Panel members and the Panel's final report.

F. Termination of the Case

1. Notification of Final Outcome of Investigation

After termination of a case, the SVPAA shall inform editors of scientific journals and program directors of scientific meetings who had been notified of the existence of an investigation, and all individuals interviewed or otherwise informed of the allegation of the outcome of the investigation.

2. Creation, Sealing, Storage of and Access to the File

The SVPAA shall ensure that the complete file, including the original records of all proceedings conducted by the Initial Inquiry Committee and by the Investigative Panel, and copies of all documents and other materials furnished to the Committee and the Panel, is sealed and retained indefinitely in a locked confidential cabinet in the Office of the SVPAA. Access to materials in the file shall be available only upon authorization of the SVPAA for exceptional cause.

3. Return of Sequestered Data and Other Materials

The SVPAA shall decide on a case-by-case basis when the research records, original data and other original materials sequestered during the initial inquiry or investigation may be returned. Among the determining factors in this decision are the requirements of pertinent government agencies or other sponsor.

G. Investigation by Federal Agencies

Under 42 CFR Part 50, Subpart A and 45 CFR Part 689, federal agencies have reserved the right to perform their own investigation in cases involving federally funded research at any time prior to, during, or following the University's investigation, and to impose corrective actions of their own in addition to those imposed by the University.

H. Immediate Notification of Funding Agency

At any time during the course of the inquiry, investigation or otherwise, the ORI (in the case of research conducted under a PHS grant) or another funding agency or sponsor shall be immediately notified by the SVPAA as soon as it appears that there is substantial evidence that:

1. there is an immediate health hazard to patients, human research subjects, laboratory workers or staff;

2. there is an immediate need to protect federal or other funds or equipment;

3. there is an immediate need to protect the human or animal subjects of the research;

4. there is an immediate need to protect the interests of the person making the allegation or of the individual who is the subject of the allegation as well as his/her co-investigators and associates, if any;

5. the alleged incident is expected to be publicly disclosed; or

6. there is an indication of possible criminal violations (notification of which must take place within 24 hours).

The Initial Inquiry Committee or Investigative Panel may also recommend to the SVPAA that interim administrative action be taken in situations such as those described in 1-6 above.

In addition, at any time during the course of the inquiry, investigation or otherwise, the ORI or the pertinent funding agency or sponsor shall be apprised of any facts that may affect current or potential federal or other funding for the respondent, or that the ORI or another pertinent funding agency or sponsor needs to know to ensure appropriate use of federal or other funds and otherwise protect the public interest.

I. If Respondent leaves the University

If the respondent leaves the University prior to the completion of the initial inquiry or investigation, the inquiry and investigation, if any, shall nevertheless continue according to the procedures described above, and the respondent shall be afforded full opportunity to participate. The SVPAA may inform the respondent's new employer, if any and if known, of the existence and status of investigation and of the final findings of the investigation.

J. Admission of Misconduct by Respondent

If the respondent admits to misconduct in science prior to the completion of the initial inquiry or investigation, an investigation shall nevertheless be conducted and continued to conclusion in order to discover the scope of the misconduct or other problems and make recommendations to the SVPAA. The initial inquiry and investigation shall be conducted according to the procedures described above.

K. Withdrawal of Allegation by Complainant

If the complainant withdraws his or her allegation prior to the completion of the initial inquiry or investigation, the proceedings shall continue if sufficient information is available to warrant such continuance.

L. Confidentiality

The confidentiality and privacy of the respondent, the complainant and all others involved in the procedures undertaken under this policy; the confidentiality of the Initial Inquiry Committee's and Investigative Panel's proceedings, files, reports and records; and patient confidentiality in the case of clinical research shall be maintained to the extent possible and permitted by law. However, confidentiality may not be maintained if the allegation is determined to be false and is found to be made in bad faith (see definition, Section IV.F). Protection of confidentiality does not preclude disclosures that are necessary in the process of handling allegations of misconduct; are in the public interest; are required by statute, regulations or rules of the research sponsor; or are a component of sanctions and/or corrective actions in the resolution of allegations of misconduct.

By Direction of the President:

_____

Vice President for Academic Affairs

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

CONFIDENTIAL

June 22, 2001

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB F-452
Newark, NJ 07103

Dear Dr. Hill:

The Newark Campus Committee on Research Integrity has completed its initial inquiry prompted by your allegations of potential misconduct in science against Dr. Anupam Bishayee, Ph.D., regarding experiments conducted in September/October 1999 and during March 26-30, 2001 in the laboratory of Dr. Roger Howell at UMDNJ-New Jersey Medical School.

The Committee unanimously concluded that there is no cause, based upon insufficient definitive evidence, to warrant further misconduct-in-science proceedings in this case. The Committee has submitted its recommendations to Dr. Robert A. Saporito, Senior Vice President for Academic Affairs, who will make the final decision pursuant to University policy.

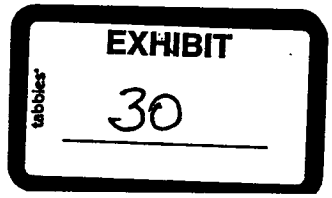
Thank you very much for your assistance in the conduct of the initial inquiry.

Sincerely yours,

Elizabeth Raveché

Elizabeth Raveché, Ph.D.
Chair, Newark Campus Committee on Research Integrity

c: Dr. Russell Joffe, Dean, UMDNJ-New Jersey Medical School



UMDNJ
University of Medicine & Dentistry of New Jersey

Graduate School of Biomedical Sciences
New Jersey Dental School
New Jersey Medical School
Robert Wood Johnson Medical School
School of Health Related Professions
School of Nursing
School of Osteopathic Medicine
School of Public Health

Robert A. Saporito, DDS
Senior Vice President for Academic Affairs

CONFIDENTIAL

July 2, 2001

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB F-452
Newark, NJ 07103

Dear Dr. Hill:

The University of Medicine and Dentistry of New Jersey has completed its proceedings prompted by your allegations of potential misconduct in science against Dr. Anupam Bishayee, Research Associate in Dr. Roger Howell's laboratory in the department of Radiology at UMDNJ-New Jersey Medical School. The Newark Campus Committee on Research Integrity charged with reviewing these allegations conducted an Initial Inquiry in accordance with federal regulations and University policy. After considering over a period of two months the testimony of relevant witnesses, and reviewing original research data, written testimony, the grant proposal, published papers and abstracts, photographs, and other documents and materials concerning the allegations and the research in question, the Committee unanimously concluded that there is no cause to warrant further misconduct-in-science proceedings with regard to the allegations. After reviewing the Committee's report and the minutes of the Committee's meetings, along with the attachments thereto, I have accepted the Committee's findings.

Thank you for your assistance in the conduct of these proceedings.

Sincerely yours,

Robert A. Saporito, D.D.S.
Senior Vice President for Academic Affairs

c: Dean Russell Joffe
Dr. Stephen Baker
Dr. Roger Howell
Dr. Elizabeth Raveché

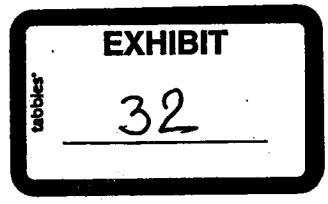
EXHIBIT
31

65 Bergen Street, Room 1441 • Newark, NJ 071 • Fax: (973) 972-7621 • E-mail: saporito@umdnj.edu

List of recipients of notice about Roger's appointment as Chief of the Division of Radiation Research and the demise of the Section of Cancer Biology

----- Original Message -----
From: "karel campbell" <campbeka@UMDNJ.EDU>
To: "Hani Abujudeh" <judehmd@yahoo.com>; "Terry Aquino" <aquino@umdnj.edu>; "Sohail Contractor" <sohailcontractor@hotmail.com>; "Robert Fountila" <robertfountila@aol.com>; "Devang Gor" <roentgen1895@yahoo.com>; "Clay Hinrichs" <yalcnih@aol.com>; "David Hirschorn" <hirschor@umdnj.edu>; "Mary Hu" <beston2@yahoo.com>; "Chris Mele" <mele@umdnj.edu>; "Jane Rothenberg" <drjane01@aol.com>; "Effi Samuels" <ephraimfishel@yahoo.com>; "Kartik Shah" <shahka@umdnj.edu>; "Vivek Sharma" <sharmav60@hotmail.com>; "George Visvikis" <george9899@aol.com>; "Al Ybasco" <ybasco@yahoo.com>; "Joy Anderson" <andersje@umdnj.edu>; "Marcia Blacksin" <blacksin@umdnj.edu>; "Charles Cathcart" <cathcacs@umdnj.edu>; "Kyunghee Cho" <chokc@umdnj.edu>; "Helene Hill" <hill@umdnj.edu>; "Roger Howell" <rhowell@umdnj.edu>; "Andrew Kalnin" <kalninaj@umdnj.edu>; "Huey-Jen Lee" <leehu@umdnj.edu>; "Kristine Mosier" <kmrad@aol.com>; "Ronald Wachsberg" <wachsbrh@umdnj.edu>; "Leo Wolansky" <lwolansky@aol.com>; "Wen-Ching Liu" <wliu@umdnj.edu>; "Loren Godfrey" <godfrelo@umdnj.edu>; "Robert Halvorsen" <halvorra@umdnj.edu>; "Andre Holodny" <holodnai@umdnj.edu>; "Jeffrey Farkas" <farkasje@umdnj.edu>; "Corey Eber" <ebercd@umdnj.edu>; "Edouard Azzam" <Azzamei@umdnj.edu>; "Philip Bahramipour" <bahramph@umdnj.edu>; "Daniel Contractor" <contrada@umdnj.edu>; "Sosamma Methratta" <methrast@umdnj.edu>; "Jill Siegel" <siegeljr@umdnj.edu>; "Marc Simmons" <simmonmz@umdnj.edu>; "Malti Trivedi" <trivedma@umdnj.edu>; "Cornelia Wenokor" <wenokoco@umdnj.edu>; "Jutta Greweldinger" <greweldinger@aol.com>; "Helene Goldfarb" <goldfahe@umdnj.edu>; "Joel Bloom" <bloomjo@umdnj.edu>; "Debra Green" <green.debra_j@east-orange.va.gov>
Sent: Wednesday, July 11, 2001 5:25 PM
Subject: Announcements

> I am pleased to announce that Dr. Roger Howell has accepted my
> invitation to serve as the Chief of the Division of Radiation Research.
> Please see the attached memo.
>
> Stephen R. Baker, MD
> Professor and Chair
>



July 2, 2001

Roger Howell, PhD
Professor
Department of Radiology
Division of Radiation Research

Dear Dr. Howell:

It gives me pleasure to appoint you the Chief of the Division of Radiation Research in our department. If you accept, your leadership responsibilities will encompass the previous division of Radiation Research and the section of Cancer Biology, now to be combined into one division under the appellation Radiation Research. I hope you will take on this position. I eagerly look forward to a written confirmation of that fact from you.

Sincerely,

Stephen R. Baker, MD
Professor and Chair
Department of Radiology

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

Division of Radiation Research
Department of Radiology
Phone: (973) 972-5323/5067
Fax: (973) 972-6474
E-mail: raodv@umdnj.edu
rhowell@umdnj.edu

MSB F-451
185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

MEMORANDUM

Date: 7/30/01

To: Edouard I. Azzam, Ph.D., Assistant Professor of Radiology
Sonia M. deToledo, Ph.D., Adjunct Assistant Professor of Radiology
Helene Z. Hill, Ph.D., Professor of Radiology

From: Roger W. Howell, Ph.D. RWH
Professor and Chief of Radiation Research

Re: Assignment of laboratory space

Effective August 3, 2001, office and laboratory space in the Division of Radiation Research will be assigned as follows:

Office

MSB F-453	R.W. Howell
MSB F-452	H.Z. Hill
MSB F-466	E.I. Azzam

Laboratory

MSB F-468	Shared between H.Z. Hill and E.I. Azzam
MSB F-451a	R.W. Howell
MSB F-451b	E.I. Azzam
MSB F-451	R.W. Howell

The laboratories and offices will be rekeyed. Faculty will be provided keys to access their assigned areas only. Post-doctoral fellows will only be provided keys to the laboratories of their mentors.

cc. Stephen Baker, M.D., Professor and Chairman of Radiology

EXHIBIT
tabbies
33

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

Division of Radiation Research
Department of Radiology
Phone: (973) 972-5323/5067
Fax: (973) 972-6474
E-mail: raodv@umdnj.edu
rhowell@umdnj.edu

MSB F-451
185 South Orange Avenue
University Heights
Newark, NJ 07103-2714

MEMORANDUM

Date: July 31, 2001

To: Helene Z. Hill, Ph.D.
Professor of Radiology

From: Roger W. Howell, Ph.D. RWH
Professor and Chief of Radiation Research

Re: Access to laboratory space

Thank you for your inquiry regarding access to MSB F-451 as a consequence of the recent reorganization of the laboratory space within the Division. You have requested clarification of your privileges to access laboratory space within the Division. As stated in my memorandum of July 30, 2001, you have been assigned laboratory space in MSB F-468. The laboratory MSB F-451 has been assigned to R.W. Howell. According to the memorandum, you will be denied access to MSB F-451 as of August 3, 2001. By virtue of the layout of the laboratory space, this will also restrict your access to MSB F-451a and MSB F-451b. To facilitate continuation of your collaborative work with Drs. Azzam and deToledo, I have assigned MSB F-468 as shared laboratory space with Dr. Azzam. Equipment of yours that is necessary for your work will be moved to MSB F-468.

You have also asked for access to MSB F-451 during my vacation so that you can work closely with Dr. deToledo during my absence. Drs. Azzam and deToledo have also informed me that cells will require refeeding during their absence in the latter part of August. Accordingly, access to MSB F-451 is granted during my absence through August 28, 2001. The keys of Drs. Azzam and deToledo can be used for this purpose.

cc. Edouard I. Azzam, Ph.D.
Sonia M. deToledo, Ph.D.
Stephen Baker, M.D.

EXHIBIT
tabbies
34

Department of Radiology
NJ Medical School
Tel: 973-972-3421
Fax: 973-972-5592

FACSIMILE COVER PAGE

To: M.D. Karen Putterman	From: Helene Z. Hill, Ph.D.
Fax #: 2-7596	Fax #: 9739725592
Company:	Tel #: 973-972-3421
Subject:	
Sent: 8/13/01 at 10:22:48 AM	Pages: 3 (including cover)

MESSAGE:

WinFax PRO Cover Page

EXHIBIT
35

UMDNJ
NEW JERSEY
MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

185 South Orange Avenue, Newark, NJ 07103-2714 • Phone: (973) 972-3421 • Fax: (973) 972-5592

CONFIDENTIAL

MEMO

Date: Monday, August 13, 2001

To: Karen Putterman, M.D., Vice-President for Academic Affairs

From: Helene Z. Hill, Ph.D. Professor of Radiology

Re: Retaliation

Mark Schorr, Esq., attorney for the AAUP, suggests that I give the University every opportunity to make things right without going through the formality of the grievance process. I presume that you will do your best to rectify this matter and assume that I have your assurance that this will be the case. He further suggests that I send you a copy of the grievance that I have prepared in order to properly record the events and my desired remedy. Please note that I have not included attachments as you have those documents in your possession already. However, if you believe I need to file a grievance to set things right, please let me know.

Text of the proposed Grievance

On April 10, 2001, I reported to the Chairman of the Newark Campus Committee on Research Integrity, Dr. Elizabeth Raveché, acts committed by a Fellow in the laboratory of Dr. Roger Howell that I believed satisfied the University definition of Scientific Misconduct. University guidelines require Faculty Members to report suspicions of misconduct (cf the white tag we are required to wear) and my report was made as per these requirements. University policy also protects the reporter of possible scientific misconduct from retaliation. On June 22, 2001, Dr. Raveché reported to me that there was 'insufficient definitive evidence to warrant further misconduct-in-science proceedings in this case'.

On July 30, 2001, Dr. Howell sent me a memo (attached), and on July 31, 2001, a second memo (attached) that have barred my access to the laboratories of the Division of Radiation Research thereby preventing me from effectively carrying out my research in collaboration with Drs. Edouard Azzam and Sonia de Toledo. It needs to be understood that, since I am not allowed to enter MSB-F451 – the door must be locked at all times because of the radioisotopes that are contained therein -- I cannot gain access to MSB-F451b where they do most of their work. This will seriously impair our collaborative endeavors. Furthermore, although Dr. Howell has offered to move my equipment (2 large incubators) into the small laboratory assigned to me – MSB-F468, this is not feasible as there is no more room in that laboratory. There are a number of other shared instruments and fume hoods that I would not be able to use as they would remain in MSB-F451. Dr. Howell has not given me any reason for his action. He did, however, on July 6, 2001 say to me 'I do not

want to have anything more to do with you'. This was followed on July 11 by a copy of a letter to Dr. Howell from my department Chairman, Dr. Baker, sent to all the members of my department which was obviously designed to chastise, demean and humiliate me (attached). My name was not mentioned, but the intent of this memo is clear. This letter effectively put Dr. Howell in complete control over my activities in the Division of Radiation Research.

I reported what I believed was misconduct in good faith. It was my understanding that I could do no less as a responsible member of the faculty and the University community. I am deeply saddened that the result has been what appears to be retaliation that impedes my own research.

I believe that Dr. Howell, supported by Dr. Baker barred me from the Division Laboratories in retaliation for my report regarding his Fellow. I know of no other possible explanation.

Requested Remediation:

I ask only the restoration of the *status quo* with regard to access to the Division Laboratories as it existed before the memos of July 30 and July 31. That is, that I be issued a key to MSB-F451 so that I can enter and leave that suite of laboratories at will and that I be able to continue to use instruments and equipment that I had access to before that time. I further ask that Dr. Howell shall no longer be my supervisor.

Helene Z. Hill, Ph.D.
3 Silver Spring Road
West Orange, NJ 07052-4317
Tel/Fax: 973-736-0738
hzhill@home.com

Thursday, August 23, 2001

Dr. Kay Fields
Division of Investigative Oversight
Office of Research Integrity
5515 Security Lane
Suite 700
Rockville, MD 20852

Dear Dr. Fields,

As per our conversation last week, I am sending you material regarding scientific misconduct at the New Jersey Medical School. I believe that the acts observed by myself and my colleague, Dr. Marek Lenarczyk, constitute misconduct in science as defined in our University policy as 'fabrication, falsification. plagiarism or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting or reporting research'. I believe that the findings of the initial investigation of 'insufficient definitive evidence' for scientific misconduct were in error. I present to you the material that I provided to the Committee and I also relate that in the aftermath, the key experiments cannot be replicated and the person that I accused of misconduct was forced to resign and has been black-balled from obtaining jobs in the relevant field.

The grant in question is R01CA83838, Roger W. Howell, Ph.D., Principal Investigator, 'Effects of non-uniform distributions of radioactivity', total costs requested for 5 years $1,358,075. I am listed as a co-Investigator on this grant.

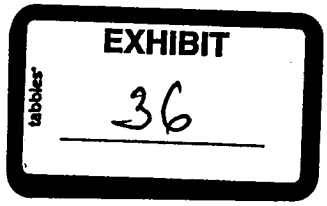
If, after you review the material that I provide and that the University will provide you, and you agree with me, I ask that the inquiry be reopened and proceed to the second – investigative – phase as described in our University guidelines for misconduct.

During your initial investigation of this matter, I prefer to remain anonymous.

Thank you for your patience with me. I have every hope that we will eventually get to the truth of this matter.

Sincerely yours,

Helene Z. Hill, Ph.D.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Office of Public Health and Scier
Office of Research Integrity
5515 Security Lane, Suite 700
Rockville MD 20852

Tel: 301-443-5330
Fax: 301-594-0043

CONFIDENTIAL/SENSITIVE

August 27, 2001

Helene Z. Hill, Ph.D.
3 Silver Spring Road
West Orange, NJ 07052-4317

Reference: DIO 2352

Dear Dr. Hill:

This will acknowledge receipt of your August 23, 2001, letter to Dr. Kay Fields of the Office of Research Integrity (ORI) regarding an allegation of possible scientific misconduct involving Public Health Service (PHS) grant R01 CA83838, Roger W. Howell, Ph.D., Principal Investigator at the University of Medicine & Dentistry of New Jersey.

Scientific misconduct is defined in PHS regulations (42 C.F.R. 50) as "fabrication, falsification, plagiarism, or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting, or reporting research. It does not include honest error or honest differences in interpretations or judgments of data." To establish ORI authority to investigate an alleged act of scientific misconduct, we must determine that: (1) the allegation of possible misconduct meets the definition of scientific misconduct above; and (2) that the allegation is related to PHS- supported research or an application for PHS support.

This matter will be assigned to an investigator who will contact you if it is determined that additional specific information is necessary for us to complete our review.

Sincerely,

Alan R. Price, Ph.D.
Director
Division of Investigative Oversight
Office of Research Integrity

EXHIBIT
37
tabbies

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Office of Public Health and Science
Office of Research Integrity
5515 Security Lane, Suite 700
Rockville MD 20852
Phone: 301-443-3400
FAX: 301-594-0043

SEP - 5 2001

CONFIDENTIAL/SENSITIVE

Dr. Helene Z. Hill
3 Silver Spring Road
West Orange, NJ 07052-4317

Re: ORI 2001-28

Dear Dr. Hill:

The Division of Investigative Oversight (DIO) of the Office of Research Integrity (ORI) has completed its oversight of the University of Medicine and Dentistry of New Jersey's (UMDNJ) inquiry into allegations that you forwarded to ORI of possible scientific misconduct involving alleged falsification of research included in National Cancer Institute (NCI), National Institutes of Health (NIH), grant application 1 R01 CA83838-01A1.

ORI concurred with the institution that there is insufficient evidence to warrant an investigation.

Consistent with Federal law and ORI policy, inquiries that result in a recommendation that no investigation is warranted remain confidential.

We appreciate your bringing these allegations to our attention.

Sincerely,

/S/ Pascal

Chris B. Pascal, J.D.
Director
Office of Research Integrity

EXHIBIT
38

Helene Z. Hill, Ph.D.
3 Silver Spring Road
West Orange, NJ 07052-4317
Tel/Fax: 973-736-0738
hzhill@home.com

Saturday, November 03, 2001

Kay L. Fields, Ph.D., Scientist-Investigator
Division of Investigative Oversight
Office of Research Integrity
5515 Security Lane
Suite 700
Rockville, MD 20852

Reference: DIO 2352

Dear Dr. Fields,

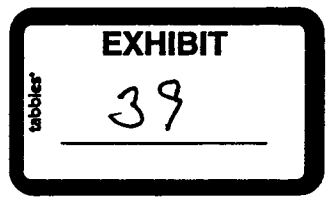
It is now more than two months since I sent you the material regarding my allegation of scientific misconduct at the New Jersey Medical School. I hope that you will not think me presumptuous if I ask to know what is your progress and/or your time line.

I am in contact with Dr. Bishayee and I think it is important to inform you that he has not been successful in finding another job and is having financial difficulties. He has changed his visa from H to visitor or tourist and I understand that if he does not find a sponsor, he will have to return to India sometime in January or February.

I find myself becoming increasingly uncomfortable when other scientists refer to the work on which I am a co-author which I now know not to be true. I am loathe to take any action on this matter until I know the outcome of my request to the ORI to reinvestigate this misconduct.

Sincerely yours,

Helene Z. Hill, Ph.D.



Email sent to Fields on Wed., Dec 12, 2001, 11:21 PM
(fr. hzhill@hsm.com)

Dear Dr. Fields,

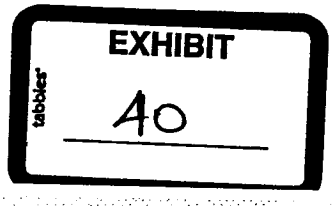
You asked me to verify the Figures in the two papers that contained data that could not be reproduced (Bishayee, et al., Rad Research 152: 88-97, 1999; Bishayee, et al., Rad Research 155: 335-344, 2001). These would, indeed, be Figures 3 and 6 in the earlier paper and 2A in the more recent paper. Also, Figure 7 in Dr. Roger Howell's grant application depicts the data in the 1999 experiment that I believe to have been fabricated.

As far as I know, the experiment that was repeated many times without showing a bystander effect only involved incubating 50% radio-labeled cells with non-labeled cells in the absence of any modifiers such as lindane and DMSO. The results did not show a rapid decline in survival to 50% followed by a slower decline. They showed a rapid decline to about 50% and then little or no further killing. The other conditions -- +/- lindane, +/- DMSO would only be relevant if there were a bystander effect and a decline in survival after 50% in the master experiment.

Dr. Marek Lenarczyk should have the data from the experiments that he did. I am sure that he would be glad to talk with or correspond with you. His email address is mlenarczyk@pzh.gov.pl.

I would like to ask you if you could send me a copy of the first letter that I received about the misconduct before the investigation began that says that I would get a copy of the report. As I told you, I could not find my copy and have no idea where it can be. Also, would you be able to send me a copy of the report. I am sure I would have some observations about it that might be useful.

Along those lines, I have thought about what both Drs. Anupam Bishayee and Howell said about a second experiment and a second cell line. As you can see from the plan of the lab, quarters are small and Marek and Anupam spent most of their time together in the inner lab. If there had been a second experiment, Marek would certainly have known about it. Roger's routine, which I never knew to vary, was such that cells were harvested for rolling on either Monday or Thursday and they rolled overnight to be harvested on Tuesday or Friday. They then incubated at 10.5 degrees until the next Friday or Monday. We know that there was no experiment started on Thursday, March 29 because my notes show that the rollers were empty in the morning before Anupam came in. Furthermore, if Anupam had processed cells for a new experiment which appeared in the 10.5 degree incubator at the very time that the previous experiment would have disappeared from the same incubator, he would have had to be a miracle worker because he had plenty to do that whole morning getting cells ready for the FACS. There was no protocol for such an experiment at the time that we copied pages from the notebook to make our report and there is no evidence for such an experiment in the radioactivity record. We know that the tubes that remained in the incubator were radioactive because we counted them later (see notes from March 31). The second cell line would almost certainly have been AG1522 which Anupam was growing in a T175 flask in his incubator. The flask was not contaminated but on Friday, the day the putative experiment would have gone into the 10.5 degree incubator, that flask was in the trash. I remember looking at the cells. They had not been trypsinized but were floating in sheets. They had apparently overgrown and detached – a common problem with human fibroblasts. Furthermore, at the time that Anupam told Marek that he was working with clusters, those very clusters were still sitting in the incubator.



Helene Z. Hill, Ph.D.
3 Silver Spring Road
West Orange, NJ 07052-4317
Tel/Fax: 973-736-0738
hzhill@comcast.net

Thursday, August 22, 2002

Alan R. Price, Ph.D., Director
Division of Investigative Oversight
U.S. Department of Health and Human Services
Office of Research Integrity
5515 Security Lane, Suite 700
Rockville, Maryland 20852

Re: 200126

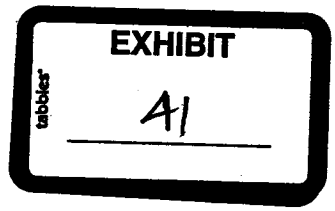
Dear Dr. Price,

I would like to add information that may not be known to you to that which you already have concerning this case:

1.) Analysis of all the Coulter counts that I have in my notebook for the period of 9/13/1999 – 2/28/2000 employing the method outlined in the recent article by Mosimann, *et al.* (J.E. Mosimann, Dahlberg, J.E., Davidian, N.M. and Krueger, J.W. Terminal digits and the examination of questioned data. Accountability in Research, 9, 75-92, 2002). *See attached Table.*

Dr. Bishayee recorded 198 Coulter counts and I recorded 160. Dr. Bishayee counted a smaller volume than I did so his numbers had only 3 digits of which I analyzed the units position. Some of my numbers had 4 digits so I analyzed both the units and the tens positions. There is one chance in 56.8 billion that Dr. Bishayee's 198 digits are randomly distributed and one chance in 4.26 that my 160 digits are randomly distributed. The p-value for Dr. Bishayee's data (0.0000000000176) is highly significant, while that for my data (0.235) is not significant by usual statistical standards. You should, of course, check out these numbers with your own statistician.

The Coulter counts are important because they are used in the mutagenesis arm of these experiments to determine how many cells to reseed in order to obtain enough cell doublings for mutation expression as well as to determine how many cells to seed for plating efficiencies and for quantitation of the frequency of mutants resistant to the challenge drug. The protocol that was followed in these experiments is known as the Banbury Protocol (J.P. O'Neill and A.W. Hsie, The CHO/HGPRT Mutation Assay: Experimental Procedure in A.W. Hsie, J.P. O'Neill and V.K. McElheny, eds. *Banbury Report 2, Mammalian Cell Mutagenesis: The Maturation of Test Systems* Cold Spring



Harbor Laboratory, 1979, pages 55-69). The data for 9/13, 9/17,10/1 and 10/4/1999 were included in the material that I sent to the ORI last year. The data for 12/6, 12/20/99 and 2/28/00 are in my notebook. However, I was never asked to submit any additional results. Copies of these experiments are attached.

2.) Dr. Bishayee lived in an apartment that I rented for my son who has been hospitalized for some time, thus I am knowledgeable as to his immigration situation both before and after the University made its decision. Dr. Bishayee was promoted from part-time Post-Doctoral Fellow to Research Associate in the summer of 2000 after Dr. Howell started his R01 grant. His salary went from less than $20,000 with health insurance only to about $35,000 with full benefits. At that time, he changed his visa from a J1 to an H1 and began an application for a green card. He left the Laboratory of Radiation Research at the end of July, 2001. You may have been told that he left to pursue an interest in Molecular Biology. However, he was handed a letter of resignation which he was told to sign.

On or about the end of August, because he no longer had a sponsor, Dr. Bishayee changed his visa from an H1 visa to a Visitor's visa, good for 3 months. When it expired, he renewed it for an additional (final) 3 months. Employment is not permitted on such a visa and he was unemployed during this time. His visa status during this period can be verified by contacting the INS. In December, he was no longer able to pay for the apartment and did not do so again until March. For 3 months, he did not pay me because, he told me, he was out of money. In March, he found employment in Dr. Pain's laboratory and was able to change his visa back to an H1. Dr. Pain came from the University of Pennsylvania and did not set up his laboratory until October.

It seems unlikely that Dr. Bishayee would voluntarily leave a high salaried job to become unemployable, subject to deportation if he could not find a new sponsor, and no longer eligible to apply for a green card.

Please take these facts in to consideration when you make your final decision regarding the disposition of this case.

Thank you.

Helene Z Hill

Helene Z. Hill, Ph.D.

CC: Chris B. Pascal, Director, J.D., Director, ORI
Attachments

Bishayee's hand writing

TABLE-3

Expt. # : 1 **Date/Time :** 02/28/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 / Cells/ml]	KBq/cell [pCi/cell x 1/37]
1	511, 526, 532				
2	546, 530, 519				0.0408
3	520, 540, 543	534	2137333	0.0027	~~0.0048~~ ~~0.136~~
4	475, 495, 482	484	1936000	0.0042 ~~0.5165~~	0.626
5	531, 521, 547	533	2132000	0.0041	0.6101
6	480, 499, 501	493	1973333	0.0067	0.99
7	507, 519, 489	505	2020000	0.0083	1.23
8	465, 472, 482	473	1892000	0.0102	1.52
9	555, 532, 521	536	2144000	0.0092	1.37
10	411, 408, 429	416	1664000	0.0123	1.82
11	460, 469, 472	467	1868000	0.0159	2.36
12	422, 410, 404	412	1648000	0.02	3.05

Hill's handwriting

12/6/99		Tubes rec'd fr Anupam										
d0		Counts (Coulter)			Avx400 = cells/ml					Vol	# cells plated	
M	1	801	784	832	806 ±	$3.2 \times 10^5 \pm 0.87$				0.65ml	2.1×10^5	
	2	829	836	827	831 ± 4.73	$3.3 \times 10^5 \pm 0.57$				0.75	2.5	
	3	759	747	732	$3.0 \times 10^5 \pm 0.54$					0.75	2.3	
	4	745	713	696	$2.9 \times 10^5 \pm 0.895$					0.65	1.9	
	5	824	782	807	$3.2 \times 10^5 \pm 0.85$					0.63	2.0	
	6	808	792	771	$3.2 \times 10^5 \pm 0.44$					0.73	2.3	
Th 3.7	7	728	752	702	$2.9 \times 10^5 \pm 0.10$					0.80	2.3	
lt 63.8	8	648	648	651	$2.6 \times 10^5 \pm 0.007$					0.80	2.1	
	9	725	723	713	$2.9 \times 10^5 \pm 0.026$					0.67	1.9	
	10	656	647	634	$2.6 \times 10^5 \pm 0.0044$					0.75	2.0	
											Doublings	
12/9	1	farm	249 195	2.2×10^6	$\xrightarrow{0.45}$	10^6	x3ml	$= 6.6 \times 10^6$			5.0	
Th d3	2	"	171 x2 168 x2	3.4×10^6	$\xrightarrow{0.29}$			$= 1.02 \times 10^7$			5.4	
	3	"	150 89	1.2×10^6	$\xrightarrow{0.83}$			$= 3.6 \times 10^6$			4.0	
	4	"	75 66	7.0×10^5	$\xrightarrow{1.43}$			2.1×10^6			3.5	
	5	feed										
	6											
	7											
	8											
	9											
	10	↓										
											New Doub	Total
12/13	1	Farm	253 cl 141 139 189	1.8×10^6	$\xrightarrow{0.59}$	10^6	x4ml	$= 7.1 \times 10^6$			2.8	7.8
M d7	2		238 186	2.1×10^6	$\xrightarrow{0.48}$			8.5×10^6			3.1	8.5
	3		127 x4 117 x4	4.9×10^6	$\xrightarrow{0.20}$			2.0×10^7			4.3	8.3
	4		164 x4 162 x4	6.5×10^6	$\xrightarrow{0.15}$			2.6×10^7			4.7	8.2
	5		99 x4 137 x2	2.4×10^6	$\xrightarrow{0.42}$			9.4×10^7			8.9	8.9
	6		142 x2 115 x2	2.6×10^6	$\xrightarrow{0.38}$			1.0×10^7			5.4	5.4
	7		206 175	1.9×10^6	$\xrightarrow{0.53}$			7.6×10^6			5.0	5.0
	8		172 176	1.7×10^6	$\xrightarrow{0.59}$			6.7×10^6			5.0	5.0
	9		76 75	7.6×10^5	$\xrightarrow{1.32}$			3.0×10^6			4.0	4.0
	10	↓	69 88	7.9×10^5	$\xrightarrow{1.27}$			3.1×10^6			4.0	4.0

Analysis of coulter counter counts by Bishayee and Hill

If the numbers had 3 digits, units were analyzed
If the numbers had 4 digits, units and tens were analyzed

Exp		DIGITS	0	1	2	3	4	5	6	7	8	9	Total	p =
Bishayee	9/24/99	Coulter-units	2	6	5	0	0	1	1	5	2	8	30	0.00549
Bishayee	9/27/99	Coulter-units	0	9	7	1	1	4	4	0	0	4	30	0.000439
Bishayee	10/1/99	Coulter-units	1	5	8	1	1	3	4	2	0	5	30	0.028181
Bishayee	10/4/99	Coulter-units	2	3	11	2	0	3	3	1	2	3	30	0.001588
Bishayee	12/20/99	Coulter-units	7	6	3	4	3	5	2	2	3	7	42	0.534146
Bishayee	2/28/00	Coulter-units	6	6	7	1	1	4	2	2	1	6	36	0.087929
TOTAL			18	35	41	9	6	20	16	12	8	33	198	1.76E-11
Hill	9/13/99	Coulter-units	4	3	3	2	5	5	2	2	2	2	30	0.862344
Hill	9/13/99	Coulter-tens	3	1	5	3	3	3	3	2	5	2	30	0.862344
Hill	9/17/99	Coulter-units	3	0	2	4	7	7	2	7	3	0	35	0.025193
Hill	9/17/99	Coulter-tens	4	6	1	3	2	8	2	4	2	3	35	0.238562
Hill	12/6/99	Coulter-units	0	3	6	3	3	2	3	4	4	2	30	0.602458
TOTAL			14	13	17	15	20	25	12	19	16	9	160	0.235285
Expected:	9/24, 9/27, 10/1, 10/4, 9/13, 12/6/99		3	3	3	3	3	3	3	3	3	3		
	9/17/99		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
	2/28/00		3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6		
	12/20/99		4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2		
	Total for Hill		16	16	16	16	16	16	16	16	16	16		
	Total for Bishayee		19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8		

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Office of Public Health and Science
Office of Research Integrity
5515 Security Lane, Suite 700
Rockville MD 20852

Ph. 301-443-5330
FAX 301-594-0043
email: aprice@osophs.dhhs.gov
ORI Web site: http://ori.hhs.gov

CONFIDENTIAL / SENSITIVE

September 5, 2002

Dr. Helene Hill
3 Silver Spring Road
West Orange, New Jersey 07052-4317

RE: DIO 2001-28

Dear Dr. Hill:

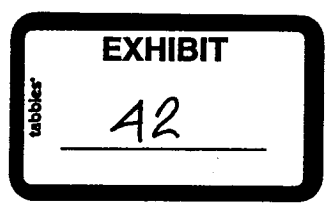
The Division of Investigative Oversight (DIO), Office of Research Integrity (ORI), received late last week your letter dated August 22, 2002. Since Dr. Kay Fields told me that she encouraged you, when you called her last month, to submit any new allegations or evidence directly to the University of Medicine and Dentistry of New Jersey, the institution responsible for investigating the referenced case, I assume you have done so.

I also assume that Dr. Fields told you to contact me, since she had removed herself from the case. When you called me on August 14, 2002, I told you that I thought Mr. Chris Pascal would be making the decision for ORI in the next couple of weeks, and he did so, before your recent letter arrived. The closeout letters have been mailed, so you should be receiving one from him soon.

Dr. Fields may have discussed with you that she had already done a similar analysis as part of her earlier oversight review in this case, and it was considered by DIO and ORI in the discussions of how to handle this matter.

Sincerely,

Alan R. Price, Ph.D.
Director, Division of Investigative Oversight
Associate Director
Office of Research Integrity



UMDNJ
SCHOOL OF NURSING
University of Medicine & Dentistry of New Jersey

Office of the Assistant Dean

CONFIDENTIAL

November 25, 2002

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB E-586g
P.O. Box 1709
Newark, New Jersey 07101

Dear Dr. Hill:

Pursuant to the UMDNJ policy on Misconduct in Science (copy enclosed), this letter is to inform you of an Initial Inquiry into an allegation of misconduct in science opened today, November 25, 2002, by the Newark Campus Committee on Research Integrity. You are the complainant and the respondent is Anupam Bishayee, Ph.D., formerly Research Associate III in the Department of Radiology.

The allegation involves possible falsification and/or fabrication of data for NIH grant RO1 #CA83838.

You are being given the opportunity to be heard today, November 25, 2002 and will be expected to cooperate fully in this and any subsequent proceedings. Pursuant to University policy, confidentiality will be maintained to the extent possible and permitted by law. The policy states that appropriate action will be taken against those who attempt to retaliate against those reporting misconduct in good faith. The policy also states that appropriate action will be taken against individuals found to have made an unsubstantiated allegation which the complainant knew or had reason to know was false, or an allegation made with reckless disregard for or willful ignorance of facts that would disprove the allegation.

Sincerely yours,

D. Anthony Forrester, Ph.D., R.N.
Chair, Newark Campus Committee on Research Integrity

Enclosure: University Policy on Misconduct in Science

EXHIBIT
tabbies
43

UMDNJ
UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY

UNIVERSITY POLICY

SUBJECT: ACADEMIC AFFAIRS **TITLE:** MISCONDUCT IN SCIENCE

CODING: 00-01-20-60;00 **ADOPTED:** 07/15/89 **AMENDED:** 10/18/02

I. PURPOSE

To establish policy and procedures for the University's response to allegations and apparent occurrences of misconduct in science for which the University is the grantee, or which is conducted by or under the direction of any employee or agent of the University in connection with his or her institutional responsibilities. The objective of this policy is to ensure the prompt and appropriate investigation of alleged or apparent misconduct while protecting the rights of individuals, both those who report misconduct and those about whom allegations are made.

This policy is intended to implement the Federal Law 42 U.S.C. Section 289b and the regulations promulgated pursuant thereto, 42 CFR Part 50, Subpart A and 45 CFR Part 689.

II. APPLICABILITY

This policy applies to faculty members, housestaff, trainees, students (including postdoctoral fellows), volunteers, attending physicians and staff members.

III. ACCOUNTABILITY

Under the direction of the President, the Senior Vice President for Academic Affairs shall ensure compliance with this policy. The Vice President for Academic Affairs shall implement this policy.

IV. DEFINITIONS

A. **"Misconduct in science"** - fabrication, falsification, plagiarism or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting or reporting research. Misconduct does not include those factors intrinsic to the process of science, such as honest error, conflicting data, or differences in interpretations or judgments about data or experimental design.

Other research practices or activities that deviate from those commonly accepted within the scientific community, and civil or criminal misconduct and regulatory violations occurring in the course of research (such as theft or other financial misfeasance, harassment, discrimination, and violations of regulations or University policies governing human subjects, animals, recombinant DNA or use of hazardous materials) shall be addressed by the University administratively or through laws and regulations.

B. **SVPAA** - shall mean the Senior Vice President for Academic Affairs.

C. **VPAA** - shall mean the Vice President for Academic Affairs.

D. **Complainant** - the individual who made an allegation of misconduct in science.

EXHIBIT
tabbies
44

E. **Respondent** - the individual against whom the allegation was made.

F. **Allegation made in bad faith** - the intentional filing of an allegation which the complainant knew or had reason to know was false; or an allegation made with reckless disregard for or willful ignorance of facts that would disprove the allegation.

V. POLICY

A. UMDNJ faculty, administration, staff, students and volunteers have an important responsibility to maintain high ethical standards in scientific research that is conducted on University premises by University personnel. These standards, based upon well-established principles of scientific research, include validity, accuracy and honesty in proposing and performing research, in collecting, analyzing and reporting research results, and in reviewing the research of others. Failure to observe these principles that results in misconduct in science damages the University's image, the general public trust and the entire scientific community. In addition, University personnel who commit research misconduct breach their obligations to the University.

UMDNJ faculty, administration, staff, students and volunteers also have the responsibility to report known or suspected instances of misconduct in science to the appropriate Campus Committee on Research Integrity (see Section V.D. below).

B. The University shall make diligent efforts to assure that:

1. those reporting alleged misconduct in good faith are protected from retaliation;

2. appropriate action will be taken against individuals who attempt to retaliate against those reporting misconduct in good faith;

3. appropriate action will be taken against individuals found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F);

4. the reputations of those unfairly accused are not damaged or are restored.

C. Immediate Notification

At any time during the course of the preliminary assessment, initial inquiry, investigation or otherwise, the following notifications shall immediately be made:

1. If the Campus Committee or Investigative Panel becomes aware of a risk to human subjects or deviations in an Institutional Review Board (IRB)-approved protocol or other breach of University policy regarding human subjects research, the chair of the Committee or Panel shall notify the Executive Director of Human Subjects Protection and the Campus IRB Chair.

2. If the Campus Committee or Investigative Panel becomes aware of the commission of a criminal act, the Chair shall notify Public Safety.

3. If the Campus Committee or Investigative Panel becomes aware of incidents of harassment or discrimination, the Chair shall notify the Office of Affirmative Action/Equal Employment Opportunity.

4. If the Campus Committee or Investigative Panel becomes aware of non-compliance with federal or state law or regulation or with University policy, the Chair shall notify the Office of Business Conduct and the Office of Legal Management.

5. If the Campus Committee or Investigative Panel becomes aware of any facts that may affect current or potential federal or other funding for the respondent, or facts that the funding agency or sponsor needs to know to ensure appropriate use of federal or other

funds and otherwise protect the public interest, the Chair shall notify the SVPAA who shall apprise the Office of Research Integrity (ORI) or the pertinent funding agency or sponsor.

6. If there is found to be substantial evidence of any of the following, the SVPAA shall be informed and shall notify immediately the ORI (in the case of research conducted under a Public Health Service grant or if the research results were used in a Public Health Service grant, fellowship or contract application), or another funding agency or sponsor:

   a. There is an immediate health hazard to patients, human research subjects, laboratory workers or staff.

   b. There is an immediate need to protect federal or other funds or equipment.

   c. There is an immediate need to protect the human or animal subjects of the research.

   d. There is an immediate need to protect the interests of the person making the allegation or of the individual who is the subject of the allegation as well as his/her co-investigators and associates, if any.

   e. The alleged incident is expected to be publicly disclosed.

   f. There is an indication of possible criminal violations (notification of which must take place within 24 hours).

D. Campus Committees on Research Integrity

Three Campus Committees on Research Integrity shall be established, one each for Newark, Piscataway/New Brunswick and Camden/Stratford. These Committees shall be called together by the Chairperson or his/her designee on an as-needed basis to review allegations and reports of misconduct in science and apparent instances of misconduct, but in any event at least semiannually.

1. Membership

Membership of the Campus Committees shall consist of tenured faculty members representing the schools on that campus. Members shall represent a mixture of the basic and clinical sciences, and shall have strong research experience and other appropriate qualifications to judge the issues raised by allegations of misconduct in science. The members should be of appropriate status and stature with regard to their school's research enterprise in order to be effective in carrying out initial inquiries.

   a. The Newark Committee shall have seven members, two faculty members from New Jersey Medical School (one of which shall be from the basic sciences and the other from the clinical sciences), and one faculty member each from the Graduate School of Biomedical Sciences-Newark Division, New Jersey Dental School, School of Health Related Professions, School of Nursing, and School of Public Health.

   b. The Piscataway/New Brunswick Committee shall have six members, three faculty members from Robert Wood Johnson Medical School (representing both the basic and clinical sciences) and one faculty member each from the Graduate School of Biomedical Sciences-Piscataway Division, School of Health Related Professions, and School of Public Health.

   c. The Camden/Stratford Committee shall have six members, one faculty member each from Robert Wood Johnson Medical School-Camden, School of

Osteopathic Medicine, Graduate School of Biomedical Sciences-Stratford Division, School of Nursing, School of Health Related Professions and School of Public Health.

2. Appointment

Members shall be appointed by the SVPAA upon the recommendations of the Deans following consultation with their faculties.

3. Term of Appointment

Members of the Campus Committees shall serve for terms of three years which may be renewed. In the event of an extended absence or resignation of a Campus Committee member, an alternate to serve out the term shall be appointed by the SVPAA in the same manner as original appointments..

4. Chair

Each Campus Committee shall elect a chairperson who should be at the rank of full professor. The Chairperson or designee shall call all meetings, whether regularly scheduled or in response to the receipt by any member of the Campus Committee of a report or allegation of misconduct in science.

5. Functions

The functions of the Campus Committees shall be to:

a. receive reports or allegations of misconduct in science from any source within or external to the University about University individuals working on that campus or whose primary academic appointment is at a school on that campus; however, when appropriate, any given allegation may be assigned by the Campus Committee for action to another Campus Committee;

b. conduct initial inquiries of allegations of misconduct in science, and send resulting reports to the SVPAA; and

c. supply the VPAA with the information needed to make the University's annual submission to the ORI of the Office of the Secretary for Health pursuant to 42 CFR Part 50.

6. Expenses of the Campus Committees

Expenses related to the general functioning and training of the Campus Committees shall be borne by the schools on that campus.

E. Initial Inquiry

The initial inquiry shall involve information gathering and initial fact finding to determine whether an allegation of misconduct in science or apparent instance of misconduct warrants further investigation.

1. Preliminary Assessment of Allegation

The Campus Committee shall perform a preliminary assessment of an allegation or report to determine if an initial inquiry is warranted. This determination shall be limited to whether the allegation meets the definition of misconduct in science as set forth in Section IV.A, and whether there is or could be adequate information available to proceed with an initial inquiry. This determination shall take place within ten (10) working days

of the Committee's receipt of the allegation or report. In the case of research disputes when an initial inquiry is not felt to be warranted, the Committee may recommend the services of the School's research ombudsperson. When an initial inquiry is not felt to be warranted, the Committee's reasons shall be documented and the complainant shall be informed. The identification of the respondent shall be kept confidential from everyone without a need to know.

2. Initiation of Initial Inquiry

The Campus Committee (hereinafter the Initial Inquiry Committee) shall meet to begin the initial inquiry within ten (10) working days of its determination that the allegation warrants an initial inquiry.

3. Conflict of Interest/Bias

It is the responsibility of each member of the Initial Inquiry Committee to divulge potential conflicts of interest. In the event that any member of the Initial Inquiry Committee has any real or apparent personal or professional conflicts of interest or bias with respect to the respondent, complainant or case, that member shall be recused. Such conflicts include, but are not limited to, involvement with the research in question, competition with the respondent, and a previous or ongoing close professional or academic relationship with either respondent or complainant.

4. Notification of Initial Inquiry

Within fourteen (14) calendar days of the initiation of the initial inquiry, the respondent, the complainant, the Dean of the appropriate School, the President/CEO of the pertinent patient care unit or the Vice President of the pertinent administrative unit (in the case of a non-faculty respondent who is an employee of such unit), and the VPAA shall be notified in writing of the inquiry by the Chairperson of the Initial Inquiry Committee. Under certain circumstances set forth in Section V.C., the Office of Research Integrity (ORI) in the case of research conducted under a PHS grant or another pertinent funding agency must be immediately notified.

5. Rights and Obligations of the Respondent

The respondent shall be informed of the charges, of the opportunity to be heard, as well as the obligation to cooperate fully, and that unreasonable refusal to supply relevant material or other uncooperative behavior shall constitute violation of this policy. Legal counsel may not participate in the initial inquiry.

6. Sequestering of Data and Other Materials

At the time, the respondent is notified of the inquiry, the Initial Inquiry Committee shall, with the assistance of the Dean's or Vice President's office and/or of campus security and/or Information Services & Technology (IST) personnel if necessary, take custody of and sequester any original data, research records and other material and documents necessary to the conduct of the initial inquiry and potential future investigation. An inventory shall be made of each item removed. This inventory shall be signed by the Initial Inquiry Committee Chairperson, and a copy given to the respondent. Efforts should be made to permit the research to continue while the initial inquiry and other procedures go forward. If original materials are required for the proceedings, the Committee Chairperson or designee shall give the respondent reasonable access to original or unduplicatable materials during the proceedings. In addition, copies of original documents and records shall be made for the respondent. Materials sequestered shall be stored in a manner to ensure their preservation.

7. Consultants and *Ad Hoc* Members for Initial Inquiry Committee

For purposes of the initial inquiry, the Initial Inquiry Committee in its discretion, may seek expert scientific advice and/or decide to add *ad hoc* members such as experts in a particular field, student representatives or postdoctoral fellow representatives, especially if a student or postdoctoral fellow is the respondent.

8. Duration of Initial Inquiry

The Initial Inquiry Committee shall complete the inquiry and prepare a written report summarizing the conduct of the initial inquiry and its conclusions for the SVPAA within sixty (60) calendar days from the date the initial inquiry began. If circumstances warrant a longer period, the records shall include documentation of the reasons for exceeding the 60 day period and the Initial Inquiry Committee may request an extension of time from the SVPAA. If such an extension is granted, the respondent shall be so notified.

9. Decision of Initial Inquiry Committee

The Initial Inquiry Committee shall decide by majority opinion whether the initial inquiry reveals:

a. finding of no cause, i.e., insufficient credible evidence of misconduct in science to warrant further investigation. The reasons for this decision shall be documented in sufficient detail to permit later assessments of this decision if necessary. A written report summarizing the conduct of the initial inquiry and its conclusions shall be prepared for the SVPAA. The respondent shall be given a copy of the report, and the respondent's comments, if any, shall be made part of the record. The complainant and the appropriate Dean or Vice President shall be notified in writing of the Committee's decision. The Initial Inquiry Committee may also make recommendations to the SVPAA regarding actions to restore the reputation of the respondent, and may consult with the respondent in this regard. The Initial Inquiry Committee may also make recommendations to the SVPAA concerning actions against a complainant found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

b. finding of cause, i.e., of the existence of credible evidence of misconduct in science sufficient to warrant further investigation. In this event, the Initial Inquiry Committee shall recommend to the SVPAA that an investigation be initiated to formally examine and evaluate all relevant facts to determine if misconduct has occurred.

A written report summarizing the conduct of the initial inquiry and its conclusions shall be prepared for the SVPAA. The respondent shall be given a copy of the report, and the respondent's comments, if any, shall be made part of the record. Comments of the respondent about the Committee's decision must be filed with the Committee within five (5) working days of receipt of the report. The complainant and the appropriate Dean or Vice President shall be notified in writing of the Committee's decision.

If the Initial Inquiry Committee finds there is a high probability that false or misleading information has been or may be disseminated to the scientific community and that such dissemination could cause significant harm, the Committee may recommend that the SVPAA, if he or she initiates an investigation, inform the following individuals of the existence and status of the investigation: (1) editors of scientific journals in which articles or other publications concerning the research under investigation have been published or are pending publication; and (2) program directors of scientific meetings at which the research under investigation is scheduled to be presented.

10. Decision and Actions of the SVPAA

The SVPAA has the sole discretion to accept, reject or modify the recommendations of the Initial Inquiry Committee. The SVPAA shall make a decision concerning the recommendations of the Initial Inquiry Committee within ten (10) working days of receipt of the Committee's report.

a. If the SVPAA accepts the recommendation of the Initial Inquiry Committee that there is insufficient credible evidence of misconduct in science to warrant further investigation, the case shall be closed.

The SVPAA shall notify in writing the respondent, the complainant, all individuals interviewed or otherwise informed of the allegation, and the appropriate Dean or Vice President of the disposition of the allegation. In the event that the ORI or another pertinent funding agency or sponsor was notified during the initial inquiry, under the circumstances enumerated in Section V.C., the same shall be informed by the SVPAA of the finding of no cause following the inquiry and that the University considers the case closed. When necessary and in consultation with the respondent, diligent efforts shall be undertaken fully to restore the reputation of the respondent.

If the SVPAA finds that the allegation was made in bad faith (see definition, Section IV.F), pursuant to a recommendation of the Initial Inquiry Committee, he/she shall determine whether and what administrative actions should be taken against the complainant pursuant to applicable University policies, procedures or contracts.

All research records, original data and other original materials sequestered by the Initial Inquiry Committee from the respondent or furnished by others shall be returned.

The Chairperson of the Initial Inquiry Committee shall gather the original records of the proceedings of the initial inquiry and copies of all documents and other materials furnished to the Committee. This file shall be sent to the SVPAA who shall seal it and retain it in a locked confidential cabinet for at least five (5) years and preferably indefinitely. The documents shall, upon request, be provided to authorized personnel representing the funding agency or sponsor. Otherwise, access to materials in the file shall be available only upon authorization of the SVPAA for exceptional cause.

b. If the SVPAA accepts the recommendation of the Initial Inquiry Committee that there is credible evidence of misconduct in science sufficient to warrant further investigation, the SVPAA shall initiate an investigation. All files accumulated by the Initial Inquiry Committee in this matter shall be transferred to the Office of the SVPAA.

The SVPAA shall provide notice of the investigation in writing to the respondent, the complainant, the appropriate Dean or Vice President, the Vice President of the Office of Legal Management, and the Director of the Office of Research Integrity (ORI) if the research in question was funded by the Public Health Service or if the research results were used in a Public Health Service grant, fellowship or contract application, or the director of another pertinent funding agency or sponsor if required (see paragraph below). Notification should include the name of the respondent, the nature of the charges, and the specific applications or grant numbers involved. The SVPAA may also decide to notify certain editors of journals or program directors of scientific meetings pursuant to a recommendation from the Initial Inquiry Committee.

Under certain circumstances set forth in Section V.C., the funding agency must be immediately notified.

c. If the SVPAA for good reason cannot accept the recommendation of the Initial Inquiry Committee, the SVPAA shall document his or her reasons and communicate these in writing to the Committee, the respondent, the complainant, and the appropriate Dean or Vice President. In accordance with the SVPAA's decision, either the procedure outlined in Section V.E.10.a or Section V.E.10.b shall be followed.

11. Expenses of the Initial Inquiry

Expenses of initial inquiries shall be borne by the Dean or Vice President in whose School or Unit the respondent's research in question has been or is being conducted.

F. Investigation

The investigation shall be a formal examination and evaluation of all relevant facts to determine if misconduct in science has occurred. It shall include interviewing the complainant and the respondent as well as others who might have relevant information; reviewing original data, research records and other necessary material and documents; talking with experts; considering materials and/or comments submitted by the respondent and complainant; reviewing relevant literature, publications, correspondence, memos, etc.

1. Formation of Investigative Panel

An investigative panel shall be appointed by the SVPAA, consisting of three scientists with strong research experience and other appropriate qualifications to judge the issues raised in the investigation. These individuals may be internal to the University or external. University faculty serving on investigative panels must be tenured. Members of the Initial Inquiry Committee shall not be appointed to the Investigative Panel.

2. Conflict of Interest/Bias

In making appointments to the Investigative Panel, precautions shall be taken against real or apparent personal or professional conflicts of interest or bias with respect to the respondent, complainant or the case. For example, Panel members should not be involved with the research in question, should not be professional competitors with the respondent, and should not have a previous or ongoing close professional or academic relationship with either respondent or complainant.

3. Rights and Obligations of Respondent

The respondent shall be notified of the charges, the opportunity to be heard, and the obligation to cooperate fully with the investigation. Such notice shall inform the respondent that the investigation may determine: (a) whether or not misconduct has occurred; and/or (b) if the actions or conduct investigated are/is otherwise unacceptable within the University for proposing, performing or reviewing research or reporting research results. The respondent shall also be informed that unreasonable refusal to supply relevant material or other uncooperative behavior constitutes violation of this policy.

4. Objections to Proposed Investigative Panel Members

The respondent and the complainant shall be informed of the proposed membership of the Investigative Panel. If the respondent or the complainant objects to the participation of any member of the Investigative Panel based upon personal or professional conflict of interest or bias with respect to the respondent, complainant or the case, this objection

must be made within five (5) working days in writing to the SVPAA who shall decide whether to replace the challenged member. The decision of the SVPAA shall be final. Such challenges to the membership of the Investigative Panel must be resolved prior to the official appointment of the members by the SVPAA.

5. Appointment of and Charge to Investigative Panel

The SVPAA shall appoint the members of the Investigative Panel and shall administer the charge to the Panel. The official date of the initiation of the investigation shall be the date of the first meeting of the Investigative Panel. This shall be within thirty (30) calendar days of the completion of the initial inquiry (transmission of written report to SVPAA by Initial Inquiry Committee).

6. Chairperson of Investigative Panel

The Investigative Panel shall choose its chairperson at its first meeting.

7. Staff to Investigative Panel

The SVPAA and the Vice President for Legal Management shall assign staff to assist the Investigative Panel. Staff shall consider themselves and their activities for the Investigative Panel as strictly confidential.

8. Protection of Complainant from Retaliation

The SVPAA shall coordinate the University's efforts to protect the complainant from retaliation during and after the inquiry and investigation, working with all relevant University offices in these efforts.

9. Conduct of Investigation

a. Procedural Protection

Every effort shall be made to ensure a comprehensive, fair and expeditious investigation. The respondent shall have the opportunity to examine all evidence forwarded to the Panel, to be represented by legal counsel, to present evidence to the Panel, including witnesses on the respondent's behalf, and to cross-examine witnesses, including the complainant. Anonymous third-party statements are not admissible as evidence.

b. Confidentiality

The respondent and the complainant shall be afforded confidential treatment to the extent possible and permitted by law. Files shall be kept in a central location in a locked cabinet accessible only to the appropriate individuals taking part in the investigation.

c. Testimony before the Investigative Panel

Tape recordings shall be made of all testimony given. Documentation (including original data) substantiating the Investigative Panel's findings will be carefully secured, prepared and maintained. Transcriptions of the taped interviews shall be provided to the persons interviewed for comment or revision and included as part of the investigative file.

d. Sequestering of Additional Data and Material

The Investigative Panel may secure and sequester additional pertinent original research data, records, documents and other material from the respondent or others, using inventories and signed receipts for all material taken. If additional original materials are required for the proceedings, the Panel chairperson or designee shall give the respondent copies of original documents and records and reasonable access to original or unduplicatable materials during the proceedings. Materials sequestered shall be stored in a manner to ensure their preservation.

e. Consultants and Assistance for Investigative Panel

The Investigative Panel may seek additional expert scientific advice and/or the advice of students or postdoctoral fellows as appropriate to the status of the respondent.

f. Broadening/Change in Subject Matter of Investigation

If, during the investigation, information becomes available which the Panel considers substantially related to the original charge from the SVPAA, the Panel may broaden the scope of its charge and give written notice to the respondent of the new scope. If the Panel does not consider the new information substantially related to the original charge, the Panel may refer the new information to the Campus Committee as the basis of a new allegation.

10. Decision of Investigative Panel

In reaching a conclusion on whether there was misconduct in science, the burden of proof is on the University to support its case by a preponderance of the evidence.

The Investigative Panel's decisions shall be the majority opinions. There may be a minority report. The results of any vote taken shall be made known to the SVPAA in the written report of the Investigative Panel.

11. Duration of Investigation

The investigation shall be completed within ninety (90) calendar days of its initiation date to allow sufficient time for review of the Investigative Panel's report by the complainant, respondent and the SVPAA, and submission of the University's report, including the decision of the SVPAA, to the funding agency, within a total of one hundred and twenty (120) calendar days of the initiation of the investigation. If the investigation cannot be completed within these time limits, the University may request an extension of time from the ORI (in the case of research conducted under a PHS grant or if the research results were used in a PHS grant, fellowship or contract application) or from another pertinent funding agency or sponsor if required. If such an extension is granted, the respondent shall be so notified.

12. Report of the Investigative Panel

Upon conclusion of its investigation, the Investigative Panel shall prepare a written report for the SVPAA. A copy of the report shall be given to the respondent, the Chairperson of the Initial Inquiry Committee, and the appropriate Dean or Vice President. The complainant may be provided with those portions of the report that address his/her role and opinions in the investigation. Comments, if any, must be filed with the Panel within ten (10) working days of receipt of the Panel's report. These comments shall be made part of the report and considered by the SVPAA.

The report shall describe the policies and procedures under which the investigation was conducted and how and from whom information relevant to the investigation was obtained. It shall also include a recommendation as to whether a finding of misconduct

should be made, the basis for that recommendation as well as a recommendation about the appropriate corrective measures to be taken if any.

The report may also include recommendations that a finding be made that the respondent has engaged in practices that are unacceptable within the University for proposing, performing or reviewing research, or reporting research results, but which do not constitute misconduct in science as defined in Section IV of this policy. The report may make recommendations about corrective actions, if any, to be taken under these circumstances.

The report may also include the Panel's concerns that violations of other University policies or of Federal or state regulations may have occurred, with recommendations to refer these concerns for administrative action.

In addition, the Panel may make recommendations concerning notification of law enforcement agencies, professional societies, licensing boards, journal editors, collaborators of the respondent or other concerned parties of the outcome of the investigation.

In the event of a recommendation that there be no finding of misconduct, the Investigative Panel, after consultation with the respondent, may make recommendations to the SVPAA regarding actions to restore the reputation of the respondent. The Investigative Panel may also make recommendations to the SVPAA concerning actions against a complainant found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

13. Expenses of the Investigation

The expenses of the investigation, including external consultants' fees if any, shall be borne by the pertinent Dean or Vice President.

14. Decision and Actions of the SVPAA

The SVPAA shall review the final report of the Investigative Panel and shall make a final decision in writing on behalf of the University. If the SVPAA for good reason cannot accept the recommendation of the Investigative Panel, the SVPAA shall document his or her reasons and communicate these in writing to the Panel, the respondent, the complainant and the pertinent Dean or Vice President.

The SVPAA may make one of the following decisions:

a. finding of no misconduct: When necessary, diligent efforts shall be undertaken in consultation with the respondent fully to restore the reputation of the respondent, and appropriate action shall be taken against complainants found to have made unsubstantiated allegations in bad faith (see definition, Section IV.F).

b. finding of misconduct: The decision shall include the SVPAA's determination about the appropriate corrective actions. The SVPAA shall either accept the Investigative Panel's recommendation about corrective actions or impose alternatives. If the SVPAA decides to seek the termination of a faculty member with a full title, he or she shall initiate "Termination for Cause Proceedings" pursuant to the University Bylaws, Article VI, Title C. In these circumstances, the President shall conduct the meeting with the respondent to ascertain the validity of the charges as prescribed by the University Bylaws. Evidence gathered during the investigation, recommendations of the Investigative Panel, and the decision of the SVPAA shall be forwarded to all individuals involved in the termination-for-cause proceedings for their consideration. Other discipline imposed for misconduct in science shall be exempt from grievance and

arbitration proceedings. The SVPAA may withdraw from publication all pending abstracts and papers that are considered to be of questionable scientific validity as a result of the finding, and may notify the editors of journals, books and other publications in which the respondent's previous papers and abstracts have appeared during the preceding five years.

c. finding that actions or conduct investigated are/is unacceptable within the University for proposing, performing or reviewing research or for reporting research results, but do/does not constitute misconduct in science as defined in Section IV of this policy: The decision shall include the SVPAA's determination about appropriate corrective actions.

15. Notification of Decision of SVPAA

The SVPAA shall provide a copy of his/her final decision to the respondent, the complainant, the Chairperson of the Initial Inquiry Committee, the Investigative Panel, the pertinent Dean or Vice President, and the Vice President for Legal Management.

The SVPAA shall forward to the ORI (in the case of research conducted under a PHS grant or if the research results were used in a PHS grant, fellowship or contract application) or to another external funding agency or sponsor a copy of his/her final decision, along with the names of the Investigative Panel members and the Panel's final report.

G. Termination of the Case

1. Notification of Final Outcome of Investigation

After termination of a case, the SVPAA shall inform editors of scientific journals and program directors of scientific meetings who had been notified of the existence of an investigation, and all individuals interviewed or otherwise informed of the allegation of the outcome of the investigation.

2. Creation, Sealing, Storage of and Access to the File

The SVPAA shall ensure that the complete file, including the original records of all proceedings conducted by the Initial Inquiry Committee and by the Investigative Panel, and copies of all documents and other materials furnished to the Committee and the Panel, is sealed and retained indefinitely in a locked confidential cabinet in the Office of the SVPAA. Access to materials in the file shall be available only upon authorization of the SVPAA for exceptional cause.

3. Return of Sequestered Data and Other Materials

The SVPAA shall decide on a case-by-case basis when the research records, original data and other original materials sequestered during the initial inquiry or investigation may be returned. Among the determining factors in this decision are the requirements of pertinent government agencies or other sponsor.

H. Investigation by Federal Agencies

Under 42 CFR Part 50, Subpart A and 45 CFR Part 689, federal agencies have reserved the right to perform their own investigation in cases involving federally funded research at any time prior to, during, or following the University's investigation, and to impose corrective actions of their own in addition to those imposed by the University.

I. If Respondent leaves the University

If the respondent leaves the University prior to the completion of the initial inquiry or investigation, the inquiry and investigation, if any, shall nevertheless continue according to the procedures described above, and the respondent shall be afforded full opportunity to participate. The SVPAA may inform the respondent's new employer, if any and if known, of the existence and status of investigation and of the final findings of the investigation.

J. Admission of Misconduct by Respondent

If the respondent admits to misconduct in science prior to the completion of the initial inquiry or investigation, the admission must be in writing and must detail the full scope of the misconduct. An investigation should ordinarily be conducted and continued to conclusion in order to discover the scope of the misconduct or other problems and make recommendations to the SVPAA. The initial inquiry and investigation shall be conducted according to the procedures described above. If the initial inquiry committee believes that no purpose will be served by an investigation, it may make that recommendation to the SVPAA.

K. Withdrawal of Allegation by Complainant

If the complainant withdraws his or her allegation prior to the completion of the initial inquiry or investigation, the proceedings shall continue if sufficient information is available to warrant such continuance.

L. Confidentiality

The confidentiality and privacy of the respondent, the complainant and all others involved in the procedures undertaken under this policy; the confidentiality of the Initial Inquiry Committee's and Investigative Panel's proceedings, files, reports and records; and patient confidentiality in the case of clinical research shall be maintained to the extent possible and permitted by law. However, confidentiality may not be maintained if the allegation is determined to be false and is found to be made in bad faith (see definition, Section IV.F). Protection of confidentiality does not preclude disclosures that are necessary in the process of handling allegations of misconduct; are in the public interest or in the University's interest; are required by federal or state statute or regulations, University policy or rules of the research sponsor; or are a component of sanctions and/or corrective actions in the resolution of allegations of misconduct.

By Direction of the President:

_____

Vice President for Academic Affairs

UMDNJ
University of Medicine & Dentistry of New Jersey

Graduate School of Biomedical Sciences
New Jersey Dental School
New Jersey Medical School
Robert Wood Johnson Medical School
School of Health Related Professions
School of Nursing
School of Osteopathic Medicine
School of Public Health

Robert A. Saporito, DDS
Senior Vice President for Academic Affairs

March 21, 2003

Helene Z. Hill, Ph.D.
Professor
Department of Radiology
UMDNJ-New Jersey Medical School
185 South Orange Avenue, MSB F-452
Newark, NJ 07103

Dear Dr. Hill:

The University of Medicine and Dentistry of New Jersey has completed its proceedings prompted by your allegation of potential misconduct in science against Dr. Anupam Bishayee, currently Radiation Safety Specialist in the UMDNJ Office of Radiation Safety Services. Your allegation related to research Dr. Bishayee conducted when he was Research Associate in Dr. Roger Howell's laboratory in the department of Radiology at UMDNJ-New Jersey Medical School. The Newark Campus Committee on Research Integrity charged with reviewing these allegations conducted an initial inquiry in accordance with federal regulations and University policy. After considering your testimony and submissions, and other documents and materials concerning the allegations and the research in question, and after reviewing original research data, the Committee unanimously concluded that there is no cause to warrant further misconduct-in-science proceedings with regard to the allegation. This conclusion was based on the Committee's findings that:

1. statistics, alone, regarding the "randomness" or "uniformity" of the data in question are not sufficient evidence to warrant further investigation of this matter; and

2. the lack of appropriate independent control data with which to compare the experimental results generated by Dr. Bishayee renders the questions raised by your allegation scientifically unanswerable.

After reviewing the Committee's report, I have accepted its findings and have closed this case.

Thank you for your assistance in the conduct of these proceedings.

Sincerely yours,

Robert A. Saporito, D.D.S.
Senior Vice President for Academic Affairs

c: Russell Joffe, M.D., Dean, UMDNJ-New Jersey Medical School
D. Anthony Forrester, Ph.D., R.N., Chair, Newark Campus Committee on Research Integrity
Karen Putterman, M.D., M.P.H., Vice President for Academic Affairs, UMDNJ

65 Bergen Street, Room 1441 • Newark, NJ 07107-3001 • Phone: (973) 972-3645 • Fax: (973) 972-2621 • E-mail: saporito

EXHIBIT
45