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H.H. ROSSI, Director

E.J. HALL, Associate Director

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Radiological Research Laboratory
Department of Radiology
Columbia University
New York, New York

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INTRODUCTION

The Radiological Research Laboratory (RRL) has for many years been devoted to investigations concerning the effects of ionizing radiations on biological systems. Research in dosimetry, microdosimetry and cellular and tissue radiobiology has been carried out toward that end. An increasing fraction of the effort is concerned with the subject of cell transformation which is being studied as a model of carcinogenesis in general. This development has been formally recognized by the designation of the RRL as one of the seven sections of the Cancer Center of the Columbia Presbyterian Medical Center.

Some of the research is basic in nature, and addresses fundamental questions concerning the distribution and separation of the initial lesions produced by the deposition of radiant energy in biological materials. Other projects are directed toward practical questions that require immediate answers; these include, on the one hand, the study of carcinogenesis by densely ionizing radiations, which is vital to the ERDA mission, and, on the other hand, the comparison of high LET radiations used for human cancer therapy. An essential component of this effort is the Radiological Research Accelerator Facility (RARAF) jointly operated at the Brookhaven National Laboratory by RRL and the BNL Medical Department.

Physical measurements of energy deposition continue to be an important concern of this laboratory, because they represent the basis for understanding the biological effects of ionizing radiations. Especial attention is given to fundamental problems in dosimetry and microdosimetry. The track segment and molecular ion experiments performed at RARAF are designed to provide basic information concerning the physical events that lead ultimately to the expression of biological damage.

A significant development has been the increased interest on the part of

experimenters from other universities to use RARAF. A small, but active, outside users group is being built up.

In the summer of 1976 we were joined by Dr. Richard Miller from the University of Iowa, who is using cells in vitro to investigate the rate of neoplastic transformation produced by both radiation and drugs. This increase in staff was designed to strengthen and broaden our experimental competence to investigate cell transformation.

Dr. Dusan Srdoc from the Institute Ruder Boskovic in Yugoslavia joined the staff for a one-year visit to perform microdosimetric measurements at the RARAF facility. Dr. Srdoc has worked in this laboratory before and actively collaborates in our research program.

Experiments of direct relevance to radiotherapy have been conducted at the neutron facilities at the Naval Research Laboratory, Washington, D.C.; the Texas A & M Variable Energy Cyclotron; the University of Washington, Seattle; the University of Maryland; Hammersmith Hospital, London; the Fermilab, Batavia, Illinois; and at the National Institute for Radiological Sciences, Chiba, Japan. The last mentioned effort was sponsored by the U.S.-Japan Cooperative Cancer Research Program. Measurements with protons have been made at the Harvard Cyclotron, with Pi mesons at Los Alamos, and with accelerated heavy ions at the BEVALAC. In all of these instances, an attempt has been made to bring to bear the expertise in both biology and physics available in this laboratory, to provide information relating to the RBE and OER of the various heavy particles. These data are of immediate practical use in the implementation of these radiations in radiotherapy.

An area that has advanced rapidly, and merits further effort, has been the development of electron affinic drugs that both radiosensitize hypoxic cells and also are cytotoxic to cells deficient in oxygen. The use of these

drugs in combination with radiation and hyperthermia will continue to be a major thrust of this laboratory in the coming year.

Overall, there has been a slight expansion of the laboratory during the past year, specifically in the area of transformation. The decrease in effective funding from the ERDA contract has been balanced by newly acquired funds from the National Cancer Institute.

ACKNOWLEDGEMENTS

Much of the research effort of this laboratory centers around the Radiological Research Accelerator Facility at the Brookhaven National Laboratory, where a number of members of our staff are located. In addition, many members of the Brookhaven staff collaborate on several different projects. We would not miss this opportunity to thank Dr. V.P. Bond and Dr. E. P. Cronkite who have been primarily responsible for this active cooperation.

Because of our interest in high LET radiations, we have used many accelerators in various parts of the U.S. as well as abroad. We are grateful to the following individuals who made this possible: Drs. Bondelid, Theus and August at the Naval Research Laboratory, Washington, D.C.; Drs. Almond, Hussey and Smathers at the Texas A & M Variable Energy Cyclotron; Drs. Parker and Wooten at the University of Washington Cyclotron, Seattle; Drs. Harrison and Holgren at the University of Maryland; Drs. Bewley and Field at the Hammersmith Hospital; Drs. Misono, Inada and Urano at the National Institute for Radiological Sciences, Chiba, Japan.

At the BEVALAC much of our work in microdosimetry could not have been done without the active cooperation of Drs. Howard and Llacer. We also wish

to thank Mr. Glen White and the staff of the BEVALAC shop for construction of special apparatus for us.

In the area of electron affinic drugs, Roche Products has generously supplied substantial amounts of the nitroimidazoles, while Dr. Ged Adams has kindly provided small amounts of newly synthesized compounds.

Most of the instruments and equipment used in the research described in this report were built in the Instrument Shop of the Radiological Research Laboratory. The skills of the shop personnel, under the direction of Mr. Rudolph Gand, have been essential, not only in the construction of apparatus, but in many important features of design.

A significant portion of the work described in this report was supported by additional funds from other sources. In particular, grants from the National Cancer Institute, DHEW, supported in part the investigations described in the following sections:

CA 12536 - PD-1, PM-1,2, BP-1, RB-4,5,6,7,10,11

CA 15307 - PD-2, RB-9,12,13,14

CA 18506 - RB-1,2,3,8

CA 09053 - RB-5,6

SCIENTIFIC STAFF

HARALD H. ROSSI, Ph.D., Professor of Radiology (Director)
ERIC J. HALL, D.Phil., Professor of Radiology (Associate Director)
ALBRECHT M. KELLERER, Ph.D., Consultant
RICHARD P. BIRD, Ph.D., Research Associate
CARMIA BOREK, Ph.D., Associate Professor of Clinical Pathology (in Radiology)
ROBERT D. COLVETT, M.S., Research Associate
RUDOLPH GAND, Staff Associate (Design and Development)
CHARLES R. GEARD, Ph.D., Assistant Professor of Radiology
LEON J. GOODMAN, B.S., Research Associate
PAUL J. KLIAUGA, Ph.D., Research Associate
ANDRE A. KOSIARA, Ph.D., Research Associate
YUK-MING P. LAM, Ph.D., Research Associate
RICHARD C. MILLER, Ph.D., Post-Doctoral Fellow
EDITH H. QUIMBY, Sc.D., Emeritus Professor of Radiology
LAURIE ROIZIN-TOWLE, M.S., Staff Associate
DUSAN SRDOC, Ph.D., Staff Associate
ALAN G. UNDERBRINK, Ph.D., Research Associate (resigned November, 1976)

SUPPORTING STAFF

MYLES ASTOR, B.A.
DELIA BROWN
ROBERT COFFEY, B.A.
STEPHEN MARINO, M.S.
HERBERT MASON
ROBERT MILLS, B.S.
CYNTHIA PAIN, B.A.

SUPPORTING STAFF (continued)

MARGIT PAULUSSON-ODENHAGEN, M.S.

STEPHANIE POVLAS, B.A.

FRANK RINI, M.A.

PEETER TEEDLA, M.S.

JAMES TRANCHINA, B.S.

JOHN VARGA, B.A.

INSTRUMENT SHOP

GARY JOHNSON, A.A.S.

ADOLF NAPHOLZ, A.A.S.

SECRETARIAL STAFF

MARY LEE BOONE, B.A., Administrative Assistant

KATHERINE JOHANNY, Administrative Consultant

LOUISE HO

CONSTANCE J. KRAMER

SUSAN MUNSTERMAN

GERDA OSBORN

RADIOLOGICAL PHYSICS

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D. Srdoc

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R.E. Mills, R.D. Colvett and L.J. Goodman

A) DOSIMETRY

PD-1 Specialized dosimetry at RARAF

S. A. Marino, R. E. Mills, and L. J. Goodman

A. Dosimetry for mammalian cell transformation studies

The study of the neoplastic transformation of C3H mouse cells was completed. The apparatus and techniques used for neutron dosimetry and cell irradiations were described in previous Annual Reports (COO-3243-3, -4).

Twenty-five groups of eight flasks each were irradiated with 0.43 MeV neutrons to an absorbed dose of 10 mGy (1.0 rad). Forty groups of five flasks each were irradiated with 210 kV x rays to an absorbed dose of 100 mGy (10 rad). The methods and apparatus used for dosimetry and irradiation were described in last year's Annual Report (COO-3243-5).

B. Neutron dosimetry for rat irradiations

Monoenergetic neutron irradiations of rats described in previous Annual Reports (COO-3243-1 through -5) were continued in collaboration with members of the Medical Department of Brookhaven National Laboratory. Two sets of irradiations were performed using 0.43 MeV neutrons, each consisting of two groups of 24 rats which received an absorbed dose corresponding to 40 mGy (4.0 rad) of total tissue kerma in free air at the center of the animal positions. These are part of a larger, split-dose experiment to be completed over a period of about two months and consisting of another 16 groups of rats, each receiving either the same or twice the absorbed dose above.

C. Neutron dosimetry for *Drosophila* irradiations

The study of the genetic effects of monoenergetic neutrons on *Drosophila melanogaster* being conducted in collaboration with members of the Zoology Department of the University of Wisconsin was continued.

For the irradiations performed using 15 MeV neutrons, the equipment and methods employed for dosimetry and irradiation were the same as were used for the 6 MeV irradiations described in Annual Report COO-3243-1. A total of 46 groups of approximately 250 flies each were given absorbed doses of 15, or 30 Gy (1500 or 3000 rad).

Flies were also irradiated with neutrons having energies of 0.43 and 0.67 MeV. Apparatus and procedures for dosimetry and irradiation were described in Annual Report NYO-2740-5. Twenty-four groups of approximately 500 flies each were given absorbed dose of 5, 10, or 15 Gy (500, 1000, or 1500 rad) of 0.43 MeV neutrons. Twelve groups of flies were given absorbed doses of 10 or 15 Gy (1000 or 1500 rad) of 0.67 MeV neutrons.

D. Neutron dosimetry for human stem cell irradiations

Studies of the effects of monoenergetic neutrons on the survival and proliferation of human hematopoietic stem cells were begun in collaboration with members of the Medical Department of Brookhaven National Laboratory and the Division of Toxicology of the Norwegian Defence Research Establishment, Kjeller, Norway.

Cells were irradiated with monoenergetic neutrons having mean energies of 0.44, 6, or 15 MeV. Total absorbed doses were measured using the 6 mm diameter tissue-equivalent multiplication chamber described in Annual Reports NYO-2740-3, -4, -6, and -7. Gamma-ray absorbed doses were measured using the compensated Geiger-Müller counter described in Annual Report NYO-2740-8.

Irradiations with 6 and 15 MeV neutrons were performed at 0° relative to the charged particle beam direction with a target-to-specimen distance of 10 cm. For 0.44 MeV, specimens were irradiated at 120° and 5.1 cm in order to achieve sufficient absorbed dose rate and to allow for simultaneous irradiation of two specimens.

One or two groups of cells were each given an absorbed dose of 0.25, 0.5, 1.0, 2.0, or 3.0 Gy (25, 50, 100, 200, or 300 rad) at each neutron energy employed.

PD-2 Charged particle beams for radiobiology at RARAF

R. D. Colvett, N. Rohrig, and S. A. Marino

Two charged particle beam irradiation facilities are in routine use at RARAF. The molecular ion beam has been described extensively in previous Annual Reports (COO-3243-2, -3, and -4). The Track Segment Facility, described in last year's Annual Report (COO-3243-5), is now in routine use. The biological investigations include cell survival (RB-13), chromosome aberration induction (RB-7), and DNA strand breakage. No further work has been done on the charged particle microbeam facility beyond the proposed design described last year (COO-3243-5).

A. Molecular ion beam: physical data

Previously described measurements of the multiple coulomb scattering of protons and deuterons (COO-3243-2, -3) employed only monoatomic ions. The data were used to calculate the spacing of paired ion tracks when molecular beams penetrated Mylar foils. The vacuum tank equipment of the ABC experiment (PM-4) provided a convenient opportunity to explore the following question: To what extent does the internal structure of a molecule affect the separation of its constituent atoms after the molecule dissociates? Is separation increased by the mutual repulsion of the associated ions, or does some persistence of the molecular structure decrease separation by holding the ions together for a short time in the Mylar? To answer this question, the scattering behavior of 1.25 MeV protons, 2.5 MeV diatomic protons, and 3.75 MeV triatomic protons, 2.5 MeV diatomic protons, and 3.75 MeV triatomic protons was measured using the same techniques as before. If molecular effects are negligible, as has been previously assumed, then the observed scattering widths will be the same. The experimental data, fitted by least squares to a gaussian expression

(Figure PD-2-1) shows that the three species of ions have essentially indistinguishable scattering parameters thus the original assumption of independent scattering was appropriate.

B. Track segment dosimetry

Track segment dosimetry uses an ionization chamber, a silicon particle counter, and a tissue-equivalent gas-filled LET chamber as described previously (Figure PD-2-2 is repeated here from COO-3243-5, pp. 38-49). Figure PD-2-3 shows representative LET spectra of several ions in the LET chamber. In each case there is a major peak corresponding to the LET of the chosen irradiation conditions and an upper limit of the spectrum corresponding to the maximum energy that the particle can deposit along a 10 μm path length. This maximum energy is characteristic of the particle type, providing a calibration point for the spectrum. (Since the LET counter measures ionization, not energy, the apparent energy of the spectrum edge used for calibration is displaced by the varying $\bar{W}$ for heavy ions.) Thus, the LET chamber is essentially self-calibrating and gives absorbed dose by providing both LET and fluence, independent of the more conventional instrumentation. To calculate LET's in different portions of the biological sample, in the ion chamber, and in the LET counter, a consistent set of range and energy loss tables has been derived from tabulated values of Janni (1), Northcliffe and Schilling (2), and Ziegler and Chu (3).

C. Proton penetration studies

Measurements to determine the depth distribution of radiation sensitive sites in Chinese hamster cells have begun. Cole and co-workers (4,5) have described radiobiology studies with electrons and alpha particles which partially penetrate the cell. Similar studies with protons using the Molecular Beam Facility should provide better control of depth dose than with the electrons

Figure PD-2-1

Scattered intensity vs lateral distance (at 46 cm flight path) for beams of 1.25 MeV monoatomic deuterons (circles), 2.5 MeV diatomic deuterons (diamonds), and 3.75 MeV triatomic deuterons (squares) after passing through a Mylar foil of nominal thickness 0.9 mg/cm^2 . The intensities are normalized at 0 cm. The solid line is a gaussian fitted to the data inside 2 cm. The departure at large angles results from single large angle coulomb collisions. Deuterons originating from a diatomic beam are spread somewhat more than those from mono or triatomic beams as expected. For our purposes this difference is insignificant.

Fig. PD-2-1

Figure PD-2-2

The three track segment dosimeters are mounted together on a wheel that supports them on the track segment drive mechanism, in the same way that specimen wheels support cell dishes for irradiation.

a) Self-calibrating LET chamber. An ungridded pulse ionization chamber to measure the energy deposited along a path length in tissue-equivalent gas corresponding to 10 μm path in unit density tissue. b) Solid state detector for determining fluence and uniformity of fluence along the irradiation window. Apertures for devices a) and b) are nominally 0.04, 0.13, or 0.3 mm diameter depending on the dose rate. c) Ionization chamber. A guarded, parallel-plate chamber for measuring absorbed dose. Electrical conduction across insulators is obtained where necessary with a very thin coating of colloidal graphite.

(a) LET CHAMBER

(b) MOUNT FOR SOLID STATE DETECTOR

(c) IONIZATION CHAMBER

Fig. PD-2-2

Fig. PD-2-3. Spectra from LET counter. Sections a, b, and c show energy losses of various energy deuterons in the LET counter. The indicated edges correspond to the maximum energy which can be lost in 1 mg/cm^2 of tissue equivalent gas. This energy is calculated for deuterons to be 720 keV so the equivalent LET is $72 \text{ keV}/\mu\text{m}$. The deuteron energies before passing through the Havar foil, air gap, and Mylar window were 2.2, 1.9, and 1.7 MeV, yielding LET's at $5 \mu\text{m}$ depth in tissue of 34, 44, and $54 \text{ keV}/\mu\text{m}$ for spectra a, b, and c respectively. Spectrum d was made by helium-3 particles with 6.2 MeV incident energy. The gain is reduced by a factor of 2 for spectra d. The LET for the edge is $189 \text{ keV}/\mu\text{m}$ so the main peak is at $105 \text{ keV}/\mu\text{m}$. The deuteron contaminant peak at $40 \text{ keV}/\mu\text{m}$ contributes 7 percent of the fluence and 2-1/2 percent of the dose. Noise resolution is adequate for $11 \text{ keV}/\mu\text{m}$ proton spectra.

or alpha particles used by Cole. One motivation for these experiments is to associate loss of cell reproductive integrity with particular depths of a cell at different stages of the cell cycle. A more specific need is in connection with the molecular beam experiment. The mean separation of the associated particles depends on the amount of material between the damage site and the break-up point of the molecule. Since the cells are of comparable thickness to the Mylar substrate, interpretation of molecular beam results depends on the position of the radiation sensitivity throughout the cell depth, or more specifically as a function of distance away from the Mylar window. The depth sensitivity measurements with the proton Bragg peak should provide this information.

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PD-3 Investigation into the thermal defect of tissue-equivalent plastic

L. J. Goodman

A. Introduction

Last year's Annual Report (COO-3243-5) outlined planned studies of the thermal defect of tissue-equivalent plastic. This work is being done in collaboration with the Biophysics Laboratory of the Memorial Sloan-Kettering Cancer Center, New York, NY.

The aim of the investigations is to determine the thermal defect, defined as the fraction of absorbed radiation energy which is not converted to heat in the absorber, for samples of A-150 tissue-equivalent plastic obtained from different laboratories and molded under diverse conditions, and to examine the dependence of the thermal defect on the energy and type of the irradiation particles. This information is needed to utilize this plastic for calorimetric neutron dosimetry.

Several calorimeter absorber discs have been contributed by each of the laboratories listed in Table PD-3-1. Unless otherwise indicated, each laboratory molded the discs according to its routine procedures. Although discs were supplied by four widely separated laboratories, it should be realized that each laboratory obtained the virgin A-150 material from the Physical Sciences Laboratory where it is manufactured.

Some discs obtained from supplier No. 1 were molded at the lowest and at the highest temperatures and pressures deemed feasible by the instrument maker responsible for the work. These discs will be used to assess the degree to which the thermal defect of the plastic depends on molding techniques.

The uniformity of the material in the molded absorbers obtained from the four suppliers was examined by measuring the

Table PD-3-1

Laboratories which supplied calorimeter absorber discs

<u>No.</u>	<u>Supplier</u>	<u>Disc labels</u>
1	Radiological Research Laboratory New York, NY, U.S.A.	RP1 - RP12 (Note 1) RL1 - RL3 (Note 2) RH1 - RH3 (Note 3)
2	Radiobiological Institute, TNO Rijswijk, The Netherlands	T1 - T5
3	EG&G Corporation Goleta, CA, U.S.A.	E1 - E4
4	Physical Sciences Laboratory Lisle, IL, U.S.A.	P1 - P5

- Notes:
1. Routine molding temperature and pressure:
160° C and 5.2 MPa (750 psi)
 2. Low molding temperature and pressure:
150° C and 2.1 MPa (300 psi)
 3. High molding temperature and pressure:
200° C and 21 MPa (3000 psi)

densities of all discs and by performing chemical analyses on samples from each group. Uniformity within and between sample groups would portend similar agreement in the thermal defects to be measured.

B. Density of discs

The density of each disc was calculated as the quotient of its mass by its volume, as determined by measurements of the diameter and thickness. A balance¹ having a resolution of 0.1 mg was calibrated using masses of $(10 \pm 0.02 \text{ max})$ mg and $(100 \pm 0.025 \text{ max})$ mg. Each disc was then massed once, except that several massings were made for two discs to assess the random uncertainty. It was concluded that the random uncertainty of each measured mass, nominally 50 mg, has a standard deviation of about 0.2 percent.

Linear dimensions were determined using instruments and gauges at the Inspection Department of the Brookhaven National Laboratory Central Shops. The 1.4 cm nominal diameter of each disc was measured using a microscope with a moveable stage.² The translation control has a resolution of 0.25 μm and was calibrated using 1.3 cm gauge block having a maximum error of $\pm 0.15 \mu\text{m}$. Measurements made across three diameters spaced equiangularly were averaged. The slightly ragged edge of each disc resulted in a random uncertainty having a standard deviation of about 0.2 percent.

The largest contribution to the overall uncertainty of the

¹Sartorius Type 2462

²Gaertner toolmakers microscope

density was made by variations in the 0.025 cm nominal thickness of each disc. An optical thickness gauge³ with a resolution of 0.25 μm was calibrated using 0.25 and 0.50 mm gauge blocks having maximum errors of +0.1 and -0.05 μm . The thickness of each disc was measured five times, once in the center and once near the periphery along each of four radii spaced equiangularly. The mean disc thickness, used to compute the density, had a standard deviation which ranged from 0.3 percent to almost 4 percent.

The calculated densities are tabulated in Table PD-6-2. As mentioned previously, the standard deviations of the individual densities are dominated by nonuniformity in the thickness of each disc. The weighted mean density for each group and its standard deviation are also shown in the Table. Student's t test was applied to all combinations of the six groups taken two at a time and showed no significant density difference between any pair of groups even at the 50 percent probability level. Therefore, all values were pooled to give a weighted mean density having a 99 percent confidence interval of 1.126 to 1.135 g cm^{-3} .

Table PD-3-2 also shows five densities of A-150 plastic listed by Smathers, et al. (1). The methods of measurement were not described, and for only one value was an uncertainty indicated. The mean of these densities is 1 percent less than found for the absorber discs.

C. Elemental composition of discs

Eight discs were cut in half and either one-half (A) or both halves (A and B) underwent analyses⁴ for all the elements shown in Table PD-6-3, except oxygen which was assumed to be the

³Bausch and Lomb Optical Gauge DR-25B

⁴Schwartzkopf Microanalytical Laboratory, Inc., Woodside, NY

Table PD-3-2

Computed densities of A-150 tissue-equivalent plastic calorimeter discs

Disc	Density, $g\ cm^{-3}$	Percent Std Dev	Mean density, $g\ cm^{-3}$	Percent Std Dev of mean
RP1	1.135	1.1		
RP2	1.138	0.8		
RP3	1.127	1.8		
RP4	1.115	0.6		
RP5	1.111	1.1		
RP6	1.138	2.1	1.132	0.2
RP7	1.130	0.3		
RP8	1.139	0.3		
RP9	1.129	1.5		
RP10	1.114	1.4		
RP11	1.061*	1.5		
RP12	1.120	1.4		
RL1	1.139	0.4		
RL2	1.118	0.9	1.132	0.3
RL3	1.115	0.9		
RH1	1.154	1.6		
RH2	1.140	1.7	1.135	0.8
RH3	1.122	1.2		
T1	1.115	0.7		
T2	1.120	1.1		
T3	1.118	0.9	1.121	0.4
T4	1.142	1.1		
T5	1.123	1.0		
E1	1.136	0.8		
E2	1.130	0.6	1.130	0.3
E3	1.125	0.5		
E4	1.137	0.7		
P1	1.120	3.5		
P2	1.132	2.7		
P3	1.123	3.9	1.124	1.4
P4	1.120	2.7		
P5	1.124	3.2		
Smathers	1.10	-		
et al.	1.12	(± 0.005)		
(1977)	1.12	-	1.119	0.5
	1.13	-		
	1.125	-		

*Anomalous value not used to compute means

balance of each sample. Variances for each elemental analysis were calculated for discs RP11 and RP12 (samples from the same supplier for which replicates were analyzed) and compared to the variances for the remaining six discs taken as a group. This test showed no evidence that the precisions of the two sets differ at the 5 percent level and, therefore, no statistically significant difference is discernable in the elemental compositions of the discs. On this basis, the data was pooled to yield the means and confidence limits shown in the Table and compared to the elemental composition calculated from the plastic formulation (1).

It is worth noting that the last batch of A-150 plastic was manufactured in 1970 (2), and the discs were all molded in 1975. Thus, it is very likely that all four laboratories used material from the same mix to mold the discs. Coupled with the consistent results of the analyses, the important general conclusion can be drawn that the material supplied to the molders was of uniform composition and that differences in their molding techniques, including extremes of temperature and pressure, had no significant effect on this uniformity.

Smathers, et al. (1) recently reviewed several analyses of the composition of A-150 plastic. Since these analyses were made at different times and by different laboratories, it was not possible to arrive at a definite conclusion regarding the uniformity of composition in the end product. However, "based on chemical analyses and on judgments influenced by knowledge of the materials from which the plastic is formulated," these authors arrived at the recommended composition shown at the bottom of Table PD-3-3. These values agree well with those of the present analyses for hydrogen and carbon. For the other four elements significant differences are apparent which, in part, could be reconciled by differences in absorbed water. Systematic errors in analyses performed at different laboratories probably account

Table PD-3-3

Analyses of A-150 tissue-equivalent plastic absorber discs

<u>Disc</u>	<u>H</u>	<u>C</u>	<u>Percent mass</u>		<u>F*</u>	<u>Ca</u>
			<u>N</u>	<u>O</u>		
RP10A	10.31	76.98	3.39	7.61	0.34	1.34
RP11A	10.37	76.94	3.26	6.71	1.40	1.32
RP11B	10.16	76.82	3.24	5.53	2.93	1.32
RP12A	10.32	76.91	3.18	6.10	2.14	1.35
RP12B	10.24	76.49	3.46	7.68	0.90	1.23
RL3A	10.49	76.78	3.23	7.07	0.92	1.51
RH3A	10.36	76.99	3.37	6.59	1.20	1.49
T5A	10.34	77.12	3.41	7.68	0.14	1.31
E4A	10.45	76.75	3.34	7.72	0.74	1.45
P5A	10.23	77.52	3.11	7.13	0.69	1.32
Means	10.33	76.93	3.30	6.94	1.14	1.37
95% confidence limits	10.40	77.12	3.38	7.45	1.74	1.43
	10.26	76.74	3.22	6.43	0.54	1.30
Calculated	10.13	77.55	3.50	5.23	1.74	1.84
Smathers et al. (1977)	10.2 <u>±0.1</u>	76.8 <u>±0.5</u>	3.60 <u>±0.2</u>	5.9 <u>±0.2</u>	1.7 <u>±0.1</u>	1.8 <u>±0.1</u>

*Fluorine is acknowledged to be difficult to determine in this mixture.

for the remaining disparities.

The uniformity of density and composition of the A-150 plastic is important because this material is widely employed in dosimetry. For the application of the plastic to calorimetric neutron dosimetry, these results prompt the expectation that the thermal defects to be measured will show similar consistency.

D. Instrumentation

A FOCAL language program for the RARAF PDP-8/e minicomputer has been written, debugged, and satisfactorily tested. This program provides control, data logging, and analysis of measurements made with the calorimeter, as follows:

- a) Tests for absorber temperature stability.
- b) Starts and stops absorber heating by an electrical heater or by the charged particle beam.
- c) Controls the cooling cycle required to return absorber to starting temperature.
- d) Calculates all required temperatures and charges from transducer responses.
- e) Calculates regression lines for time versus absorber temperature for data taken before, during, and following heating.
- f) Calculates regression lines for time versus energy deposited in absorber by beam for data taken before, during, and following heating.
- g) Calculates mean drift slopes, net slopes, quotient of rate of absorber temperature change by rate of energy deposition, and total energy deposited.
- h) Provides graph of absorber temperature versus time showing data points and regression lines on a CRT terminal and/or a hard copy plotter.
- i) Stores all primary data on magnetic tape for possible future reanalysis.

Absorber temperatures are computed by adding the small temperature difference between the absorber and the adiabatic jacket, as sensed by a thermopile, to the temperature of the adiabatic jacket determined by the response of a thermistor. The thermistor resistance is measured by a digital ohmmeter having a resolution of 0.001 percent. An interface which allows the computer-controller to acquire resistance values from the ohmmeter has been constructed and is described in Section PA-2B.

Uniform deposition of beam energy over the surface of the calorimeter absorber is achieved by electrostatically sweeping the beam in a raster pattern. Feedback control of one of the sweep amplitudes is used to regulate the beam current at the absorber. Further discussion of this instrumentation is given in Section PA-2A.

E. Preliminary measurements

Measurements made while the calorimeter was at a stable temperature have shown that the standard deviation of a measurement of absorber temperature is 2 mK. Preliminary measurements have also shown that the quotient of absorber temperature rise by absorber energy input is about 1 K/J. Thus, a power input to the absorber of 2 mW for 400 s should result in a temperature rise of about 0.8 K. Regression line analysis of the data should permit determinations of the temperature-energy quotient with a resolution less than 0.2 percent.

Whether this can be achieved will depend on the extent to which thermal losses can be minimized and made reproducible for successive heating cycles. Preliminary trials have indicated reproducibilities from 0.3 to 0.5 percent, depending on specific conditions. These results were obtained without a foil at the beam entrance to the adiabatic jacket. It is expected that the addition of a thin nickel foil to shield the absorber from small

heat exchanges with uncontrolled external temperatures will improve reproducibility. Judicious adjustment of rate and time constants in the temperature controller of the adiabatic jacket should help to minimize heat losses. Routine measurements to determine the thermal defects of the A-150 absorber discs will follow these modifications.

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PD-4 Measurements of $\bar{W}$ in methane-based tissue-equivalent gas

N. Rohrig and R. D. Colvett

Using the apparatus and techniques described in last year's Annual Report (COO-3243-5, pp. 84-95) measurements of $\bar{W}$ in methane-based tissue-equivalent gas have been extended to carbon and helium ions in the energy range specified in Table PD-4-1. As previously described, absolute measurement of $\bar{W}$ is made by measuring the particle energy, the number of incident particles, and the ionization charge collected on a capacitor ($Q = CV$). The measured values of $\bar{W}$ vary smoothly with incident particle energy increasing at low energies. Systematic errors in the measurements are being analyzed prior to publication but are equal to or less than 1 percent in most cases. The largest uncertainty results from the dead time correction on the measured number of particles.

Table PD-4-1

$\bar{W}$ in methane-based tissue-equivalent gas

<u>Ion</u>	<u>$E_{\min}$ (keV)</u>	<u>$\bar{W}$ (eV)</u>	<u>$E_{\max}$ (MeV)</u>	<u>$\bar{W}$ (eV)</u>
Proton	150	30.9	2.7	30.5
Helium	360	35.4	7.8	31.0
Carbon	120	46.9	5.6	35.3

PM-1 Microdosimetry at the BEVALAC: Preliminary Results

P. Kliauga, Y-M.P. Lam, D. Colvett and L.J. Goodman

I. Introduction

As noted in last year's Annual Report (1, 2), microdosimetric measurements on energetic heavy ions from the BEVALAC had been planned in support of biology experiments performed there in January 1976, utilizing ^{40}Ar at 450 MeV/A (3-6). The first microdosimetry run was completed in January 1977 using two sphere-within-a-sphere (S&S) type wall-less proportional counters (2). This report will review the experimental procedures and data analysis of that experiment and discuss results which are available as of the date of writing (April 1, 1977).

II. Experimental Procedures

The experimental program actually carried out differed considerably from that which had been planned. This was necessitated by the unavailability of the BEVALAC Biomed computer for data storage and analysis. The original plan called for simultaneous irradiation of both S & S counters, with each counter feeding up to three independent analog-to-digital converter (ADC's), each ADC accepting data from the counter at a different amplifier gain setting so that a wide dynamic range could be covered with good resolution. This would have required six independent ADC's. A melding program was written to join the individual spectra into one continuous spectrum, plot the distribution functions, and calculate various moments. Unfortunately, while the necessary hardware was available, an error in the software necessary for the data handling routines made the computer unavailable for that purpose during the time of our visit. As a result, we were forced to implement a much less ambitious program, which was successful because of the efforts of Drs. Jerry Howard and

Jorge Llacer, who contributed much time and effort to see that we were provided with two pulse height analyzers for data storage.

The availability of only two independent ADC's made simultaneous irradiation impractical. It was decided to irradiate only one counter at a time and utilize the pulse height analyzers to store data from that counter at two different amplifier gains. The ratio of the high gain to low gain varied somewhat, but was generally about 10.

Each counter was fitted with a spherical cap of TE plastic positioned normal to the beam direction. The purpose of the cap was to ensure that all particles entering the counter traversed identical thicknesses of plastic regardless of their position relative to the center of the counter.

The location of the Bragg peak was determined by using the variable water absorber provided at the Biomed facility and observing the counter pulses on an oscilloscope. The sharp transition from the peak, characterized by many large pulses, to the steep drop-off toward the tail, characterized by relatively few large pulses and many small ones, is readily detectable. It was apparent from repeated trials that this transition region, the back edge of the peak, could be determined to within an accuracy of ~ 0.5 mm of water. This experimentally determined peak was used as the reference point for all irradiations.

The irradiations on the ^{40}Ar beam were performed at the same relative locations on the Bragg curve as the biology experiments referred to earlier (7). These will be referred to as plateau, ascending, peak, and tail, respectively. In addition, some data were taken with a ridge filter often used at the Biomed facility for biological irradiations. The full scope of the experiment included data on the ^{12}C beam at 400 MeV/A as well as the ^{40}Ar beam at 450 MeV/A. Only the ^{40}Ar data will be discussed at this time,

since the ^{12}C data have not yet been analyzed.

Because of the reduction in the experimental program due to circumstances already discussed, and due further to the unavoidable loss of run time because of machine and/or computer malfunctions, the amount of usable data is less than had been anticipated. Since cuts were mandatory, it was decided to make them in the number of simulated site diameters (proportional to gas pressure) for each counter. One of the more interesting features of this two-counter experiment is a comparison of microdosimetric spectra between the 1 inch and the 1/4-inch counter at a given simulated diameter, i.e., with the 1/4 inch having four times the gas pressure of the 1 inch. Any differences are presumably due to the differences in energy deposition by delta rays, i.e., a "wall effect" (8, 9). In order to preserve this feature, data at all four Bragg curve positions were taken first with both counters simulating 1 μm site diameters (17.5 Torr in the 1 inch counter, 70 Torr in the 1/4-inch counter, standard propane base TE gas). A few points at other pressures were also obtained.

For calibration, a mercury pulser of known stable output was employed, as well as an ^{241}Am source. The pulser output was fed into the counter preamplifier, the output of which served as source for two amplifiers whose gain ratios were ~ 10 . Part of the data analysis problem, to be discussed later, was to meld these two spectra into a single unified spectrum with a constant "bin" size, i.e., fixed energy increments.

The LET of ^{40}Ar at $\sim 430 \text{ MeV}/\mu\text{m}$, which is the energy upon entrance into the counter at the plateau, is $\sim 100 \text{ keV}/\mu\text{m}$; at the peak, it is $\sim 3000 \text{ keV}/\mu\text{m}$. The large amounts of ionization created in the counter by these high LET particles required that the counters be operated at gas gains of ~ 10 to 100, as opposed to ~ 1000 as is normally the case, to avoid saturating the pre-

amplifier. The smallest number of ionizations observable is determined by the gas gain and preamplifier noise (10). Ordinarily, these counters are capable of detecting single ionization events. However, the low gas gains employed, along with the high-noise characteristics of an accelerator environment did not permit detection of events less than ~ 2 keV, and often much above that. Techniques for extrapolation to small event sizes are discussed in the section on data analysis.

Because of the uncertainty in determining the energy which the ^{241}Am source deposits in the counters at different pressures, it was decided to perform the calibration by correlating the known pulser outputs with an x-ray source (Al K_α) at our home laboratory, while duplicating the pressures, gas composition and potentials used at the BEVALAC. The use of reproducible pulser signals makes the calibration independent of amplifier gains and analyzer conversion gains. All analyzers used were checked for linearity and zero offset, and corrections applied where necessary.

III. Data Analysis

A. Calibration

Consider, in the following schematic setup,

an event which deposits energy e into the counter causes a count to appear in the P channel of the pulse height analyzer PHA. If V is the height of the input pulse into the PHA, then P is related to V by the linear equation

$$P = a + bV \quad (\text{III.1})$$

where a and b are constants. Let

c = conversion factor from energy deposited in the counter
to the output charge pulse from the counter,

g = preamp gain factor

G = amp gain factor.

The conversion factor depends on the size and pressure of the counter; and also its voltage supply.

Then $V = e c g G$, (III.2)

so that

$$P = a + b g G c e. \quad (\text{III.3})$$

Calibration of the setup then consists of determining the constants a and $b g G c$, which can be accomplished in three steps.

Step 1: Replace the counter by a pulser, and for each pulser output p_i measure the corresponding peak channel P_i in the PHA. Then

$$P_i = a + b g G p_i. \quad (\text{III.4})$$

Doing this for more than one i allows us to determine the constants a and $b g G$. To determine the remaining conversion factor c , two more steps are required.

Step 2: In the previous setup, replace the PHA at Berkeley with one at RRL which we will call the standard PHA, and expose the counter to an x-ray of known energy e^S , which is soft enough that all the energy deposited by a photon will be collected by the counter.

Then the observed channel p^S is related to the input standard energy e^S by

$$p^S = a^S + b^S g G c e^S \quad (\text{III.5})$$

where a^S and b^S are the constants for the standard PHS.

Step 3: As in Step 1, replace the counter by a pulser and for each pulser output p_i^S measure the corresponding peak channel P_i^S . Then, because of the relation:

$$P_i^S = a^S + b^S g G p_i^S, \quad (\text{III.6})$$

we may measure the constants a^S and $b^S g G$.

Substituting these values into equation (III.5) will allow us to determine the remaining constant c .

This calibration was performed for both the high-gain and low-gain PHA's, and enables the spectra to be interpreted as frequency distributions in energy deposited in the counter by each event. For any experiment, the gain factors had been so arranged that the high-gain distribution overlapped with the low-gain distribution, and the next subsection will indicate how to meld these two overlapping spectra into a single normalized distribution in lineal energy.

B. Melding

The noise regions of both the high-gain and low-gain data are first discarded. Then for each experiment, the low-gain data are multiplied by a

constant so chosen that both the high-gain data and the resulting low-gain data have equal area in the region of their overlap. They are then combined and recollected into a histogram of constant bin width extending from the lowest energy in the high-gain data to the highest energy in the low-gain data. In this collection, data in the overlap region are taken only from the high-gain data, since they have the higher resolution. This histogram represents a frequency distribution of energy deposited in the counter by an event.

Important contributions from delta rays may have been masked by the noise of the electronics, and therefore may have been discarded together with the noise. As an attempt to recover their contributions, we use the last two bins in the histogram to extrapolate linearly backwards to the minimum ionization energy of 30 eV. Then the histogram, together with this extrapolation, is normalized to unit area to give the $f(y)$ distribution after having changed the variable from energy to lineal energy y . ($y = \text{energy}/(2d/3)$, where d = simulated diameter of counter.) These distributions are shown in Figs. PM-1-1 through PM-1-6.

C. Moments

The distributions of dose in lineal energy density are shown by plotting $y d(y)$ against y in a logarithmic scale, where

$$d(y) = \frac{y f(y)}{\int dy' y' f(y')}$$

is the dose distribution of y . The moments of interest are

$$\bar{y}_F = \int dy y f(y)$$

and

$$\bar{y}_D = \int dy y d(y) .$$

When one considers saturations in biological effects, as discussed by Kellerer and Rossi (11), the relevant quantity to be considered in place of $\bar{y}_D$ is

$$\frac{\bar{y}}{y}^* = \frac{\int dy y_0^2 (1 - e^{-y^2/y_0^2})}{\int dy y f(y)} ,$$

where y_0 is a constant taken to be 125 keV/ μ m.

D. Computer program

The data were analyzed on the PDP-11/45 computer of the Cancer Research Center. A program was written not only to realize the above scheme, but also to plot the spectra $f(y)$ and $yd(y)$ on a Tektronix-4012 graphics terminal. Hard copies of these graphs were made and assembled into Figs. PM-1-1 through PM-1-6.

IV. Discussion

The analysis following the scheme laid down as above is summarized in Tables PM-1-I through PM-1-V, while the computer-generated plots are displayed in Figs. PM-1-1 through PM-1-6. Due to the limited time available up to the preparation of this report, only data for argon ions of 450 MeV per nucleon have been analyzed. Carbon data will be similarly treated in the near future.

As has already been mentioned in the preceding section, electronics noise in the accelerator environment masked away a good deal of the low energy events, thereby introducing considerable uncertainty in recovery of the full spectrum by linear extrapolation. The effect of this is an uncertainty in the low end of the distribution $f(y)$ in lineal energy y .

- Fig. PM-1-1. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A in the plateau region of the Bragg curve. Simulated site diameters are $1\ \mu\text{m}$ and $4\ \mu\text{m}$.
- Fig. PM-1-2. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A in the ascending region of the Bragg curve. Simulated site diameters are $1\ \mu\text{m}$ and $4\ \mu\text{m}$.
- Fig. PM-1-3. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A in the peak region of the Bragg curve. Simulated site diameter is $1\ \mu\text{m}$.
- Fig. PM-1-4. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A in the peak region of the Bragg curve. Simulated site diameters are $0.25\ \mu\text{m}$ and $4\ \mu\text{m}$.
- Fig. PM-1-5. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A in the tail region of the Bragg curve. Simulated site diameters are $1\ \mu\text{m}$ and $4\ \mu\text{m}$.
- Fig. PM-1-6. Microdosimetric distributions $f(y)$ and $y \cdot d(y)$ for ^{40}Ar at ~ 430 MeV/A utilizing a ridge filter. Positions are $1\ \text{cm}$ and $4\ \text{cm}$ upstream of the drop-off. Site diameter is $1\ \mu\text{m}$.

Figure PM-1-1

Figure PM-1-2

ARGON, 1.47 COUNTER, PEAK, SITE DIAMETER 1.00 MICRONS

ARGON, 1.47 COUNTER, PEAK, SITE DIAMETER 1.00 MICRONS

ARGON, 1.0 COUNTER, PEAK, SITE DIAMETER 1.00 MICRONS

ARGON, 1.0 COUNTER, PEAK, SITE DIAMETER 1.00 MICRONS

Figure PM-1-3

Figure PM-1-4

Figure PM-1-5

Figure PM-1-6

Plateau	$\bar{y}_F$ (keV/ μm)	$\bar{y}_D$ (keV/ μm)	$\bar{y}^*$ (keV/ μm)
1.00 μm site diameter 0.25 inch counter	14	82	42
1.00 μm site diameter 1.00 inch counter	31	132	62
4.00 μm site diameter 1.00 inch counter	7	88	48

Table PM-1-I Plateau

Ascending	$\bar{y}_F$ (keV/ μm)	$\bar{y}_D$ (keV/ μm)	$\bar{y}^*$ (keV/ μm)
1.00 μm 0.25 inch	38	242	46
1.00 μm 1.00 inch	29	289	39
4.00 μm 1.00 inch	190	342	49

Table PM-1-II Ascending

Peak	$\bar{y}_F$ (keV/ μm)	$\bar{y}_D$ (keV/ μm)	$\bar{y}^*$ (keV/ μm)
0.25 μm 0.25 inch	90	538	34
1.00 μm 0.25 inch	142	554	34
1.00 μm 1.00 inch	103	529	32
4.00 μm 1.00 inch	146	501	36

Table PM-1-III Peak

Tail	$\bar{y}_F$ (keV/ μm)	$\bar{y}_D$ (keV/ μm)	$\bar{y}^*$ (keV/ μm)
1.00 μm 0.25	40	299	41
1.00 μm 1.00 inch	49	371	42
4.00 μm 1.00 inch	73	330	48

Table PM-1-IV Tail

Ridge Filter	$\bar{y}_F$ (keV/ μm)	$\bar{y}_D$ (keV/ μm)	$\bar{y}^*$ (keV/ μm)
1 cm upstream	12	133	35
4 cm upstream	19	134	43

Table PM-1-V Ridge Filter, 1 μm site simulated by a 1/4-inch counter

Consequently, the resulting values of the mean $\bar{y}_F$ cannot be considered meaningful. However, since $\bar{y}_D$ is essentially the mean of y^2 , uncertainty in the low end of $f(y)$ is expected to cause only a relatively small perturbation. For the same reason, the effect on the mean $\bar{y}^*$ is not expected to be large, although greater than that on $\bar{y}_D$. This has, in fact, been observed by using different extrapolation methods and noting that $\bar{y}_D$ only changes by a few percent.

The results of the analysis will now be briefly discussed.

1. Plateau

Inspection of Fig. PM-1-1 reveals that all three experiments show a strong peak in the $y_d(y)$ plot at about 200 keV/ μm , to be compared with the predicted (12) LET of about 100 keV/ μm for argon ions of 450 MeV per nucleon. The data taken with the 1/4-inch counter also show a strong secondary peak at ~ 20 keV/ μm , indicating a very sizable contribution from delta rays and possibly spallation products. This is of course expected from such high energy argon ions. Unfortunately, this contribution fails to be revealed in the other two measurements, because, as had been mentioned earlier, the large amount of electronics noise made the detection of small energy events impossible.

Despite this, data from the 1-inch counter do hint at the presence of a secondary peak at the same y , but of a smaller magnitude. This behavior is compatible with the wall effect, which amounts to a larger suppression of low energy delta rays for the 1-inch counter relative to that of the 1/4-inch counter. Because of this, $\bar{y}_D$ for the 1-inch counter is boosted up more than that for the 1/4-inch counter, when simulating the same diameter. This wall effect is minimal when the range of delta rays is short compared to the spacing between the counter and the shell containing it. Hence, when the

1-inch counter is used to simulate a $4\text{ }\mu\text{m}$ site, we expect minimum boosting of $\bar{y}_D$, and so the $\bar{y}_D$ here should be smaller than that for the 1/4-inch counter simulating a $1\text{ }\mu\text{m}$ site diameter. It may be mentioned at this point that these inequalities due to wall effects are seen to be substantiated by data taken not only here at the plateau, but also at the tail.

2. Ascending

Fig. PM-1-2 shows a main peak at about $400\text{ keV}/\mu\text{m}$ in all plots of $y_d(y)$. However, measurements taken with the 1/4-inch counter simulating a $1\text{ }\mu\text{m}$ site show a small secondary peak at $100\text{ keV}/\mu\text{m}$. This may be due to the secondary peak at the plateau region which may have moved up towards higher values of y as we moved up from the plateau towards the peak. Nonetheless, it is mysteriously absent in the other two measurements, and this is as yet unexplained. Another point that defies explanation at this time is that the wall-effect inequalities in $\bar{y}_D$'s discussed earlier are not observed in the $4\text{ }\mu\text{m}$ site size.

3. Peak

All plots of $y_d(y)$ are about as closely identical as may be expected. There is only one peak centered at the very high value of $600\text{ keV}/\mu\text{m}$. Since the delta rays in this region have ranges much smaller than the simulated site size, no wall effects are to be expected, and this is experimentally confirmed (Figs. PM-1-3 and PM-1-4).

4. Tail

The presence of spallation products is amply displayed by the gentle tails in the plots of $y_d(y)$ in Fig. PM-1-5, where the main component, namely argon ions, has all disappeared. The wall effects are as expected, while $\bar{y}_D$

has decreased due to the absence of the main component. The low counting rate of the experiment produced large fluctuations in $y_d(y)$, as evidenced in the high y regions of the graphs.

5. Ridge Filter

Data were taken with the 1/4-inch counter simulating a 1 μm site downstream of the ridge filter. A secondary peak of delta rays is evident in the plot of $y_d(y)$ (Fig. PM-1-6) taken at 4 cm upstream of the fall-off point. This is similar to the plateau data. When the counter was placed at 1 cm upstream of the fall-off, the gains were unfortunately improperly set so that the high end of the spectrum above 495 keV/ μm was cut off. This is evident in both the $f(y)$ and $y_d(y)$ plots. Because of this cutoff, the measured $\bar{y}_D$ should have been higher than it is now.

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PM-2 Microdosimetry at the BEVALAC: Future plans

P. Kliauga

Another section of this report, (PM-1, hereafter referred to as I), deals with experiments performed at the BEVALAC with wall-less counters mounted inside of tissue-equivalent plastic shells. This section is a review of progress on preparations for a more complex and detailed series of experiments designed to explore the microdosimetric characteristics of the energetic heavy ions available at the BEVALAC Biomedical Facility.

The basic features of these experiments have been outlined in last year's Annual Report (1, 2). The essence of the proposed experiment is twofold: 1) to measure microdosimetric spectra with a wall-less counter in a container large enough so that wall effects are essentially eliminated, and 2) to measure these spectra as a function of the distance of closest approach of the ion to the counter, i.e., the impact parameter. The first point will resolve the question of wall-effect perturbations on the spectra measured in I, while the second point will yield detailed experimental evidence concerning the relative importance of the delta ray, or "indirect" contribution to the biological effect of these heavy ions. At the same time, it may be possible to explore the contribution of fragmentation products to the total γ -spectra.

The size of the container required to produce an error of less than 5% in the measurement of the ionization produced by a parallel beam of Argon ions of 450 MeV/ μ at a site simulating $\sim 1/4 \mu\text{m}$ diameter has been estimated in Ref. 1. If the container is assumed to be a cylinder with the ^{40}Ar beam parallel to its axis, it was found that a radius of $\sim 30 \text{ cm}$ is sufficient to meet the above specifications. The length of the cylinder is not a sensitive

parameter, provided that the length is ≥ 100 cm.

The BEVALAC shop (through the cooperation of Glenn White and staff) has constructed an experimental tank meeting these specifications. Parts were obtained on the surplus market to reduce costs. The tank consists of two sections of surplus ventilating pipe welded together to form a cylinder 96 inches long. The outside diameter is 24 inches, but, due to the presence of internal flanges, the usable inside diameter is 20.38 inches - still adequate for our purposes. The front end has a thin window of sail cloth and mylar for the beam entrance port, while the rear of the tank will provide O-ring sealed plates containing all required electrical and gas connections.

Instrumentation for the experiment was briefly discussed in Ref. 2. The basic instrumentation required to do the experiment consists of a wall-less counter to obtain microdosimetric spectra and a device to measure the impact parameter of each particle.

A 1/4 inch cylindrical counter has been constructed and tested with x-rays and α particles. A schematic of the counter showing the basic geometry and proportions is shown in Fig PM-2-1. The collecting volume is defined by the volume within the helix and between the ends of the field tubes. This defines a right circular cylinder 1/4 inch in diameter. In order to insure a cylindrically symmetric field throughout the collecting volume, the potentials on helix, anode and field tube must obey certain constraints dictated by the geometry. If the field tube is at ground potential and the anode and helix positive and negative, respectively, relative to the field tube, then a simple calculation based on cylindrical field geometry shows that $V_H = -0.866 V_A$, where V_H is the helix potential and V_A the anode potential.

Fig. PM-2-1. Schematic diagram of cylindrical proportional counter showing basic geometry and dimensions. All dimensions are in inches. Anode wire is 0.001 inch stainless steel, helix wire is .0025 inch stainless steel.

Fig PM-2-2 shows the resolution of the counter to aluminum x-rays as a function of V_H/V_A . The potential difference $V_A - V_H$ was held constant throughout these tests. Two pressures of TE gas were utilized, 35 Torr and 70 Torr, simulating a $1/2 \mu\text{m}$ and $1 \mu\text{m}$ diameter cylinder. In both cases, the minimum in resolution is rather broad, and seems to fall at slightly smaller values of V_H/V_A than the theoretically predicted value, 0.866.

The resolution obtained is not, in general, as good as can be obtained with the TE spherical grid counters, but it is adequate. The main advantage this counter has for the experiment under discussion is its relatively "open" construction, with less material required to define the collecting volume.

An apparatus has been constructed to move a collimated α source parallel and perpendicular to the axis of the cylinder. By mapping the collecting volume limits, it is hoped to determine the optimum pitch of the helix, i.e., the minimum number of turns required to properly delineate the collecting volume.

The other device basic to the successful performance of the experimental program at the BEVALAC is an instrument which can furnish information about the position of a particle which deposits energy in the counter relative to the counter itself. If the particle does not pass through the counter, such energy deposition must be accomplished by means of energetic secondaries, i.e., delta rays.

Although many such devices already exist, e.g., multiwire proportional counters, position sensitive detectors, etc., the constraints of cost and efficiency dictate a simpler system. For this purpose our present plans call for a "drift chamber." The drift chamber consists of a cylindrical

Fig. PM-2-2. Resolution of cylindrical counter to Al K_α x-rays as a function of $-V_h/V_a$, where V_h is helix potential and V_a is anode potential. Two pressures are shown. $V_a - V_h = 750$ volt for 34 Torr, $V_a - V_h = 840$ volt for 68 Torr. Theoretical minimum is at $-V_h/V_a = 0.86$.

parallel plate chamber operated as a counter. The counter wire is parallel to the beam direction, and the "drift time", i.e., the time required for the charge cloud to drift from its origin to the wire is a function of the distance between the location of the original ionization and the collecting wire. If the collecting wire is placed along the axis of the experimental tank, along with the 1/4 inch cylindrical proportional counter, this time is a function of the desired impact parameter.

A prototype of this device has been built (2). The prototype consists of two circular lucite plates separated by 1/2 inch. A series of 18 holes have been drilled through the lucite in a spiral pattern, with adjacent holes separated by 1/4 inch along the radial direction. The holes are covered with thin (.0025 inch) mylar, and serve as entrance ports for an α source and a detector (solid state detector) on the opposite face. By measuring the time interval between the solid state detector pulse, which signals passage of an α particle through the chamber, and the counter pulse, which signals arrival of the charge cloud at the collecting wire, one can determine experimentally the form of the function relating drift time and impact parameter.

It is desirable that this function be linear, or nearly so. As reported earlier (2), preliminary tests indicated (as was expected) that the chosen cylindrical geometry prohibits linearity. In fact, for distances ≥ 2 inches from the center wire, the time response flattens out and the counter signals becomes progressively weaker. These symptoms are due to the poor field characteristics necessitated by the small ratio of length to diameter of the cylindrical collecting volume. Not only does the field drop off rapidly (as $1/r$) but static charges develop over the interior surfaces of the wall, causing excessive field "fringing" and consequent

loss of collection efficiency; hence the loss of signal.

To remedy these characteristics, a series of 18 concentric circular annuli were scribed into the interior surfaces of the lucite plates. These annuli were made conducting by application of "Aquadag" and polishing. The resulting conducting rings were 3/16 inch wide and separated by 1/16 inch non-conducting rings. The conducting rings were connected to a potentiometer - voltage divider circuit so that potentials on the rings could be varied at will. Rings opposite each other on the opposing faces were at the same potential.

For a true cylindrically symmetric field, the first ring, which is 5/8 inch from the center wire, would be at 0.24 times the potential of the center wire. An optimum value for the ring potential can be determined experimentally by observing the intensity and pulse height of pulses from events located $\gtrsim 2$ inches from the center. In general, this value is in good agreement with what one would expect based on the geometry, although in many cases a slightly weaker field is adequate.

Results with this modified form of the drift chamber yielded a nearly linear time dependence for distances $\gtrsim 2$ inches, but strong variations in the counting rate at different positions from the center were noted. These variations were reproducible and not a monotonic function of distance. It was deduced that fluctuations in the surface charge were responsible. In order to minimize the effect of surface charges and field fringing on the collection efficiency, the spacing between the circular faces of the drift chamber was doubled. The original prototype had a 1/2 inch spacing between opposite faces, while the present version uses a one inch spacer.

Some results with this chamber are shown in Fig. PM-2-3. At distances $\gtrsim$

Fig. PM-2-3. Drift time as a function of radial distance for drift chamber at two voltages and pressures. The first position is $\sim 1/2$ inch from center, successive points are $1/4$ inch apart.

one inch from center, the time dependence is reasonably linear with distance. At smaller distances, the dominant $1/r$ field is apparent. The vertical time scale is only approximate, representing a given fraction of full scale on the time-to-amplitude converter. These are, however, sufficiently close to the true drift times. The horizontal scale represents the position of the source from the center wire, the distance between adjacent points being $1/4$ inch, and the first position $\sim 1/2$ inch from center. In all cases a linear voltage distribution was used for the conducting rings. Some deviations from non-linearity have been tried without substantial improvement in signal strength or resolution.

Some conclusions can be drawn from the tests conducted so far: 1) The drift chamber is a useable instrument to perform the measurements for which it has been designed. Time resolution is quite adequate for our purpose, i.e., we should be able to determine impact parameters to within ~ 3 mm without difficulty. This represents an accuracy of $\sim 1\%$ of the full range of impact parameters. 2) A pressure of ~ 100 Torr is the maximum at which the present instrument can be used. This is due to degradation of signal at large distances for high pressures. At 20 Torr, the pulse height varies only by a factor of ~ 3 to 1 over the full scale of the drift chamber, while counting rate varies not at all. At 80 Torr, the amplitude can vary by 70 to 1 and the signal is obscured by noise at the extreme outer positions. It should be noted, however, that no special precautions have been taken with the present set-up to reduce preamplifier noise and this can be reduced by an order of magnitude in the actual set-up. That this effect may be

partly due to surface degassing of the lucite plates was demonstrated by repeating runs after a long period of pump-down (at least a week). Recent experiments conducted at 70 Torr show only ~ 30 to 1 amplitude variation. There is no change in counting rate with position of the source. 3) While operation of the drift chamber prototype is satisfactory, it has yet to be demonstrated for a final design under accelerator conditions; in particular there is the question whether beam intensity can be made low enough so as not to overload this rather large cross-section counter. The actual physical design of an instrument to be used at the BEVALAC also has yet to be developed.

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PM-3 Microdosimetry in the nanometer region

A. Kosiara and H.H. Rossi

Microdosimetry in a region comparable to the dimensions of the DNA (1 nm) is of obvious interest. In this experiment we are using a new approach, with which we hope to avoid difficulties encountered in previous work on this problem in our laboratory (1, 2). The scheme of the system is shown in Fig. PM-3-1. A charged particle from source S passes through the TE gas at pressure of approximately 0.1 Torr (1 nm of tissue is equivalent to 0.043 Torr x cm of propane base TE gas). The creation of the track is signified by a pulse from a solid state counter D1. The track is viewed at right angle by a scintillation detector D2. An orifice in front of this detector selects a segment of the track which is equivalent to 1 nm. Positive ions are extracted from the track by a weak electric field set up within the parallel plate capacitor C, and drift toward the detector D2. No gas multiplication is expected. After passing through the orifice the positive ions will encounter a strong, localized electric field created by a spherical electrode E held at negative potential. They will be accelerated rapidly in a small region pumped on differentially by a diffusion pump. Upon impact on the electrode the positive ions will produce secondary electrons which will be intercepted by a scintillator positioned around the electrode. The light from the scintillator is converted into electrical pulses (energy and timing) by the photomultiplier.

The object of this experiment is to determine the number of electron-ion pairs created in a simulated 1 nm region. The electronics is shown in Fig. PM-3-2. It is based on a digital-to-analog converter ("ion counter") system which generates a discrete pulse proportional to the number of positive ions

Fig. PM-3-1. Scheme of the system. Positive ions are extracted from the track by an electric field set up in the capacitor C. Secondary electrons produced upon impact of those ions on the electrode E are intercepted by the scintillator and the light is converted into electrical pulses by the photomultiplier.

Fig. PM-3-2. Electronics for the determination of the number of electron-ion pairs created by a charged particle.

registered by the photomultiplier within a "gate open" period. A spectrum showing the number of events resulting in production of 0, 1, 2, 3 etc. electron-ion pairs will be then produced by the multichannel analyser. The gate is opened by a signal from the solid state counter. By changing the width and delay of the gate pulse, one will be able to sample regions of variable dimensions located at various points along the viewing axis of the scintillation detector, and investigate ion production by the primary particle and by delta rays. The spatial resolution of the system is determined by the drift velocity of positive ions in the electric field (approx. 10^5 cm/sec) and by the pulse pair resolution of the digital-to-analog converter (100 nsec) and should be better than 1 Angstrom.

At this time most of the hardware and some of the electronics is in place. The single photon grade photomultiplier tube is selected for low dark noise ($< 2 \times 10^{-17}$ W equivalent anode dark current input) and high quantum efficiency. A spectrum of 5.8 keV x-rays following the electron capture in Fe^{55} is shown in Fig. PM-3-3a. The exponentially rising part at low energies is due to cascading low energy x-rays. The noise is shown in Fig. PM-3-3b, and is quite low in the 1 keV region (approx. 1/6 of the peak). We anticipate being able to deposit at least that much of the secondary electron energy into the scintillator. If necessary the noise could be lowered by cooling the photomultiplier.

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Fig. PM-3-3a. Spectrum of 5.8 keV X-rays from Mn^{55} obtained with an NE102 scintillator.

Fig. PM-3-3b. Background from the photomultiplier under the same conditions is approximately 2 cpm at the 1 keV level.

PM-4 Radial energy deposition profile of proton tracks

R. E. Mills

The early stages of the investigation of radial energy distribution about a proton beam were described in last year's Annual Report (COO-3243-5), together with the "ABC" chamber and instrumentation used for the measurements. Several equipment modifications have been effected to improve the accuracy and ease of acquisition of data. An additional external stage of signal amplification has been included to provide gain and remote adjustments of voltage offsets. Conditioning of the signal obtained from an electrometer which samples the proton beam incident upon an aperture upstream of the gas-filled tank is accomplished in the same chassis. Adequate range switching and offset adjustment is provided for the latter. The four voltages thus developed are converted to pulse trains having proportional frequencies, which are then used as inputs for computer scalars. Thus, the entire measurement can be performed under the control of the RARAF PDP-8/e minicomputer.

Due to nonuniformities of the proton beam resulting from differing conditions of steering and focusing, the beam current measured as described above does not have a constant relation to the current traversing the "ABC" chamber. It is, nonetheless, suitable for normalizing chamber ionization currents and spectral count rates measured simultaneously since the relationships of charge collected in the three chamber regions is of primary interest.

Measurement of the tank gas pressure has been improved by

the installation of an MKS Baratron type 221 pressure transducer having an accuracy of 1 percent for pressures up to 10 torr. For this pressure range, a switching regulator has been provided which maintains the gas pressure in the tank at a value determined to within about 0.03 torr of the selected pressure. Excessive gas leakage from the tank into the evacuated beam transport system is obviated by differential pumping between 130 (upstream) and 180 μ m diameter apertures, thereby permitting operation with tank gas pressures as high as about 150 torr.

Some difficulties have been encountered in the course of obtaining data both at high and low gas pressures. At 80 torr scattering of protons by the gas in the tank between the last collimating aperture and the "ABC" chamber results in a gaussian fluence distribution having a size (width at half-maximum) comparable to the diameter of the central chamber region. The effect of gas scattering, which varies as the square root of pressure, is minor for pressures below 30 torr.

Figure PM-4-1 shows ionization current in region B of the chamber, normalized to incident proton beam current, versus collecting voltage for two low values of tank pressure. For either polarity of collecting potential, the ionization collected using low voltages (less than about 5 V) is relatively high. As stated previously, this is assumed to result from the drift of low energy ions into the chamber volume; higher potentials effectively inhibit such drift. Although the curves for positive polarity quickly obtain a steady level, it can be seen that with increasing negative collecting potential, the ionization current collected continues to increase, but at a decreasing rate. This is not characteristic of the behavior which would be expected to result from gas multiplication. Curves for region C are of the same form. Region A, which includes the chamber center, exhibits a decrease in collection with increasing negative collecting

potential, in nearly the amount required to account for the increase in the other regions. The effect thus appears to be a defocusing of ionization using negative polarities, but the cause is not known. Its relative importance decreases with increasing gas pressure, and is insignificant at pressures above 10 torr.

It is intended to derive the radial energy deposition profile from data relating the ionization produced within the central (12.7 mm diameter) cylindrical volume to that in the entire chamber volume. The variation of this central fraction with gas pressure is shown in Figure PM-4-2 for gas pressures below 30 torr, using 3.5 MeV protons. Because a path length of 18 mm in tissue is approximately equal to 12.7 mm in methane-compounded tissue-equivalent gas at a pressure of 1 torr, the abscissa represents site diameters ranging from 0 to about 500 nm. The figure shows that, for example, 90 percent of the ionizing events in tissue occur within a diameter of about 130 nm.

Some recent data have yielded central fractions about 10 to 15 percent lower than those described above. Subsequent investigation using a wide aperture, solid state detector revealed scattered protons (presumably scattered by foreign material at the last collimating aperture before the "ABC" chamber) in sufficient quantity to account for the observed difference. Because the intensity of these scattered protons was less than 0.5 percent of that of the central, unscattered protons, they were difficult to detect. It is possible that some scattering was present even in the data of Figure PM-4-2 resulting in calculated values of central fraction lower than those that would be obtained without scatter.

Fig. PM-4-1. Current versus voltage collection efficiency curves for region B at 2 and 5 torr. The ionization currents are normalized to incident proton beam current.

Fig. PM-4-2. Variation with pressure of the ratio of charge collected within the central volume of the chamber (calculated) to that collected in the entire chamber.

PM-5 Measurement of microdosimetric spectra using an amplifier with logarithmic response

D. Srdoc and L. Rogers*

The fact that the pulse height distribution of a typical microdosimetric measurement extends over three decades creates serious problems when standard electronic equipment is used. The microdosimetric spectra are usually measured in several sections at various amplifier gain settings. The final spectrum is subsequently reconstructed usually by the aid of the computer. However, problems such as accurate calibration of each section of the total spectrum as well as proper matching of neighboring sections have never been satisfactorily solved.

Therefore, an amplifier which can handle a broad distribution of pulse height was considered a necessity.

A 3-1/2 decade logarithmic pulse amplifier was developed for use in microdosimetry. The logarithmic conversions are performed using the exponential characteristics of a semiconductor junction in a circuit configuration described by Radeka (1).

Figure PM-5-1 is a block diagram of the instrument. The input shaping amplifier is designed to accept output pulses from a charge sensitive pre-amplifier with a maximum full scale amplitude of 8 volts. Output of the shaping amplifier is a bipolar, positive lobe leading RC shaped pulse with a maximum linear range $\approx$ 200 millivolts.

Following the logarithmic converter is a voltage amplifier which is used to increase the signal level before it is applied through the linear

*Instrumentation Division, Brookhaven National Laboratory, Upton, New York, 11973.

Fig. PM-5-1. Block diagram of the
3-1/2 decade logarithmic pulse
amplifier.

gate to the output buffer-amplifier. The signal at the output connector is a positive pulse compatible with most conventional pulse height analyzers. The gain of the linear amplifier is adjustable over 10 to 1 range by means of front panel controls.

An internal leading-edge amplifier and associated threshold discriminator are provided in order to generate a strobe pulse for the linear gate.

The fast logarithmic converter which made it possible to develop this 3-1/2 decade amplifier is described in detail in reference 1.

Figures PM-5-2 and 3 show examples of various spectra as measured with the aid of the logarithmic amplifier. Aluminum K_{α} -line calibration spectrum, single electron spectrum, and $P(Y)$ vs Y spectrum for a cylindrical wall-less counter (shown in Fig. PM-5-4) have been measured.

The logarithmic amplifier was developed by L. Rogers and V. Radeka and constructed by D. Stephani and the counter by D. Srdoc. Measurements were made by D. Srdoc.

Reference

1. Radeka, V. "Logarithmic charge amplifier for scintillation detectors." Nucl. Instr. and Meth. 113, 401 (1973).

Fig. PM-5-2. 1) Aluminum K_{α} -line calibration spectrum, $E=1486$ eV, Resolution 32.5%;
2) Single electron spectrum obtained by irradiating a semi-transparent layer of aluminum evaporated on a quartz rod with UV light.

Fig. PM-5-3. $P(E)$ vs E spectrum for a wall-less cylindrical counter, shown in Fig. PM-5-4. Gas: Propane based tissue-equivalent, 100 torr. 1) Arrows on the figure indicate calibration energy.

Fig. PM-5-4. The cylindrical wall-less proportional counter and the source to counter schematic arrangement.

PM-6 Parameters influencing electronic noise in a typical microdosimetric arrangement

D. Srdoc

The ultimate electronic noise of a microdosimetric arrangement consisting of the proportional counter, the counter base and connectors, the preamplifier, and the amplifier depends on several parameters as described below:

1. The counter collecting electrode capacitance is usually very small, considering only the active part of the central wire. However, leads, spring-loaded contacts, and pins, as well as the wiring on the preamplifier board, represent the major source of electronic noise increase as can be seen from Table PM-6-1. The noise expressed as the RMS (in number of electrons) is presented for an IH-700 preamplifier¹ with and without the added capacitance. A spherical 1 inch counter inserted in the counter base and connected to the IH-700 preamplifier will double the RMS (Table PM-6-1, Nos. 1 and 2). The increase of noise is more pronounced in a parallel plate counter. In a narrow gap parallel plate counter consisting of disc-shaped electrodes, 5 cm in diameter, the resulting noise is increased by a factor of 10 or more (Table PM-6-1, No. 3). However, by replacing the standard type 2N4416 FET transistor with a 2N4861 the noise of a large capacitance detector can be reduced. Work is in progress to minimize the counter noise by redesigning the device, the coupling,

¹Developed and made by Instrumentation Division, Brookhaven National Laboratory, Upton, New York, 11973.

Table PM-6-1

Electronic noise measurement under various experimental conditions

<u>No.</u>	<u>FET</u>	<u>RC Shaping (μsec)</u>		<u>Testing Conditions</u>	<u>Noise_</u> <u>RMS e</u>
		<u>Diff.</u>	<u>Int.</u>		
1	2N4416 standard	3.2	3.2	IH 700 preamplifier without added input capacitance	88 _± 6
2	2N4416 standard	3.2	3.2	IH 700 preamplifier with counter base and 1 inch spherical counter connected to the input	186 _± 10
3	2N4416 standard	3.2	3.2	IH 700 preamplifier with counter base and parallel plate counter connected to the input	840 _± 40
4	2N4416 "selected"	1.6	1.6	IH 700 preamplifier without added input capacitance	74 _± 5
5	2N4416 "doubly selected"	1.6	1.6	IH 700 preamplifier without added input capacitance and protective FET	62 _± 4
6	not specified	1.6	1.6	Commercial Tennelec preamplifier model TC 133 without added input capacitance	486 _± 20

and the preamplifier since it is evident that the major contribution to the preamplifier noise is due to the external capacitance connected to the input.

2. The insulating material used in counter construction plays a significant role in the resulting electronic noise as shown by Radeka (1). However, factors other than electric properties of insulators, may be decisive in selecting insulating materials. Teflon, polyethylene, and polystyrene are among the best while bakelite and polyamide (Nylon) are among the worst materials from the standpoint of introducing additional electronic noise.

3. Selecting the FET transistor² will result in significant noise reduction as shown in Table PM-6-1 (Nos. 4 and 5).

4. RC shaping of pulses has an influence on signal to noise ratio and a proper shaping constant will result in additional noise reduction. Figure PM-6-1 shows the influence of shaping constant for two types of FET transistors. The curve representing the RMS vs RC has a broad, flat minimum around 4 μ sec. However, it should be emphasized that the counting rate is limited by the RC shaping and in case of higher counting rates shorter RC constant must be applied, sacrificing the optimal signal to noise ratio.

5. Cooling of FET and feedback resistor, commonly applied in solid state detectors is limited by the nature of proportional counter and tissue-equivalent gases. Whereas a further reduction of noise can be achieved, the system is less practical for routine measurement in microdosimetry.

²The extremely low noise FET transistors have become quite sparse and the author is grateful to Mr. Dmitri Stephani of Brookhaven National Laboratory, Instrumentation Division, for supplying selected FET's.

Fig. PM-6-1. Measurement of electronic noise vs shaping time for two types of FET transistors (2N4416 and 2N4861). Preamplifier: IH 700; Solid lines: no additional input capacitance; Dashed line: standard counter base and 1 inch spherical counter (approximately 5-7 pF).

For comparison, a commercial Tennelec preamplifier, model TC 133, designed for proportional counter use has been also tested (Table PM-6-1, No. 6).

Acknowledgment

The cooperation and advice of Drs. V. Radeka and R. L. Chase and the technical help and skill of Mr. Dmitri Stephani are fully acknowledged and made the development of low noise proportional counters possible.

Reference

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PM-7 The influence of insulators adjacent to the collecting electrode in a proportional counter

D. Srdoc

At least a part of the anode in a proportional counter is surrounded by insulating material--usually at both ends of the central wire. In some cases, such as the spherical anode counter or the specially designed counter shown in Figure PM-7-1, a substantial fraction of anode surface is in contact with insulating material. It was of interest to investigate the influence of insulators on the counter performance. The results of testing and measurements described below and presented in Figures PM-7-2 to -4 show that the presence of insulating material close to the anode brings about transient phenomena, which are difficult to predict and interpret. The general conclusion is that the gas multiplication factor depends on the counting rate, the avalanche size, and the number of pulses per unit anode length per unit time (the latter definition is different from counting rate in the sense that it takes into account the fluence which may vary along the counter wire). Namely, it was observed that transient phenomena in general are much more pronounced when a relatively narrow beam of soft x rays hits a limited section of the anode as opposed to the exposure of the whole counter to a ^{60}Co source. Whereas it is obvious that transients are caused by the charge buildup on the insulator surface, more research is necessary to explain the observed phenomena. Also, it can be concluded that such counters have a limited application caused by inherent instability of gas gain.

Fig. PM-7-1. Schematic drawing of the experimental counter. 1. Anode - 25 μ m stainless steel wire glued with epoxy resin into a groove cut in the lucite support. 2, 3. Gas inlet and outlet. 4. X-ray tube.

A. Experimental

The counter shown schematically in Figure PM-7-1 was connected to a standard gas line, which provided a constant flow of propane at 100 torr. The counter anode was connected to an IH-700 preamplifier followed by a standard pulse height analyzing system. Three types of measurement have been performed, as follows:

1. Measurement of counter resolution

An x-ray tube emitting 1.5 keV Al-K α line photons was used to test the counter resolution. Figure PM-7-2 shows the spectrum obtained. A tail at the low energy side indicates regions of low electric field, probably close to the insulator surface. However, it must be emphasized that the measurement of the spectrum shown in Figure PM-7-2 was possible after a prolonged period required to achieve stability of gas gain. During this period, gas gain has dropped by a factor of 4 to 8 depending on fluence in the irradiated area. It is very likely that the rest of the anode had retained its original high gas gain.

2. Measurement of overall gas gain at low counting rate

The counter shown in Figure PM-7-1 was exposed to a weak ^{226}Ra source, and the counting rate was registered in the multiscaling mode. This method enables one to measure the counting rate as a function of time. Figure PM-7-3 shows the result. Each channel represents the total number of counts in the preset time period (0.8 second). A decrease of counting rate is evident during the first two to three minutes.

3. Measurement of overall gas gain at high counting rate

The same measurement as described in No. 2 was performed using a 50 mCi ^{60}Co source at a distance of ≈ 1 m. An unexpected increase of counting rate was obtained as shown in Figure PM-7-4, which leads to the conclusion that the involved phenomena of charge buildup and leakage are quite complex.

Fig. PM-2-2

Fig. PM-2-3.

Fig. PM-2-4

Figure PM-7-2 Resolution of the counter shown in Figure PM-7-1.
Al- K_{α} line, $E = 1486$ eV.

Figure PM-7-3 Counting rate vs time. Each channel corresponds to a 0.8 second period. Source: ^{226}Ra , ≈ 10 μCi close to the counter. Approximate counting rate: 30 pulses per second.

Figure PM-7-4 Counting rate vs time plot. Each channel corresponds to an 0.8 second period. Source: ^{60}Co , 50 μCi at ≈ 1000 pulses per second.

C) THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY (RARAF)

PA-1 Operation and development of the RARAF Facility

L. J. Goodman, N. Rohrig, and S. A. Marino

A. Introduction

Table PA-1-1 summarizes the use of the Radiological Research Accelerator Facility (RARAF) during the past five years and for the first third of 1977. The scheduled time is one shift per day (8 hours), five days per week. The category of miscellaneous projects includes health physics surveys, safety system inspections, and use of the accelerator by the Brookhaven National Laboratory Department of Applied Science.

<u>Table PA-1-1</u>						
Percent of annual total time scheduled						
<u>Application</u>	<u>1972</u>	<u>1973</u>	<u>1974</u>	<u>1975</u>	<u>1976</u>	<u>1977*</u>
Radiobiology and dosimetry	18	31	39	52	53	60
Radiological physics	28	45	10	13	27	28
Van de Graaff development and maintenance	42	17	12	5	14	2
Target development	5	3	1	2	1	1
<u>Miscellaneous projects</u>	<u>3</u>	<u>8</u>	<u>13</u>	<u>6</u>	<u>4</u>	<u>2</u>
Total time used	96	104	75	78	99	93
Idle time	4	4	25	24	14	9

*January through April only.

The sum of the total time used and the idle time exceeded

100 percent in some years due to the use of RARAF for certain projects during nonscheduled time, i.e., in the evening. The increased time during 1976 used for Van de Graaff development and maintenance was due to employing helium ions for some experiments. Routine use of helium ions requires more vacuum pump maintenance and more frequent changes of the filament in the ion source of the accelerator than is required when only hydrogen or deuterium is used.

The service of RARAF have been utilized during the past year for the following research projects:

Radiobiology

1. Genetic effects of monoenergetic neutrons on Drosophila melanogaster.

2. Induction of malignant cell transformations in cultures of hamster embryo cells irradiated with monoenergetic neutrons.

3. Induction of mammary gland neoplasms in rats irradiated with monoenergetic neutrons.

4. The effects of atomic and molecular hydrogen ion beams and of helium ion beams on mitotic delay, the induction of chromosome aberrations, and the survival of Chinese hamster lung cells in tissue culture.

5. The effects of monoenergetic neutrons on the survival and proliferation of human bone marrow stem cells.

Radiological physics

1. Instrument development and dosimetry studies made in connection with the above radiobiology investigations (Sections PD-1 and PA-2).

2. Studies of the radial energy deposition profile of proton tracks in methane-based tissue-equivalent gas (Section PM-4).

3. Measurements of $\bar{W}$, the average energy expended to create an ion pair, for protons and carbon ions in gases relevant to neutron dosimetry (Section PD-4).

4. Measurements of the thermal defect of A-150 tissue-equivalent plastic (Section PD-3).

Miscellaneous

1. Analysis of trace elements by means of charged particle excitation of x rays (Brookhaven National Laboratory, Department of Applied Science).

2. Development of neutron-producing targets and charged particle beam handling techniques for use in a variety of radiobiological and radiological physics irradiations.

B. Van de Graaff accelerator

Experience with helium beams

Over the past year experience with accelerating helium beams in the Van de Graaff has been gained as a result of some difficulties. As mentioned in the 1975 Annual Report (COO-3243-4, p. 107), striking the arc in the duoplasmatron ion source (Ortec 350, now General Ionex) on helium was not as easy as striking the arc on hydrogen. Some poisoning of the filament may occur from contaminants in the gas. This is not a problem with hydrogen because it is a strong reducing agent. Somewhat higher voltage are required in a helium arc than in a hydrogen arc so breakdown before the arc strikes is more likely. To reduce breakdown probabilities, the filament support was modified to increase the clearance between the intermediate electrode and the filament to about 1.5 mm and a nichrome wire was wrapped around the three support posts to restrain them from moving closer to the wall. In addition the source is operated with a mixture of helium and

hydrogen. This lowers the arc voltage, hopefully keeps the filament clean, and seems to increase the amount of doubly-charged helium coming out of the source. With these modifications of design and practice, the source has operated on helium about ten days in the past four months (hydrogen during remainder of schedule) without any filament changes or other difficulties.

Helium beams have been used during the past year for measurements of $\bar{W}$ and for irradiations of cells. For cell irradiations, the beam energy must be greater than 4 MeV to get through a cell and an intervening Mylar window. To obtain these energies, it is necessary to magnetically select the few doubly-charged helium ions in the beam. The ratio of doubly-charged beam to singly-charged beam is about 2×10^{-4} . Also, a collision of a singly-charged ion in the Van de Graaff column will often create a doubly-charged ion so the doubly-charged beam is not cleanly monoenergetic, but has a continuum of energies. The beam line for the molecular beam has a tight energy acceptance ($\pm 0.2\%$) so the beam current is reduced creating a problem. The beam line for the track segment experiment has a wide open energy acceptance ($\pm 3\%$) so there is adequate beam. However, there are small contaminants of protons or deuterons from ionization of residual gas in the Van de Graaff column when operating with helium-3.

Operations for track segment with helium-4 beams will be discouraged because the helium-4 beams cannot be magnetically separated from deuterons or diatomic protons coming out of the ion source. For one helium-4 experiment, one portion of the dishes (2 to 30 percent of the area) received as much dose from deuterons as from the intended helium-4 beam (3 times the fluence) which is unacceptable. The LET's obtainable with helium-3 and helium-4 ions at a particular velocity are the same. However, since the range for helium-4 ions is 30 percent more than for

helium-3 ions, some sacrifice in uniformity of LET and absorbed dose will result from using helium-3 beams.

C. Neutron-producing targets

During this past year, the two remaining targets of the five received from the Isotopes Division of Oak Ridge National Laboratory early last year were tested and found to have tritium atoms/titanium atom atomic loading ratios of 1.6 to 1.7, comparable to the first three ORNL targets tested. A second batch of ORNL targets received later last year, however, had loading ratios of 1.0.

Three targets received early last year from the Instrumentation Division of Brookhaven National Laboratory were also tested. Two had loading ratios of 0.9 to 1.1. The third target had an acceptable ratio of 1.6.

Reduction of the rate of cooling water flow through target assemblies has not been a problem this year. Most of the targets now in use were fabricated with 0.3 mm instead of 0.25 mm water gaps. This modification seems to have helped to maintain the water flow rate.

D. Future plans

The radiobiology experiments listed in Part A of this Section will be continued, except for the irradiations of hamster embryo cells which have been completed. Utilization of the molecular ion beam for irradiations of cells will continue, and similar irradiations will routinely be made using a simplified arrangement to provide track segments with selectable linear energy transfer. Work on the development of a proton microbeam has been suspended due to the lack of sufficient capital funds required to procure the critical optical components needed to per-

form feasibility studies.

The present radiological physics experiments will be continued. The measurements of $\bar{W}$ will be extended to gases other than methane-based tissue-equivalent gas, and routine measurements of the thermal defect of tissue-equivalent plastic will begin.

PA-2 Development of comprehensive dosimetry apparatus at RARAF

R. E. Mills, R. D. Colvett, and L. J. Goodman

A. Beam current regulator

An instrument to improve the constancy of charged particle beam currents was described in last year's Annual Report (COO-3243-5). Since then features have been added, and the device has been put into service for calorimetry experiments.

For the purpose of centering the swept charged particle beam accurately onto the target aperture, an oscilloscope display has been devised which shows the relationships between the sizes and center positions of the aperture and sweep amplitudes. This is accomplished with the use of the horizontal and vertical sweep driver signals as x and y inputs of an oscilloscope. The interval during which beam current passes through the aperture is sensed by AC amplification and differentiation of the beam current electrometer output signal, and the oscilloscope display is intensified during this interval. The result is a bright spot in the display of the sweep signals, and the relative sizes and positions are apparent.

At relatively high gain in the error amplifier, the overall improvement in the target current stability has been observed to be a factor of about 25, i.e., a 50 percent change in current from the accelerator results typically in a 2 percent change in target current. A difficulty exists, however, with beam intensity fluctuations having characteristic times of the order of 0.1 second. Because of time constants in the regulator

circuit, the sweep modulation is unable to keep up with such deviations and oscillation results. Improvement is expected to result from the judicious readjustment of time constants and gains.

B. Voltmeter/computer interface

To satisfy the need for accurate automatic measurements of voltage or resistance, an interface module has been designed and constructed which allows the RARAF PDP-8/e minicomputer to acquire readings from a Data Precision 3500 digital voltmeter. This is accomplished by loading a string of up/down counter integrated circuits with the binary coded decimal output from the voltmeter and then supplying a train of pulses to the count down input of the counter string. This pulse train is stopped when the count reaches zero; and, therefore, the total number of pulses (counted by one of the computer scalars) equals the reading of the voltmeter. Since the initiation of a read cycle is by computer command, a series of voltage or resistance measurements can be taken automatically with an accuracy of about 0.005 percent.

C. Printed circuit electrometers

It is frequently necessary to measure moderately small currents (μA to 0.1 nA), as for example in the determination of charged particle beam currents in physics experiments. For this purpose several electrometer circuits have been fabricated having effective feedback resistances of 10^7 to 10^{10} ohms. The principal component of each is a junction field effect transistor operational amplifier having a small (about 1 pA) input offset current. Gain and offset voltage trimming are provided, and the entire circuit is assembled on a printed circuit board measuring $5.8 \times 5.1 \text{ cm}$. Five connections are required: input, output, and power (pos., neg., com.). The output is from a fed-back emitter

follower booster and is, therefore, capable of driving long coaxial cables.

D. Computer peripherals

The versatility of the RARAF PDP-8/e minicomputer has been increased by the addition of a Digital Equipment Corporation fast character printer and a Calcomp plotter. All necessary software has been written and both are operational.

BIOPHYSICS

BP-1 Dose rate analysis according to the theory of dual
radiation action

Y-M.P. Lam and H.H. Rossi

BP-2 Analysis for the molecular beam experiment

A.M. Kellerer and Y-M.P. Lam

X BIOPHYSICS

Title

✓ BP-1

Dose rate analysis according to the theory of dual radiation action

Y-M.P. Lam and H.H. Rossi

There are ample indications that sublesions are repaired in time, and hence the number of sublesions produced at a given instant will decrease as time goes on. Then the probability that a sublesion will interact with another produced later will depend on, among other things, whether it will have been repaired or not when the other sublesion is produced. Thus predictions of the dual radiation theory inherently depend on how the sublesions are produced in the course of time, and hence on dose rates.

To put these ideas into better perspective, let $\tau(t)$ denote the probability that a sublesion survives as a viable sublesion at time t after it has been produced. Then according to the well-known principles of dual radiation action theory, the average yield of lesions as a consequence of an irradiation at dose rate $I(t)$ for a period from $t = 0$ to $t = T$ is given by

$$\frac{\epsilon}{k} = \epsilon \int_0^T dt I(t) + \int_0^T dt \int_0^T dt' \tau(|t - t'|) I(t) I(t') \quad (1)$$

where ϵ describes the dose-rate independent intratrack action and the integral describes the dose-rate dependent intertrack action.

It is reasonable to assume that the factor k , which describes the efficiency of production of sublesions, and the probability that two existing sublesions will interact to yield a lesion, is independent on dose rates. Thus by studying how the average yield ϵ of lesions varies as a function of

dose rate $I(t)$, we have the opportunity to test the theory of dual radiation action without a prerequisite knowledge of the microdosimetry quantity ξ , and also to determine which decay function $\tau(t)$ of sublesions are favored experimentally.

In 1974, Yuhas (1) reported an experiment in which ovarian tumors were induced in BALB/c female mice by various doses of ^{137}Cs gamma rays, and at various dose rates. The dose rates are not constant, because the mice were irradiated for a period of 20 hours each day at constant dose rates, followed by a 4-hour rest period. A typical plot of dose rate versus time is shown in Fig. BP-1-1. The number of tumors developed in each group of 200 mice in excess over the control is plotted against the duration of irradiation in Fig. BP-1-2, where those experiments with the same peak dose rate I_0 are joined by lines labelled with that peak dose rate.

Since we do not know the exact relation between biologically observable effects and the yield ϵ of lesions, we will therefore study groups of experiments having the same level of excess tumors. Thus ϵ/k is constant among these experiments. However, Fig. BP-1-2 shows that it is impossible to pick a set of experiments with the same level of excess tumors. To get around this difficulty we will choose a level of excess tumors and then determine a hypothetical experiment for each appropriate period of irradiation by interpolating the dose rate linearly between two neighboring real experiments with that period of irradiation. The interpolated dose rates for five levels of excess tumors chosen to be 1.7, 10.6, 23.4, 51.0, and 83.1, are shown (enclosed in parenthesis) in Table BP-1-1.

The decay function for the purpose of analysis was chosen to be the very reasonable exponentially decaying function

$$\tau(t) = e^{-t/\omega} ,$$

Fig. BP-1-1 A typical distribution of dose rate $I(t)$ in time t .

FAMILIES OF SAME DOSE RATE EXPERIMENTS

Fig. BP-1-2.

COMPARISON OF 28 PREDICTED DOSE RATES (RADS/DAY) WITH INTERPOLATED ONES (IN PARENTHESIS)

EXCESS TUMORS	IRRADIATION PERIOD (DAYS)								
	0.8750	1.7500	3.5000	7.0000	14.0000	28.0000	56.0000	112.0000	224.0000
1.70						2.45 (2.02)	1.49 (1.75)		
10.60				9.55 (7.54)	6.16 (5.77)	3.98 (4.37)	2.52 (2.67)	1.55 (1.75)	
23.40	66.79 (66.81)	37.81 (38.48)	22.51 (23.18)	14.18 (12.84)	9.29 (8.61)	6.12 (6.77)	3.99 (3.91)	2.54 (2.30)	1.56 (1.75)
51.00	102.90 (102.34)	58.41 (62.92)	34.93 (39.45)	22.16 (22.75)	14.69 (13.33)	9.84 (10.25)	6.57 (6.24)	4.32 (3.50)	
83.10		97.94 (93.75)	58.77 (56.00)	37.50 (38.85)	25.08 (26.38)				

Table BP-1-1

where ω is the mean life time of sublesions. If we had chosen the correct values of ω and ξ , then ϵ/k , when computed from Eq.1 for those experiments in the same equal-effect class will be the same. Because of statistical fluctuations, this will seldom be the case. Therefore the best fit to the experiment is obtained when the spread among these ϵ/k 's is minimum.

A convenient way of realizing this is to compute the average $\overline{\epsilon/k}$ of these ϵ/k 's and then to turn Eq.1 around to solve for that predicted dose rate, for each experiment in that class, that would yield $\overline{\epsilon/k}$. In the absence of appropriate information on statistics, χ^2 will now be obtained as usual by comparing the prediction $(\epsilon/k)_{\text{predicted}}$ against the experimental value $(\epsilon/k)_{\text{interpolated}}$:

$$\chi^2 = \sum_{\text{emp}} \frac{[(\epsilon/k)_{\text{predicted}} - (\epsilon/k)_{\text{interpolated}}]^2}{(\epsilon/k)_{\text{interpolated}}} \quad (2)$$

and the best fit is, therefore, obtained by minimizing χ^2 over various values of ξ and ω .

A simultaneous fit to five levels of excess tumors is shown in Table BP-1-1, and plotted against the irradiation period in Fig. BP-1-3, where the interpolated experimental values are shown by the respective symbols. By an act of chance, the fit is unbelievably good. χ^2 is only 2.74 for as many as 28 data points, corresponding to a total number of 21 degrees of freedom. That this analysis favours very strongly the dual radiation theory is unmistakable.

The best fit values are

$$\xi = 5.7 \text{ rads} \quad (3)$$

and

$$\omega = 15 \text{ hours.} \quad (4)$$

COMPARISON WITH 5 LEVELS OF EXCESS CASES

Fig. BP-1-3

To study the confidence region of ω , we fixed ξ at its optimum value of 5.7 rads and plotted χ^2 for various values of ω in Fig. BP-1-4. This curve with a shallow portion to the left of the optimum value of 15 hours shows that values of ω from 1 to 20 hours probably cannot be excluded with confidence.

We have also considered the possibility that sublesions are repaired at more than one rate; that is,

$$\tau(t) = \frac{1}{n} \sum_{i=1}^n e^{-t/\omega_i}, \quad n > 1. \quad (5)$$

However, this seems to be rejected by Yuhas' experiment.

References

1. J.M. Yuhas (1974) Recovery from radiation-carcinogenic injury to the mouse ovary. Radiation Research 60, 321-332.

Fig. BP-1-4 Variation of χ^2 relative to ω in an interval around the best fit values.

Introduction

In an ongoing experiment (1) mammalian cells are exposed to pairs of correlated deuterons resulting from the break-up of accelerated deuteron molecules. The purpose of the experiment is to obtain further information on the question how the microscopic distribution of energy affects the irradiated cell. By varying the average spacing of the correlated deuterons one can obtain patterns of microscopic energy distribution which are characteristically different from those in conventional experiments, where one varies the LET of the radiation. The present study deals first with the microdosimetry of the molecular beam experiment. Secondly it deals with the possible inferences, which can be drawn if the dependence of the cellular effect on the mean spacing of the deuterons is obtained.

Earlier studies (2) have shown that various effects of ionizing radiations on eukaryotes are due to the interaction of sublesions over distances of the order of a micrometer. It has not been possible to identify the nature of the sublesions, although there is strong indication that chromosome breaks and the resulting aberrations play a substantial role in various radiation induced cellular effects. Furthermore it has not been possible in previous experiments to discriminate between models in which the sublesions are formed and can combine within certain geometrically defined sites, or models where sublesions can be formed throughout the nucleus of the cell while their recombination probability depends merely on their distance. It is therefore of particular interest to determine whether the present experiment permits such a discrimination.

A general formulation of dual radiation action has been given earlier (2) and this formulation will be applied to the present case. The main result which will be used is the formula for the linear-quadratic dose dependence of the primary cellular damage:

$$\epsilon(D) = k (\xi D + D^2) \quad (1)$$

It has been found that the quantity ξ depends on three factors. The first factor is the microscopic distribution of energy transfers in the tracks of charged particles. It is described by the so-called distance distribution, $t(x)$, of the energy transfers. The second factor is the geometry of the structures in the cell in which sublesions can occur. The whole domain in which sublesions can occur is termed the site, and the geometry of the site is described by the distance distribution $s(x)$ which is, in large degree, analogous to $t(x)$. The third factor which determines the quantity ξ is the combination probability, $g(x)$, of two sublesions as a function of their distance x . The equation for ξ is:

$$\xi = \frac{\int_0^{\infty} \frac{s(x) g(x) t(x)}{4\pi x^2 \rho} dx}{\int_0^{\infty} s(x) g(x) dx} \quad (2)$$

where ρ is the density of the medium. It is convenient to link the two functions $s(x)$ and $g(x)$ into the combined function $\phi(x)$:

$$\phi(x) = s(x) g(x) \quad \Bigg/ \quad \int_0^{\infty} s(x) g(x) dx \quad (3)$$

The first part of this study will deal with the function $t(x)$ which results for correlated deuterons with different mean spacing. The second

part of the study deals with different possible mechanisms of dual radiation action and with the resulting $\phi(x)$ and ξ .

In the present phase of the experiment the observed end point is cellular survival. The simplified assumption will be made that cellular survival decreases exponentially with primary damage. The resulting equation is:

$$S(D) = e^{-k(\xi D + D^2)} \quad (4)$$

Microdosimetry of correlated deuterons

As a first step the tracks of the deuterons will be treated as infinite straight lines with continuous energy transfer. The fluctuations of energy loss, the radial extension of the tracks, and the change of stopping power, L , of the ions while traversing the nucleus will be disregarded. This has the advantage that one obtains approximate formulae which are valid regardless of the energy and type of the ions.

The influence of energy loss straggling, radial extension of the tracks, and change of LET will be considered later.

It has been shown (3) that the distance, s , between the tracks of the two members of a deuteron molecule after break-up in a mylar foil is a random variable with the distribution:

$$p_b(s) = \frac{2s}{b^2} e^{-s^2/b^2} \quad (5)$$

where b is a parameter which depends on the speed of the deuterons and is proportional to the thickness of the mylar foil. For uncorrelated deuterons, b is infinite.

The integral $T(x)$ of $t(x)$ is the expected energy imparted to a sphere

of radius x centered at a randomly selected energy transfer. With the aforementioned approximations, one can give the function $T(x)$ readily for a pair of ions each with stopping power L , travelling in parallel, and separated by a distance s :

$$T(x) = \begin{cases} 2 L x & \text{for } x \leq s, \\ 2 L (x + \sqrt{x^2 - s^2}) & \text{for } x > s. \end{cases} \quad (6)$$

Accordingly one has:

$$t(x) = \begin{cases} 2 L & \text{for } x \leq s, \\ 2 L (1 + \frac{x}{\sqrt{x^2 - s^2}}) & \text{for } x > s. \end{cases} \quad (7)$$

Integrating over Eq.(5) one obtains the function $t(x)$ for the beam of correlated ions:

$$t_b(x) = 2 L (1 + \frac{2}{b^2} \int_0^x \frac{x \cdot s}{\sqrt{x^2 - s^2}} e^{-s^2/b^2} ds) \quad (8)$$

This function is plotted in Fig. BP-2-1. For small distances $x \ll b$ it is equal to $2 L$, i.e. the contribution of the second ion disappears. For large distances $x \gg b$ the value of $t(x)$ is $4 L$, i.e. both ions make the same contribution.

Computations of the function $T(x)$ for protons or deuterons by Chmelevsky (4) have shown that the function $T(x)$ is not significantly different from $2 L x$ for $x > 50$ nm and for particle energies up to 5 MeV/nucleon. The following discussion will not refer to distances smaller than about 100 nm and to particles of energy larger than about 5 MeV/nucleon. The approximation

$$\frac{t_l}{t_l(x)}$$

which disregards the fine structure of the track will therefore be justified.

One incurs also a certain error by disregarding the change of LET of the ions while they are traversing the nucleus of the cell. Furthermore one would for a precise description have to take into account the increase of the parameter b of the two ions while they are traversing the nucleus.

Nevertheless it may be sufficient to disregard these complexities and to substitute effective mean values for L and b .

The curve in Fig. BP-2-1 can therefore be used as the basis for further considerations. It will be practical to introduce an auxiliary function $\tau(x)$

$$t_b(x) = 2 L \tau(x/b) \quad (9)$$

Argument and functional value of $\tau(x)$ are plotted on the upper abscissa and right ordinate of Fig. BP-2-1

Effective LET

If the deuterons are uncorrelated, $b = \infty$, and correspondingly:

$$\begin{aligned} t_{\infty}(x) &= 2 L \\ \xi_{\infty} &= 2 L \int \frac{\phi(x)}{4\pi x^2} dx \end{aligned} \quad (10)$$

For finite b one has:

$$\begin{aligned} t_b(x) &= 2 L \tau(x/b) \\ \xi_b &= 2 L \int \frac{\phi(x) \tau(x/b)}{4\pi x^2} dx \end{aligned} \quad (11)$$

For a finite b one can therefore define an effective LET:

$$L_b^* = L \int \frac{\phi(x) \tau(x/b)}{4\pi x^2} dx \bigg/ \int \frac{\phi(x)}{4\pi x^2} dx. \quad (12)$$

L_b^* is the LET of those uncorrelated deuterons which have the same inactivation effect as the deuterons whose parameter is b and actual LET L .

The ratio L_b^*/L is obtained in the experiment provided the theory of dual radiation action is valid. Let s_1 be the survival fraction of cells under the action of a given dose D of deuteron pairs whose parameter is b . Let s_2 be the survival fraction of cells under the action of the same dose, but of uncorrelated deuteron with the same LET L . Let s_3 be the survival fraction of cells under the action of the same dose of $^3\text{H}_e$ with twice the LET. Then the dual radiation theory says that their quadratic components are identical, while their linear components are related. In fact, we find

$$s_1 = e^{-k(\xi_b D + D^2)} \quad (13)$$

$$s_2 = e^{-k(\xi_\infty D + D^2)} \quad (14)$$

while

$$s_3 = e^{-k(2\xi_\infty D + D^2)}. \quad (15)$$

Then

$$\frac{\ln s_1/s_2}{\ln s_3/s_2} = \frac{\xi_b}{\xi_\infty} - 1 = \frac{L_b^*}{L} - 1 \quad (16)$$

$$\frac{L_b^*}{L} = 1 + \frac{\ln s_1/s_2}{\ln s_3/s_2} \quad (17)$$

Note that this ratio is independent of the dose D, providing yet another test for the dual radiation theory. Also note that

$$1 < L_b^*/L < \infty \quad (18)$$

Discrimination between different models

A model is chosen by specification (i) of the geometry of the site, and (ii) of the distance interaction function $g(x)$. One can then compute the resulting functions $\phi(x)$ and effective LET. The latter quantity can, for different values of b , be compared to the experimental observations.

These models may be classified into two classes, namely infinite-site models and finite-site models, depending on whether the site is infinite or not.

Finite site models

For these models we will take the site to be a sphere of radius a . Then the choice of the distance interaction function $g(x)$ completes the specification. We will consider two examples:

(a) Choosing $g(x) = 1$

yields what has been referred to consistently in the literature as the site model. We now find that this site model is only one of the many finite-site models. The effective LET of this model is plotted against b in Fig. BP-2-2 for four values of site radius r equal to 0.1 μm , 0.2 μm , 0.5 μm , and 1.0 μm .

(b) $g(x) = e^{-x^2/a^2}$

$$r = 3.5 \mu\text{m}$$

The distance interaction function in this model has a fast fall off for

Fig. BP-2-2 Site model

distances $x > a$, where a is a parameter, and we have chosen the site radius r to be the radius of approximately a real mammalian cell in culture. Thus we call it the realistic model, whose effective LET is shown in Fig. BP-2-3 for various values of the parameter a . It is evident here and also in subsequent discussion that a plays the role of the characteristic interaction distance. A plot of $g(x)$ against x/a is also displayed in the same figure.

Infinite-site models

(a) $g(x) = e^{-x^2/a^2}$ (Fig. BP-2-4)

(b) $g(x)$ is the 1-step function shown in Fig. BP-2-5:

$$g(x) = \begin{cases} 1 & \text{for } 0 < x < a \\ 0 & \text{for } x > a \end{cases}$$

(c) It has been speculated that the distance interaction function may have a strong core surrounded by a long but weak tail. Hence we consider $g(x)$ to be the 2-step function of Fig. BP-2-6:

$$g(x) = \begin{cases} 1 & \text{for } 0 < x < a/10 \\ 0.01 & \text{for } a/10 < x < 10a \\ 0 & \text{for } x > 10a \end{cases}$$

(d) The exponentially decaying distance interaction function of Fig. BP-2-7

$$g(x) = e^{-x/a}$$

is also considered.

Inspecting the plots of effective LET against b , it is observed that

(1) Apart from the finite-site model (b), all models give similarly looking families of curves,

(2) In particular, three models can hardly be distinguished from one another by experiments with the kind of accuracy presently attainable. They are (a), the site model, (b) the infinite-site model with $g(x) = e^{-x^2/a^2}$, and (c) the

Fig. BP-2-3. Realistic model

Fig. BP-2-4. An infinite-site model

Fig. BP-2-5. An infinite-site model

Fig. BP-2-6. An infinite-site model

Fig. BP-2-7. An infinite-site model

realistic model. In particular, it is evident from Figs BP-2-2 through BP-2-4 that a plot of L_b^*/L against b for a value of radius r in the site model is identical, as far as being relevant, to corresponding plots in the other two models with the characteristic interaction distance taking that value of r . Hence the comment: The radius of a site in the site model is equivalent to the characteristic interaction distance in a distance model.

In the future, attempts will be made to analyze the experimental data in this fashion.

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RB-1 OER Measurements at the University of Maryland Cyclotron

E.J. Hall, C.R. Geard, M. Astor and S. Povlas (in collaboration with Dr. George Harrison of the University of Maryland)

Neutron beams currently used for radiotherapy range from 16 MeV deuterons on beryllium at Hammersmith to 67 MeV protons on beryllium at the Fermilab; over this range previous measurements by this laboratory indicate that the oxygen enhancement ratio (OER) is constant at about 1.6 (Hall, 1975a & b; Hall et al., 1974b, 1975, 1977). Recent reports of studies with very high energy cyclotron-produced neutrons at the University of Maryland indicate lower OER's of 1.3 and 1.2 for 80 MeV deuterons and 101 MeV protons on beryllium respectively (Harrison et al., 1975, 1976). The possibility that higher energies may result in lower OER values is obviously of great interest and importance from the point of view of choosing the energy range for additional clinical facilities.

We have recently had the opportunity to make radiobiological measurements on the 80 MeV d^+Be beam at Maryland. Unfortunately the 101 MeV proton beam will not be available in the near future due to technical difficulties with the cyclotron.

The biological test systems chosen are well tried, and have been used to measure OER values over a wide range of neutron energies, including several of the beams currently used for radiotherapy.

First, the growth inhibition of Vicia seedlings. The seedlings were held in a specially constructed holder, fabricated of lucite but with an entrance window of tissue equivalent plastic one inch thick, in which build-up of secondary particles could occur. The holder was gassed vigorously with air or high purity nitrogen during irradiation and for 20 minutes beforehand to render the seedlings well oxygenated or hypoxic. The technique has

previously been described in detail (Hall and Cavanagh, 1967; Hall et al, 1974b). Dose response curves for root growth inhibition under aerated and hypoxic conditions are shown in Figure RB-1-1. The OER is approximately 1.7.

The second biological system used was a line of V79 Chinese hamster cells cultured in vitro. Cells were irradiated while sealed in glass ampules, which were vigorously shaken during irradiation to keep the cells in suspension and prevent them from settling out on the glass surface. Hypoxia was achieved by using a high cell concentration (2×10^6 cells in 1 ml of medium), flushing the air from the ampule with a mixture of 5% CO_2 in nitrogen, and then incubating the sealed ampule for 1 hour at 37.5°C so that residual oxygen was scavenged from the system by cell respiration. A second series of ampules contained 10^4 cells and remained aerated. This technique has also been described in detail (Hall et al., 1974a, 1975). After irradiation, the ampules were opened and aliquots of the cell suspension replated to test for colony formation. Survival curves under aerated and hypoxic conditions are shown in Figure RB-1-2. The OER is approximately 1.7.

The OER values reported here are plotted in Figure RB-1-3, together with other data obtained with the same test systems on other cyclotrons used for radiotherapy. Results from the various machines are summarized in Table RB-1-1. For comparison, the data of Harrison et al. (1976) are also shown in Figure RB-1-3. Our experiments show no indication of decreasing OER with increasing neutron energy.

With pleasure we record the help and cooperation of Dr. George Harrison, Dr. Harry Holgren and the staff of the Maryland Cyclotron in performing these experiments.

Fig. RB-1-1. Dose response curves for growth inhibition of the primary root of *Vicia* seedlings irradiated under aerated and hypoxic conditions with neutrons generated by 80 MeV deuterons on a beryllium target.

Fig. RB-1-2. Survival curves for V79 Chinese hamster cells irradiated under aerated and hypoxic conditions with neutrons generated by 80 MeV deuterons on a beryllium target.

TABLE RB-1-1

OER Measurements with Neutron Beams Produced by the $d^+ \rightarrow Be$ or $p^+ \rightarrow Be$ Process.

Accelerator	Accelerated Particle	Energy (MeV)	OER	
			Vicia	V79 Hamster Cells
Naval Research Lab.	deuteron	16*	1.6	-----
		35	1.6	1.5
TAMVEC	deuteron	50	1.6	-----
Fermilab	proton	67	1.7	1.7
Univ. Maryland	deuteron	80	1.7	1.7

*This energy was obtained at NRL, but intended to simulate the Hammersmith Cyclotron.

Fig. RB-1-3. Oxygen enhancement ratios for various neutron beams produced by bombarding a beryllium target with protons or deuterons. Open circles represent data from Harrison, et. al., 1976. Closed squares represent data reported here, or from Hall, 1975 and Hall, et. al., 1974b and 1977.

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RB-2 Intercomparison of High Energy Cyclotron Produced Beams Used For
Radiotherapy

E. J. Hall and M. Astor

The trial use of neutrons, as an alternative to x or γ -rays for the radiotherapy of human cancer, is actively underway in a number of centers around the world, and is planned or contemplated in many more. Because of the cost of implementing clinical neutron trials, the value of full cooperation between the few centers using these particles has been recognized from the outset. As of September 1976 four centers are actively engaged in treating a substantial number of patients. Several other centers have treated some patients, or plan to do so, but in the present study attention will be focussed on those which have progressed to the stage of a clinical trial. The characteristics of the accelerators used at the four major centers are listed in Table RB-2-1; all four are operated at different energies and consequently produce widely different neutron spectra.

A great deal of effort has been expended by the physicists at the various installations used for neutron therapy to achieve compatible dosimetry, and intercomparisons indicate agreement in the measurement of physical dose to within a few percent. However, compatible dosimetry does not of itself allow the radiotherapist to compare dosage schedules directly, since the varying neutron energies result in a different relative biological effectiveness (RBE) for each neutron beam.

In order to facilitate the pooling of experience and a comparison of clinical results, a number of individuals have undertaken biological intercomparisons using portable model systems. This paper reports the preliminary results. It is likely that many more biological intercomparisons will be necessary as additional treatment centers come into full-scale use, but there

TABLE RB-2-1

CYCLOTRONS INVOLVED IN THE NEUTRON INTERCOMPARISON
(USING THE $d^+ \rightarrow Be$ PROCESS)

FACILITY	LOCATION	DEUTERON ENERGY MeV	DEPTH FOR 50% DOSE CM	BIOLOGICAL INTERCOMPARISONS PERFORMED BY:
HAMMERSMITH	LONDON	16	8	W, H, M
U.W. SEATTLE	SEATTLE	22	10	W, H, M, G
NAVAL RESEARCH LABORATORY	WASHINGTON, D.C.	35	11.6	W, H, M
TAMVEC	COLLEGE STATION, TEXAS	50	15	W, H, M, G

is no justification for performing time-consuming and expensive biological measurements at any facilities until the physical dosimetry is compatible with the existing centers.

Four principal intercomparisons have been performed by various groups of investigators using different biological systems. They are summarized in Table RB-2-2. Withers, Hall and Meyn have performed experiments at all four facilities, while Geraci has used the machine at Seattle (22 MeV) and obtained the three other energies at TAMVEC.

Our own data for the three U.S. cyclotrons and Hammersmith, inter-compared using V79 Chinese hamster cells are shown in Figure RB-2-1. Two series of experiments were performed, one with attached cells and one with cells irradiated in suspension and subsequently replated. The novel feature of this study was that in order to fully exploit the precision of which the in vitro system is capable, cyclotrons were intercompared in pairs, within a given experiment on the same day. For cells in culture variations within an experiment are much smaller than between separate experiments. The NRL cyclotron always served as one arm of the experiment, and this was compared in successive experiments with the machines at College Station, Texas, Seattle, Washington and the Hammersmith Hospital in London. In each case a third arm of the experiment involved ^{60}Co gamma rays. The results of this intercomparison are shown in Figure RB-2-2 in which the RBE's of the various neutron beams are compared with TAMVEC which is arbitrarily assigned a value of unity. The two cell systems yielded slightly different results.

The results of our intercomparisons are compared with those of Withers, Meyn and Geraci in Table RB-2-3 and in Figure RB-2-3. There is a considerable spread in the results obtained with different biological systems, though the RBE scale in Figure RB-2-3 is very expanded. In looking more closely at these

TABLE RB-2-2

NEUTRON INTERCOMPARISONS

<u>PRINCIPAL INVESTIGATOR</u>	<u>BIOLOGICAL SYSTEM</u>
WITHERS	JEJUNAL CRYPT CELLS
HALL	V79 CELLS IN VITRO
MEYN	CHO CELLS IN VITRO
GERACI	MOUSE TESTES, WEIGHT LOSS, AND DNA CONTENT

Fig. RB-2-1. Survival data for V79 Chinese hamster cells irradiated with neutrons at the various facilities used for neutron therapy in the United States and the United Kingdom. Independent inter-comparisons were made with cells irradiated in suspension or attached to plastic culture flasks. Each panel represents data from a self-contained experiment in which cells from a common suspension were irradiated on the same day with neutrons from two cyclotrons and also with ^{60}Co gamma rays.

Fig. RB-2-1

Fig. RB-2-2. The Relative Biological Effectiveness of neutrons generated at Hammersmith, Seattle and NRL compared with TAMVEC, which is taken as 1.0. Based on the hamster cell data in Figure RB-2-1. In the case of Hammersmith the dose quoted is neutrons only; in addition 7% of the dose was due to gamma rays. For the other three machines the dose quoted was total dose (neutron and gamma). The biological test system used was V79 Chinese hamster cells cultured in vitro and irradiated in suspension or attached to polystyrene culture flasks.

TABLE RB-2-3

NEUTRON INTERCOMPARISON FACTORS(TAMVEC TAKEN AS 1.0).

BIOLOGIC SYSTEM	EXPERIMENTER	TAMVEC (50)	NRL (35)	SEATTLE (22)	HAMMERSMITH (16)
JEJUNAL CRYPT	WITHERS 5 FR 1 FR	1.0	1.10	1.16	1.41
		1.0	1.13	1.19	1.44
V79 ATTACHED	HALL	1.0	1.15	1.24	1.35
V79 SUSPENSION	HALL	1.0	1.15	1.29	1.40
CHO	MEYN	1.0	1.06	1.10	1.34 ⁺ (1.25)
MOUSE TESTES WEIGHT LOSS	GERACI	1.0	1.11	1.20	1.38 [*] (1.29)
MOUSE TESTES DNA	GERACI	1.0	1.11	1.15	1.40 [*] (1.31)

* DATA OBTAINED AT 16 MeV AT TAMVEC. INCREASED BY 7% TO ALLOW FOR DOSIMETRY DIFFERENCE.

⁺ DATA OBTAINED AT HAMMERSMITH BY TOTAL DOSE (NEUTRON AND GAMMA) QUOTED; INCREASED BY 7%.

Fig. RB-2-3 Summary of intercomparison measurements of the Relative Biological Effectiveness (RBE) of the various neutron beams used for radiotherapy compared with TAMVEC which is taken as 1.0. In the case of Hammersmith the dose quoted is for neutrons only; the three U.S. machines quote total dose (neutron and gamma). This figure summarizes data presented in this report, and intercomparisons by other groups listed in Table RB-2-2.

Notes.

(a) The data by Geraci for mouse testes were all obtained at TAMVEC by varying the deuteron energy. The RBE at 16 MeV was increased by 7% to allow for the Hammersmith practice of quoting neutron dose only.

(b) The data with CHO cells were obtained by Meyn at Hammersmith, but dosimetry was by the Texas physics team; the RBE at 16 MeV was increased by 7% to bring it into line with the Hammersmith practice of quoting the dose of neutrons only.

data, there are several factors which complicate the issue.

(1) It is the practice of the MRC cyclotron unit at Hammersmith Hospital to quote neutron doses only, while at the U.S. cyclotrons the total dose (neutron and gamma) is used. Withers and Hall performed experiments at Hammersmith using the local dosimetry for which Dr. Bewley is responsible. Dr. Geraci did not, in fact, visit Hammersmith but used a 16 MeV beam at TAMVEC, where the quoted doses include neutrons and gamma rays. Dr. Meyn made measurements at Hammersmith, but the dosimetry was performed by the Texas physicists who accompanied him, and who quoted total dose. In order to make a meaningful comparison, therefore, the RBE values obtained by Geraci and Meyn at 16 MeV have been increased by 7%, to bring them into line with those of Withers and Hall for which the dosimetry considered neutron dose only.

(2) The methods used to calculate the dose received by a biological specimen are different at the various installations. Some prefer to irradiate samples under conditions of minimum scatter, and attempt to measure the dose received in the experimental configuration. Others favor a set-up whereby samples are placed in a phantom of tissue equivalent material; the absorbed dose is then calculated from an ionization chamber measurement, made under conditions of minimum scatter, by the application of a backscatter factor and % depth dose obtained from tables. Consequently, it must be concluded at the present time that, while the calibration of ion chambers is standardized between the various treatment centers, the delivery and specification of dose to a biological specimen (including possibly a patient) is not. The errors are not likely to be large, but they are worth looking into. Closer collaboration is needed between the physics groups to make the biological intercomparisons more meaningful.

With these reservations in mind, it is possible to draw some conclusions from the data in Figure RB-2-3. The measurements by Meyn show consistently smaller variations of RBE between neutron energies than those of the other experimenters. Withers, Geraci and Hall agree reasonably closely at 35 MeV and at 16 MeV. They indicate that NRL neutrons are more effective than TAMVEC by about 12%, while Hammersmith neutrons are more effective than TAMVEC by about 40%. The biggest spread in the data occurs at the Seattle energy (22 MeV). The data of Withers and of Geraci imply a big RBE change between Seattle and Hammersmith and a relatively modest change between NRL and Seattle. This may be somewhat surprising in view of the energies involved. The data of Hall imply a smoother change of RBE with neutron energy. One wonders if these differences are real, and are a consequence of variations between the biological systems used, or whether it is a direct result of the problems of dosimetry eluded to above.

Choice of biological systems for intercomparisons.

RBE is a complex quantity which varies with many physical factors, such as neutron energy, dose, dose-rate, and state of oxygenation. Most important in the context of the present discussion, it varies with the biological system or endpoint used to measure it. In general, the RBE for neutrons compared with x or γ -rays is larger when measured with cells or tissues which show a large capacity to accumulate and repair sub-lethal damage, and smaller when measured with biological systems that show a small capacity to accumulate and repair sub-lethal damage. In practice this means that large RBE values are obtained with systems having a wide shoulder to their dose-response curve, e.g., jejunal crypt cells in the mouse; by contrast small RBE values are obtained for systems with a survival curve exhibiting little if any, shoulder, e.g., colony forming units in the mouse. For in

vitro systems, cell lines can be chosen which mimic either extreme (large or small shoulder) or an intermediate position. For this reason it was proposed by Dr. Jack Fowler, in the summary of part one of this meeting, that a biological system should be chosen for intercomparisons which gives RBE values similar to the "average clinical value" - and the skin reaction system in mice feet was proposed as meeting this criteria.

In fact, now that some data are available, it appears that for inter-comparing two neutron beams which differ in energy by only a modest amount, it matters little which biological system is chosen. Systems and endpoints which result in widely different values for the RBE for neutrons relative to x or γ -rays, give similar values for the difference between two closely separated neutron energies.*

The results are so striking that it is pertinent to enquire into the biophysical basis. To a first approximation, survival data can usually be fitted by an expression of the form:

$$S = e^{-\alpha D - \beta D^2}$$

where S is the fraction of cells surviving a dose D, α is a constant reflecting single event inactivation, while β is a constant relating to inactivation having a quadratic dependence on dose. Over the dose range relevant to the clinical situation (50-1000 rads) α dominates over β for neutrons, while the reverse is true for x or γ -rays. β varies widely for different biological systems and endpoints, much more so than α . Consequently, the RBE for neutrons relative to x-rays differs widely for different systems and endpoints; large RBE values when β is large, and small when β is small. By contrast, when two neutron energies are compared directly, the comparison involves primarily the α components, with the β terms of comparatively

secondary importance. Consequently, RBE differences between neutron energies is similar for different biological systems and the choice of a biological system for intercomparisons rests more on portability, repeatability and convenience than on other factors.

Eric J. Hall, Myles Astor, and Frank Rini (In collaboration with Dr. John Biaglow, Division of Radiation Biology, Case Western Reserve University, Cleveland, Ohio)

The idea that foci of hypoxic cells in some tumors may limit their radiocurability by x-rays was a consequence of the pioneering work of Gray, Read, Thomlinson and their colleagues. In the case of x-rays, the fraction of cells killed by a given dose is strongly dependent on the presence of molecular oxygen; since some tumor cells may be hypoxic they would be spared from the effects of the radiation compared with well oxygenated normal tissues.

Because many of the early radiobiologists had trained initially in physics, they naturally and logically sought a physical solution for this problem, and came up with the suggestion that x-rays should be replaced by a more densely ionizing radiation, such as neutrons, for which the cell killing effect is less dependent on the presence of oxygen. Gray was largely responsible for conceiving the cyclotron to generate neutrons at the Hammersmith Hospital, which has led directly to the worldwide effort to test the efficacy of neutrons and other high LET radiations as a potential alternative for x-rays in radiotherapy.

In more recent years radiation chemists have played an increasingly prominent role in radiation research, and have brought with them a new and different outlook. When faced with the problem of countering hypoxic cells, instead of seeking new types of radiation, they fell back on their training as chemists and sought to overcome the lack of oxygen in tumor cells by using a suitable drug which could act as a substitute for oxygen (Adams and Dewey, 1963). Many compounds of diverse chemical structure have been suggested, but

attention has focused on the nitroimidazoles which exhibit the majority of the properties necessary for a hypoxic cell sensitizer to be effective and acceptable in a clinical situation (Adams and Dewey, 1963; Adams et al, 1976). These drugs mimic oxygen in their radiosensitizing effect, but unlike oxygen they are not rapidly metabolized and consequently can diffuse from the capillaries to foci of hypoxic cells in poorly vascularized regions of a tumor. Attention has focused on the nitroimidazole, known commonly by its code name Ro-07-0582. This drug has now been used extensively in radiobiological experiments, both in vitro and in vivo, and testing has also begun in a few patients.

In the first instance, these hypoxic cell sensitizers were sought and chosen on the basis of their ability to mimic oxygen and differentially sensitize hypoxic cells to the effects of x-rays, without altering the response of the aerated cells. However, in the course of laboratory studies, it has become apparent that nitro derivatives such as the nitroimidazoles "Flagyl" and Ro-07-0582, as well as the nitrofurans nitrofurazone, are cytotoxic toward hypoxic cells. This toxic effect has been demonstrated with cells cultured in vitro (Mohnidra and Rauth, 1976; Hall and Roizin-Towle, 1975) with multicell spheroids (Sutherland, 1974) and also with transplanted tumors in animals (Brown, 1977).

The action of Ro-07-0582 in any in vivo situation is likely to reflect a combination of its radiosensitizing properties coupled with its cytotoxicity. In both regards it selectively acts against hypoxic cells, at least at low concentrations. This means, in general, that the presence of the drug will lead to an increase in the number of cells killed in tumors which contain hypoxic cells.

The present report describes experiments intended to further the understanding of Ro-07-0582 as a sensitizer. In the course of investigating the cytotoxicity of this drug, temperature was found to be an important factor (Hall and Biaglow, 1977; Stratford and Adams, 1977) and the present report describes this effect in greater detail. The mechanism of this cytotoxic effect is by no means clear, and experiments combining Ro-07-0582 with the radical scavenger cysteamine were performed to shed some light on this mechanism.

Materials and Methods

V79 Chinese hamster cells were used throughout these investigations, cultured by standard techniques, and grown in Gibco's F10 medium supplemented with 10% fetal calf serum. Cells received their treatments, with radiation or with hyperthermia, with or without the addition of the nitroimidazoles, while held in suspension in small glass ampules. Cells were made hypoxic by crowding a large number into a small volume, so that oxygen was reduced to a low level by cell metabolism and respiration. This widely used method has been described in detail elsewhere (Hall, Lehnert, and Roizin-Towle, 1974); the essential steps are as follows. Cells from a number of actively growing, partly confluent stock flasks were harvested by trypsinization, washed to remove excess trypsin, counted with a Coulter electronic counter, and prepared into a suspension at a concentration of 2×10^6 cells/ml. At this point the drug, Ro-07-0582, in appropriate amount, was added to the cell suspension to achieve the final concentration dictated by the plan of the experiment. Four drug concentrations were studied, namely 0.5, 1.0, 2.0 and 5 mM. In one series of experiments the effect of combining Ro-07-0582 and the radical scavenger cysteamine was studied, and in this case both drugs, in equal molarities, were added together at this stage.

A series of long-necked 1 ml glass ampules were filled from the cell suspension, flushed with pure nitrogen containing 5% CO₂ to remove the air from the space above the cells, and then heat-sealed. The ampules were then continuously shaken and tumbled to keep the cells in suspension, and the temperature elevated to 37.5°C for one hour to allow the residual oxygen in the medium to be consumed by cell respiration. After dilution by an appropriate factor, a parallel series of ampules were filled with cells at a concentration of 10⁴ cells/ml, and heat sealed. The lower cell density remains aerated for significantly longer periods of time because of the decreased oxygen utilization. After the procedure needed to produce hypoxia, the cell-containing ampules were then subjected to their treatment, with heat or with radiation, according to the plan of the particular experiment. For heat treatments, water baths were used, maintained at 24°C, 37.5°C, 42.5°C and 45°C; in each case the limits of control were $\pm 0.1^\circ\text{C}$. Treatment times varied from 5-25 minutes at the highest temperature, to 3-21 hours at the lowest. For experiments involving irradiation, a cobalt-60 teletherapy unit was used; at a treatment distance of 40 cm, the dose-rate was computed to be 280 rads/min.

At the conclusion of the appropriate treatments, each ampule was vigorously agitated on a vortex mixer, before being opened and various aliquots of the cell suspension replated into tissue culture flasks containing fresh growth medium. After an incubation period of 8 days at 37.5°C, the cells were fixed and stained, and the number of macroscopic colonies counted by a projection technique.

Results

In the interests of internal consistency, it would have been an advantage to compare all four temperatures (24°C, 37.5°C, 42.5°C and 45°C)

together with all four drug concentrations (0.5, 1.0, 2.0 and 5 mM) within one large self-contained experiment. This is not possible for logistic reasons, and so only one drug concentration was used in a given experiment, testing all four temperatures for several different exposure times. The raw data are shown in Figures RB-3-1 to RB-3-4. The proportion of cells killed by a given drug concentration for a given time interval increases greatly at elevated temperatures.

Figure RB-3-5 shows the results of an experiment in which cells were exposed to graded doses of cobalt-60 gamma rays under each of several conditions, namely:

(a) Aerated and hypoxic conditions. The oxygen enhancement ratio (OER), defined to be the ratio of doses under hypoxic and aerated conditions required to produce the same biological effects, is about 3.2 in this experiment.

(b) Hypoxia plus 5 mM "0582". The presence of the drug sensitizes the hypoxic cells to a point where their sensitivity approaches that of aerated cells. The enhancement ratio (defined to be the ratio of doses without and with the drug required to produce the same biological effect) is seen from Figure RB-3-5 to be about 2.5 for a drug concentration of 5 mM. In other words, the drug has mimicked 2.5/3.2 or 80% of the oxygen effect.

(c) Hypoxia plus 5 mM "0582" + 5 mM cysteamine. When the radical scavenger, cysteamine, is present at equal molarity, the radiosensitizing effect of the "0582" is almost completely reversed.

Figure RB-3-6 shows the results of an experiment to test the effect of cysteamine on the cytotoxicity of "0582". Hypoxic cells were held at 37.5°C for various times up to 5 hours with no added drug, with 5 mM "0582", or with 5 mM "0582" and an equal concentration of cysteamine. It is evident from

Fig. RB-3-1. Survival data for cells exposed for various periods of time at four different temperatures to the drug Ro-07-0582 at a concentration of 0.5 mM.

Fig. RB-3-2. Survival data for cells exposed for various periods of time at four different temperatures to the drug Ro-07-0582 at a concentration of 1.0 mM.

Fig. RB-3-3 Survival data for cells exposed for various periods of time at four different temperatures to the drug Ro-07-0582 at a concentration of 2.0 mM.

Fig. RB-3-4. Survival data for cells exposed for various periods of time at four different temperatures to the drug Ro-07-0582 at a concentration of 5 mM.

Fig. RB-3-5. Survival curves for cells irradiated with Cobalt-60 gamma rays under aerated and hypoxic conditions, and in the presence of the drug Ro-07-0582, with or without the addition of cysteamine at the same concentration.

Fig. RB-3-6. Survival data for cells maintained under hypoxic conditions at 37.5°C, and the effect of adding the drug Ro-07-0582 alone or with an equal concentration of cysteamine.

Figure RB-3-6 that the substantial cytotoxic effect of "0582" at this temperature and concentration is almost completely reversed by cysteamine.

Discussion

In a recent paper, Stratford and Adams (1977) reported the effect of hyperthermia on the differential cytotoxicity of Ro-07-0582. For several reasons it is of interest to compare these data with the results presented above.

First, the methods used to produce hypoxia were very different. Stratford and Adams (1977) grew cells in spinner culture in 250 ml flasks, and obtained hypoxia by passing a stream of 95% nitrogen + 5% CO₂ over the surface of the stirred suspension. By contrast, in the present communication hypoxia was induced by cell respiration, achieved by incubating a large number of cells in a small volume of medium sealed in glass ampules. This technique may result in an oxygen concentration which is lower and more repeatable than could be achieved by the method of Stratford and Adams. However, it also results in a nutritional trauma due to the depletion of nutrients, and a lower pH due to the accumulation of waste products. The conditions in the glass ampules which result from producing hypoxia by cell metabolism are sub-optimal from a tissue-culture standpoint, but may represent a good model of the conditions which prevail in the hypoxic regions of a tumor in vivo.

Second, the temperature ranges are different. Stratford and Adams (1977) studied a number of temperatures closely spaced around 37°C, whereas the present paper describes more widely spaced temperatures over a somewhat broader range. Figure RB-3-7 is an attempt to compare the results of the two investigations. Temperature is plotted as a function of the drug concentration necessary to reduce the fraction cells surviving to 0.1 in a treatment time

Fig. RB-3-7. Comparison of the cytotoxic effect observed for Ro-07-0582 in the present paper, with the report by Stratford and Adams (1977) from the Gray Laboratory. The drug concentration is plotted as a function of temperature required to achieve a given biological effect - chosen to be a surviving fraction of 0.1 for a treatment time of 200 minutes.

of 200 minutes. It is obvious that the data do not differ by very much, in spite of the substantial differences in experimental technique, most noticeably the methods used to induce hypoxia.

The fact that cysteamine reverses both the cytotoxic and radiosensitizing properties of "0582" suggests a common mechanism. Cysteamine is a well known radical scavenger which protects against radiation damage in the presence of oxygen. Its protective effects against "0582" and also against other electron affinic drugs (Chapman et al, 1973) is primarily due to its ability to increase the pool of radical reducing species within cells resulting in enhanced repair of free-radical damage in the targets. Both "0582" and oxygen may alter this target damage resulting in cell death.

Cysteamine may protect against the cytotoxic effect of nitro radicals or other nitro intermediates by reacting with them before they in turn can react with critical molecules within the cell. It has recently been suggested that nitro radicals or nitroso intermediates may be the cytotoxic agents (Willson, et al, 1974, 1975; Hall and Biaglow, 1977). From these preliminary experiments, it would appear that a combination of electron affinic drugs and a modest level of local hyperthermia, induced possibly by ultrasonics or microwaves, represents a most effective method to eliminate hypoxic cells, and it would merit extensive investigation with an in vivo model tumor system.

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RB-4 Bleomycin and Ro-07-0582: Studies with aerated and hypoxic cells

Laurie Roizin-Towle

Bleomycin is a chemotherapeutic drug used primarily in the treatment of squamous cell carcinoma, while Ro-07-0582 is an effective radiosensitizer and potent cytotoxic agent selectively affecting hypoxic cells (Hall and Roizin-Towle, 1976). Since the discovery of Bleomycin (Umezawa et al) in 1966, a great deal of knowledge has accumulated concerning its mode of action "in vitro" as well as in "in vivo", as a result of which it has become part of several combination chemotherapy regimes (Pouillart et al, 1976; Carter et al, 1976). Although it is considered to be cell cycle-phase non-specific, a term which is rather misleading, cells in M and G₂ are more sensitive to the drug.

Chemotherapeutic drugs exert their major toxic and antitumor effects by killing cells which undergo DNA synthesis at sometime in their life cycle. Because they are effective against actively dividing cells, but do not discriminate between normal and neoplastic tissue, it is inevitable that in the course of depopulating the tumor, they also cause a number of serious side effects resulting from damage to self renewal tissues (Blum et al, 1973). The overall aim of a successful chemotherapy regimen is to choreograph the use of various drugs in such a way that when one drug has completed its course of action, another is introduced to continue where the former left off. The majority of antineoplastic drugs kill actively dividing cells but are particularly ineffective against cells in solid tumors which are remote from the vascular supply, deficient in molecular

oxygen, and unlikely to be in active cell cycle. The nitroimidazole Ro-07-0582, which selectively affects hypoxic cells, could be very useful in a situation like this. Since Ro-07-0582 is selectively cytotoxic towards hypoxic cells, it would spare further damage to those tissues already treated with other cancer drugs. This is of utmost importance as it would not contribute to an increase of toxicity associated with damage to renewal tissues such as blood, intestinal mucosa and skin, or loss of the immune response due to bone marrow suppression. The experiments presently reported were designed to investigate the effects of Bleomycin on aerated and hypoxic cells at various temperatures, and also the combined effects of Bleomycin and Ro-07-0582 on these cells.

Materials and Methods

V79 cells were used for this investigation. The cells, contained in glass ampoules, were treated with Bleomycin at 17°C, 37.5°C, or 42.5°C. Another series of experiments were performed with Bleomycin and Ro-07-0582 in combination at 37.5°C.

Cells were treated at low concentration (10^4 cells/ml) in which case they were aerated, or high concentration (2×10^6 cells/ml) in which case they were hypoxic. The details of the method for rendering cells hypoxic with this technique have been described previously (Hall, Lehnert, Roizin-Towle, 1973).

Results

Effect of Bleomycin on aerated and hypoxic cells at three different temperatures

Figure RB-4-1 shows the results of a four-hour treatment of aerated

Fig. RB-4-1. Dose response curves for V-79 cells treated with Bleomycin for 4 hours at 37.5°C. At this temperature aerated cells are significantly more sensitive to BLM than are hypoxic cells. The most likely explanation why aerated cells are more sensitive than hypoxic cells is that they move through the cell cycle into phases where BLM is most effective.

4 HOURS TREATMENT AT 37.5°C

and hypoxic cells at 37.5°C. At this temperature, aerated cells are significantly more sensitive to BLM. At drug concentration of 5 or 10 µg/ml, the fraction of cells surviving a 4 hour treatment is lower under aerated than hypoxic conditions by a factor of three; this factor increases to 10 for a higher drug concentration of 250 µg/ml. Figure RB-4-2 shows the results of similar experiments performed at 17.5°C for 4 hours. In this case the sensitivities of aerated and hypoxic cells are reversed, with hypoxic cells being most sensitive to BLM. At 42.5°C, the hypoxic cells are again more sensitive than aerated cells as can be seen in Figure RB-4-3. The cytotoxicity of BLM is so enhanced at 42.5°C, that the treatment time was restricted to one hour and the maximum drug concentration that could be used was 25 µg/ml.

The results of experiments involving a combination of BLM and the nitroimidazole Ro-07-0582 are shown in Figure RB-4-4. In these experiments, both aerated and hypoxic cells received a four hour treatment at 37.5°C with four concentrations of BLM, with or without the addition of the nitroimidazole at a concentration of 5 mM. The nitroimidazole had no effect on aerated cells, but the surviving fraction of hypoxic cells treated with BLM was dramatically reduced when simultaneously treated with Ro-07-0582 at a concentration of 5 mM.

Discussion

Although there is a vast literature concerning the action of BLM, there appear to be no specific studies designed to investigate its effect on hypoxic cells. At 37.5°C, hypoxic cells appear to be less sensitive

4 HOURS TREATMENT AT 17.5°C

RB-4-3. Effects on V-79 cells of graded doses of BLM in combination with hyperthermia (42.5°C) for 1 hr. Under aerated and hypoxic conditions BLM exhibits a synergistic effect with hyperthermia; this reduced the treatment time to 1 hr. and the maximum drug concentration that could be used to 25 µg/ml.

Fig. RB-4-4. Effects of BLM on the survival of V-79 hamster cells treated for 4 hrs. at 37.5°C under aerated and hypoxic conditions. The addition of Ro-07-0582 at a concentration of 5mM reduces the survival of hypoxic cells treated with BLM, but has no effect on aerated cells.

4 HOURS TREATMENT AT 37.5°C

to BLM than aerated cells (Figure RB-4-1). The most likely explanation for this difference is that aerated cells at 37.5°C continue to progress through the cell cycle and can enter a more drug sensitive phase. Hypoxic cells, on the other hand, are unable to progress rapidly through the mitotic cycle. Evidence that this explanation is correct comes from the experiments at 17.5°C or 42.5°C. In either case, cell progression during the treatment is virtually eliminated. At the lower temperature (17.5°C) cells do not move through the cycle. At the higher temperature (42.5°C), the treatment time was so short (1 hour) that little movement through the cycle could occur. In either case, with cell progression eliminated, hypoxic cells are then more sensitive than aerated cells to killing by BLM.

At 42.5°C there is a remarkable increase in cytotoxicity. Hyperthermia alone kills very few cells at this temperature and treatment time (Harasiadis et al, 1975), but it greatly enhances the cytotoxicity of BLM. Not only is there a synergistic effect with hyperthermia at elevated temperatures, but a fixation of potentially lethal damage by BLM for cells treated for one hour or more at 43°C (Braun et al, 1975).

In an attempt to further reduce hypoxic cell survival at 37.5°C, Ro-07-0582 was used in combination with BLM. Although this nitroimidazole was the fruition of an intensive search for an hypoxic cell radiosensitizer, it has since been shown to be a cytotoxic agent as well - specifically with regard to hypoxic cells. When hypoxic cells treated with BLM were simultaneously treated with 5 mM Ro-07-0582 (Figure RB-4-4) their level of survival was reduced far below that of aerated cells. This could considerably augment chances for a remission or cure when treating solid

containing hypoxic cells.

The suggestion has been made more than once that Ro-07-0582 would be a logical drug for inclusion in a chemotherapy protocol. Figure RB-4-4 clearly demonstrated its enhancement of hypoxic cell killing with the widely used drug, BLM. The fact that it is cell cycle phase non-specific, and selectively cytotoxic towards hypoxic cells, means that it is effectively complementary in its action to drugs such as BLM.

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RB-5 X-radiation induced transformation *in vitro* in the C3H/10T 1/2 CL8 cell line

R.C. Miller

In vitro methods of inducing oncogenic transformations with x-radiation were first reported by Borek and her colleagues using fresh explants of hamster embryo cells and more recently, Terzaghi and Little of Harvard University (1) have used a cell line to obtain a dose-response relationship for transformation due to single and split doses of x-rays. The mechanisms of morphological transformation can be studied by eliminating immunological and hormonal variations following treatment with carcinogenic agents. Isolation of the carcinogenic action of radiation by in vitro transformation has provided a sensitive indicator of the carcinogenicity of X-radiation. Single and split dose experiments using in vitro transformations initially performed by Terzaghi and Little have been followed up in this laboratory. The C3H mouse embryo cell line (10T 1/2, clone 8) used in this laboratory was generously provided by John B. Little (Dept. of Physiology, Harvard University School of Public Health, Boston, Mass.) after permission was received from Charles Heidelberger who developed the cell line.

Cell culture method

The C3H/10T 1/2 cell line is of fibroblastic origin and was observed to be highly sensitive to postconfluence inhibition of cell division. Cells used for experiments were between passages 13 and 15. Cells were grown in Eagle's basal medium (BME) supplemented with 10% heat-inactivated fetal calf serum. Penicillin (50 units/ml) and streptomycin (50 μ m/ml) were added to

control contamination. Cells involved in each experiment were grown in 100 mm Falcon plastic Petri dishes. Experimental dishes were x-irradiated with a 300 kVp Siemens x-ray unit 18 hr after plating cells at a density such that about 400 viable cells per dish survived the various experimental treatments. Medium was changed once a week until the termination of the experiment at 5 weeks after cell plating.

Transformation evaluation

Transformed foci were evaluated using the same morphological criteria outlined by Reznikoff et al (2, 3). Three types of morphologically differing foci were observed in irradiated cell cultures. Type 1 foci appeared as groups of tightly packed cells with no other special characteristics. Type 2 foci had extensive piling up of cells and a moderate criss-crossing of fibroblast cells. Neither types 1 nor 2 were scored as transformed foci. Type 3 foci were very densely piled up stellate cells with pronounced criss-crossing and swirling of cells at the border of the clone (Fig. RB-5-1). Type 3 foci were scored as transformants. In previous reports (1, 3), type 3 foci were shown to produce tumors in 80 to 100% of the specially prepared mice. No spontaneous in vitro transformations were observed in untreated controls in any of the experiments reported, indicating that the spontaneous transformation frequency was very low.

Results

Single dose response

A dose response curve for survival is presented in Figure RB-5-2. Survival, as a function of doses above a single dose of 200 rads, decreases exponentially. At doses between 0-200 rads survival of cells decreased only slightly with increasing dose. The D_0 of the survival curve is 190 rads and

RB-5-1. Photomicrograph of a type III transformed clone of the C3H/10T 1/2 cell line.

RB-5-2. Survival curve for the C3H/10T 1/2 cell line exposed to various doses of x-rays.

the extrapolation number is 1.3. In Figure RB-5-3, data for transformation frequency is shown. The transformation frequency increases rapidly from 10 rads to about 400 rads and then reaches a plateau from above 600 rads to 1000 rads.

Split dose response

The results of a split dose experiment to determine frequency of transformation from a total dose of 30 rads up to 800 rads is shown in Figure RB-5-4. The data follows the results reported by Terzaghi and Little (4) for total doses between 150 rads and 800 rads.

Discussion

The C3H/10T 1/2 cell line presents a unique opportunity to study the in vitro carcinogenic effects of ionizing radiation. In addition to the well established cell survival technique as a means of measuring radiation damage, radiobiologists can now accurately determine the oncogenic character of radiation due to sublethal damage. Experiments originally done by Terzaghi and Little (1) were repeated. Results consistent with those reported by Terzaghi and Little (1) were obtained for dose-response survival curves and for the frequency of transformants as a function of dose. Terzaghi and Little (4, 5) reported a sparing effect when a split dose of x-rays was given as compared to a single dose in the dose range of 150 to 800 rads. Borek and Hall (6), however, found that split doses of 50 and 75 rad enhanced the transformation frequency over single doses. They interpreted their results as the consequence of fewer lethal injuries due to split dose thus the cells surviving sublethal damage can express a transformed state. Experiments are now being done to determine the effects of split doses in the low dose range.

RB-5-3. Transformation frequency of cells induced by x-radiation.

RB-5-4. X-radiation induced transformation frequency given either as a single dose or two equal fractions split 5 hrs. apart.

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R.C. Miller and E.J. Hall

The long-term effort to identify a drug that would selectively sensitize the hypoxic cells of solid tumors has met with success in recent years. First metronidazole, and later another nitroimidazole, Ro-07-0582, have been found to selectively sensitize hypoxic cells to x-rays, while having no effect on aerated cells. Concomittant with its sensitizing effects this drug was also shown to have a selective toxicity to hypoxic cells. Hypoxic V-79 hamster cells exposed for up to 20 hours in the concentration range from 0.05 to 5 mM of "0582", showed extensive cell killing, while no significant reduction of cell survival was detected in aerated cells.

Ro-07-0582 is now being used in clinical trials in Great Britain and Canada. With the increased probability of long term patient survival after the combination radiation and chemotherapy, it is increasingly important to examine the long term effects of this recently introduced drug. Specifically, the possible carcinogenicity of the various chemotherapeutic agents must be determined, so the oncologist can weigh the risks and benefits of various types of therapy. To this end, investigators have reported *in vitro* malignant transformation of a mouse fibroblast cell line designated as C3H/10T 1/2 clone 8 when exposed to chemical carcinogens (1, 2) and chemotherapeutic agents (3-5).

In the present report, the C3H/10T 1/2 cell line was used to study the effects of exposing aerated cells to the hypoxic sensitizer Ro-07-0582 using concentrations ranging from 0.4 mM to 8.0 mM and exposure times of 3 or 6 days. It is expected that this system will present us with an estimate of the carci-

nogenic risk to normal aerated tissue of this chemotherapeutic drug.

Cell culture method

The C3H/10T 1/2 cell line used was developed in the laboratory of Charles Heidelberger (McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisc.). Cells used in these experiments were between passages 13 and 15. Cells were maintained as described in the previous section. Experimental dishes were seeded with cells approximately 24 hrs before administration of "0582". Cell density was such that about 400 viable cells per dish survived the drug treatment. At 3 days or 6 days, the medium containing "0582" was replaced with fresh medium containing no drug. Medium was then changed weekly until the termination of the experiment.

Results

Growth curve of aerated cells in the presence of Ro-07-0582

Increasing concentrations of "0582" between 0.1 mM and 2mM administered continuously resulted in a decreasing growth rate as seen in Figure RB-6-1. A 5.0 mM concentration of the drug completely inhibited cell growth. When medium containing no drug was supplied to the cells, cell growth continued after a lag time of 24 hrs (Figure RB-6-1). Table RB-6-1 shows that in addition to inhibition of cell growth, cell killing as measured by cell survival techniques, also occurs when "0582" is left on aerated cells either 3 or 6 days. Inhibition of cell growth of aerated cells in vitro in "0582" for an extended period of time was also noted by Stratford and Adams 1977 (6).

Induction of transformations with Ro-07-0582

Concentrations of "0582" ranging from .4 mM to 8.0 mM were added to the cells for either 3 or 6 days to determine the carcinogenicity of this

RB-6-1. Growth curve of C3H/10T 1/2 cells exposed to various concentrations of Ro-07-0582.

Table RB-6-1. Survival of Cells in Various Concentrations of Ro-07-0582
Exposed for either 3d or 6d

<u>Concentration of "0582" (in mM)</u>	<u>Surviving Fraction after 3d or 6d exposure to "0582"</u>	
	<u>3d</u>	<u>6d</u>
.4	.68	.65
.5	.93	.78
1.0	.58	.42
2.0	.28	.31
5.0	.014	--
8.0	.008	--

Table RB-6-2. Transformation Frequency as a Function of Ro-07-0582
Concentration Exposed for either 3d or 6d

<u>Concentration of "0582" (in mM)</u>	<u>Transformation per surviving cell ($\times 10^{-4}$) after 3d or 6d exposure to "0582"</u>	
	<u>3d</u>	<u>6d</u>
.4	3.85	3.73
.5	3.30	--
1.0	2.51	--
2.0	2.40	4.26
5.0	17.0	--
8.0	10.0	--

drug; Table RB-6-2 summarizes the results. Figure RB-6-2 graphically displays the frequency of transformants per surviving cell as a function of drug concentration. The frequency of transformants induced by 2 mM 0582 is equivalent to that caused by 200 rads of x-rays.

Discussion

It has been demonstrated that the electron affinic drug Ro-07-0582, which is an excellent hypoxic cell radiosensitizer as well as being selectively cytotoxic to hypoxic cells, has the potential to induce oncogenic transformations in aerated cells. Concentrations of the drug ranging from 0.4 mM to 8 mM were capable of producing detectable transformed foci. When used clinically as a sensitizer, serum levels of 0.5 to 1 mM can be reached by 5 hours after oral administration, and this level decays with half life of about 15 hours. It is important for the clinician to realize the potential hazard of this drug in long-term survivors of radiotherapy.

RB-6-2. Ro-07-0582-induced transformation frequency plotted as a function of drug concentration and exposure time. The square symbols represent results from a 6 day exposure and the circles represent an exposure time of 3 days.

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RB-7 Biophysical studies with high energy argon ions: Chromosomal
aberrations in aerated and hypoxic Vicia faba root meristems

C.R. Geard

The traversals of energetic ions through tissue are characterized by dramatic changes in energy deposition as the particles near the end of their range. Hence, the same absorbed dose to cells from charged particles around the mid point of their range and at the Bragg peak near the end of their range is delivered by quite different numbers of traversals per cell. The observation of induced alterations of normal chromosome morphology enables the assessment of radiation effects at a sub-cellular level on a per-cell basis. Since alterations to the genetic material readily lead to loss of cell reproductive integrity and to mutations, the study of chromosomal aberrations fulfills a dual role in that they are capable of indicating radiation effects at both a sub-cellular and supra-cellular level.

The Lawrence Berkeley Laboratory BEVALAC is the only machine capable of producing heavy ions with sufficient energy to penetrate tissue to depths greater than a few millimeters. It consists of the 6 GeV Bevatron ring with heavy ion injectors. The Super-Hilac injector can deliver heavy ions up to argon with energies to 10 MeV/nucleon to the Bevatron. In the Bevatron main ring, they can then be accelerated to energies of 2 GeV per nucleon.

As part of the radiobiological assessment of a high energy argon beam (Annual Report 1976) this study presents data on the effects of argon ions on chromosomes of the broad bean Vicia faba, an organism long favored for radiobiological investigations. Roots were irradiated under aerated and hypoxic conditions since it was expected that the densely ionizing tracks of these ions, particularly at the Bragg peak, could overcome the radioprotective effect of a lack of oxygen.

Material and Methods

Selected seeds of Vicia faba (var. Prolific Long Pod) were soaked in running water for 48 hours. Fully imbibed seeds with protruding radicals were then sown in moist vermiculite for a period of 5 days. Roots selected for irradiation were between 5 and 10.5 cm in length.

Roots were placed in specially constructed lucite fixtures capable of being accurately positioned in the phantom of tissue equivalent liquid and of being gassed at all times.

Hypoxia in the root meristems was induced by bubbling pure nitrogen (<10 ppm oxygen) through the roots for at least 20 minutes before, and during irradiation. The longest time roots were maintained in hypoxic conditions was 55 mins. Aerated roots were bubbled with air throughout.

Four doses were given at each of two positions in the depth dose curve (Fig. RB-7-1), in the entrance plateau, and as close as possible to the Bragg peak. For irradiation at the peak, root tips were constrained in a volume 3.5 cms x 3.5 cms x 0.23 cms; the depth restriction being necessary in order to place the meristems as much as possible in the Bragg peak region. The mid-point of the roots was actually positioned at the Bragg peak and hence part of each meristem was in the rapidly declining region at the back of the peak. For irradiation on the plateau such constraints were unnecessary. Absorbed doses and dose rates are given in Table RB-7-I. Dose rates were averaged over the total exposure time, with the lowest being due to small scale beam loss during irradiation. Hence delivered dose rates were more uniform than indicated and differences in response for these high energy ions are unlikely to be affected by such dose rate variation in the overall range. Since particles were delivered in pulses, closer correspondence of doses between experimental regimes was limited (see Table RB-7-I).

Fig. RB-7-1 Depth dose profile for 429 MeV/AMU argon ions generated at the Bevalac. The positioning and relative depth of the Vicia faba root meristem is shown.

Table RB-7-I

Absorbed doses (rad) and averaged dose rates (rad/min.) delivered to Vicia faba meristems by 429 MeV/AMU argon ions

	Dose	Rate	Dose	Rate	Dose	Rate	Dose	Rate
Bragg peak - aeration	9.5	4.5	20.3	9.9	44.0	19.6	88.6	33.3
Bragg peak - hypoxia	10.8	2.2	21.8	1.7	48.2	6.2	80.8	8.6
Plateau - aeration	9.9	3.9	19.6	4.2	39.4	4.5	77.5	3.8
Plateau - hypoxia	9.6	3.6	20.4	8.5	40.2	1.8	78.5	4.3

Following irradiation, all roots were placed in one container of aerated culture solution at room temperature (about 23°C). Two roots were sampled at two or three hourly intervals to 12 hr. post-irradiation at the peak, and to 10 hr. post-irradiation on the plateau. Prior to root tip fixation in cold freshly prepared fixative (1 part glacial acetic acid:3 parts ethanol), metaphase cells were accumulated with 0.05% colchicine for 2 or 3 hours. Colchicine prevents formation of the anaphase spindle and hence cells accumulate in metaphase. Thereby there were five and four sequential accumulation periods to 12 and 10 hours respectively after irradiation at the Bragg peak and on the plateau for both aerated and hypoxic conditions.

After 24 hours fixation, fixative was replaced with 70% ethanol followed by refrigeration then transport to New York where Feulgen stained root tip squashes were prepared, one root tip per slide. All chromosomal alterations and the relationship between them were recorded for 50 metaphase cells from each root tip. The number of mitotic cells in samples of 600 cells from each root (3 randomly selected groups of 200 cells) provided mitotic indices for each dose-accumulation period. After the 2 or 3 hour colchicine accumulation period, no anaphases were seen although a few telophases were noted in some irradiated roots.

Results

Mitotic index determinations

In general there was a dose dependent decline in the proportion of cells in mitosis after irradiation. This was most evident in roots from the middle accumulation periods (2-8 hr.) with a recovery in the later periods. The overall means and their standard errors are given in Table RB-7-II for up to 12 and 10 hours post irradiation at the peak and on the plateau respectively.

Table RB-7-II

Mean mitotic indices for Vicia faba meristems following argon ion irradiation (standard error in brackets)

	Approximate absorbed dose (rad)*				
	0	10	20	40	80
Bragg peak - aeration 0 - 12 hr.	4.74 (0.49)	4.48 (0.19)	4.26 (0.63)	4.00 (0.48)	2.96 (0.44)
Bragg peak - hypoxia 0 - 12 hr.	3.90 (0.43)	3.68 (0.90)	3.46 (0.40)	3.38 (0.68)	1.60 (0.26)
Plateau - aeration 0 - 10 hr.	3.20 (0.07)	3.78 (0.83)	4.28 (0.58)	2.93 (0.71)	2.38 (0.55)
Plateau - hypoxia 0 - 10 hr.	4.40 (1.02)	2.60 (0.38)	4.23 (0.16)	3.35 (1.03)	3.35 (0.31)

*Actual doses are given in Table RB-7-I

Rendering the meristems hypoxic had little influence on cellular progression into mitosis although hypoxic control roots were more varied than the aerated group. Therefore, while some radiation induced mitotic delay is evidenced, this did not prevent the ascertainment of aberration levels within any dose-treatment-accumulation period.

Unaltered cells

As a first approximation to an assessment of the lethal effects of argon ions, the proportions of metaphase cells without observable chromosomal change are shown in Fig. RB-7-2.

The data for the 10-12 hr. accumulation period after argon ion peak irradiation are not shown but were very similar to those from the 0-2 hr. accumulation period. While all data appear as if they could be fitted by equations which are simple exponential functions of dose, they are somewhat inconsistent and the straight lines are eye-estimates only. Variation with time for irradiation at the peak both under aerated and hypoxic conditions is considerably less than that for irradiation on the plateau. The ratio of doses for levels of equal effect under aerated and hypoxic conditions can be equated with the oxygen enhancement ratio (OER). The OER is about 2 for peak irradiation for all stages, and though more varied after plateau irradiation, is certainly not less than 2. Apart from the accumulation period closest to mitosis, it is apparent that irradiation with equal nominal doses of argon ions on the plateau results in many more cells with changes than peak irradiation both under aerated and hypoxic conditions. That is, the RBE is lower at the Bragg peak than on the plateau.

Fig. RB-7-2 The proportion of metaphase cells without change on a log scale against absorbed dose for 429 MeV/AMU argon ion irradiation. Solid triangles, peak-aerated; open triangles, peak-hypoxic; solid diamonds, plateau-aerated; open diamonds, plateau-hypoxic. The straight line curves are eye estimates only. (There were no observable changes in 100 aerated control or 100 hypoxic control cells).

Chromosomal abnormalities

Achromatic lesions

Metaphase chromosomes examined after ionizing radiation treatments frequently show non-stained segments (gaps) along their length. That is, there appears to be a break in the continuity of the chromosome, but there is no physical displacement. Achromatic lesions usually do not result in abnormalities at the next mitotic division and their exact nature is unknown. Since true chromatid breaks may not be visually dissimilar from achromatic lesions, this class of aberrations is prone to some subjectivity in scoring. Frequencies are shown in Fig. RB-7-3. In many instances (as indicated by the shaded areas) achromatic lesions were paired, that is, they were adjacent on two sister chromatids. This category was more frequent in chromosomes after peak irradiation both under aerated and hypoxic conditions. While there were substantial variations in yields between sampling periods for both irradiation positions, these were much more pronounced after plateau irradiation. However the sensitivity pattern under aeration and hypoxia were quite different. It is possible that a differential mitotic delay is contributing to these differences. It should be noted nevertheless, that mitotic indices do not indicate a compatible heterogeneity in cell progression through the mitotic cycle. The exponents to the dose response relation $y = \alpha D + \beta D^2$ for the pooled data for chromatid gaps (single achromatic lesions) and isochromatid gaps (paired achromatic lesions) are given in Table RB-7-III. The response to Bragg peak irradiation is basically a linear increase with dose, while that for the plateau is a poor fit to both a linear and a linear-quadratic relation.

Chromosome aberrations

Aberrations were categorized as chromatid deletions (if the fragment was

Fig. RB-7-3 Frequencies of achromatic lesions from the consecutive accumulation periods after irradiation with 429 MeV/AMU argon ions. The histogram blocks correspond to approximately 80, 40, 20 and 10 rad. (Actual absorbed doses are given in Table RB-7-I). The open and shaded areas reflect the frequencies of single and paired achromatic lesions respectively.

Table RB-7-III. Values of α and β for theoretical dose-response curves fitted to pooled experimental data for chromosomal aberration frequencies after argon ion irradiation

Aberration type	Peak - aerated		Peak - hypoxic		Plateau - aerated		Plateau - hypoxic	
	α $\times 10^{-4}$	β $\times 10^{-6}$	α $\times 10^{-4}$	β $\times 10^{-6}$	α $\times 10^{-4}$	β $\times 10^{-6}$	α $\times 10^{-4}$	β $\times 10^{-6}$
Isochromatid deletions	53.1	3.6	4.5	-3.3	74.6	35.3	35.0	45.7
Chromatid interchanges	27.5	23.0	18.4	-5.7	67.6	48.9	25.4	16.0
Chromatid intrachanges II	8.8	6.2	7.1	-3.6	1.9	7.6	0.3	4.3
Chromatid intrachanges I	1.4	1.1	1.2	0.2	20.0	4.4	4.7	6.4
Triradials	9.2	1.6	4.3	-3.1	1.8	20.5	3.0	6.2
Chromatid deletions	4.0	10.5	0.8	2.1	13.3	1.4	--	11.3
Chromatid gaps	35.1	5.9	20.6	-1.5	78.6	13.7	74.5	-36.6
Isochromatid gaps	19.3	-3.2	4.0	4.5	16.4	13.9	19.5	-9.8

physically displaced one chromatid's width), isochromatid deletions, chromatid interchanges - involving interaction between chromatids of two chromosomes or chromosome arms; chromatid intrachanges I - unequal sister chromatid exchanges (SCE's) or insertions of a piece of one chromatid into its sister (3 breakage events but indistinguishable from unequal SCE's); chromatid intrachanges - the interstitial deletion of a chromatid, usually to form a minute; and isochromatid/chromatid exchanges (triradials). Dicentric and translocation aberration types were also recorded, but given the high frequency of aberrations are doubtless due to union between two isochromatid deletions rather than chromosome type aberrations. Thus all aberrations are most probably consistent with post-replication (late S and G₂) events, chromatid type, rather than pre-replication (G₁) events, chromosome type. The frequencies of the various aberration types obtained from the different accumulation periods after exposure are given in Fig. RB-7-4. As with the achromatic lesions, yield of aberrations varies between accumulation periods. However, the pattern of variation is different. Particularly for plateau irradiation the period closest to mitosis at the time of irradiation is comparatively insensitive to aberration production. The relative proportions of the different aberration types also vary between the two depth dose positions. The ratio of isochromatid deletions to chromatid interchanges is 1.29 : 1 and 1.43 : 1 for aerated and hypoxic peak irradiations, while it is 0.95 : 1 and 1.09 : 1 for aerated and hypoxic plateau irradiations. This presumably reflects the higher probability of inducing lesions in two sister chromatids, rather than one at a time, for the higher LET particles.

The frequencies of total aberrations per cell from each accumulation period are shown in Figs. RB-7-5 and RB-7-6, from which OER estimates can be derived. It is not known whether the substantial variation in yield under

Fig. RB-7-4 Frequencies of chromosomal aberrations from the consecutive accumulation periods after irradiation with 429 MeV/AMU argon ions. The histogram blocks correspond to approximately 80, 40, 20 and 10 rad. (Actual absorbed doses are given in Table RB-7-I). For the lower three blocks and 80.8 rad peak hypoxic, frequencies of isochromatid deletions, chromatid interchanges and other aberrations are shown.

Fig. RB-7-5 The frequencies of total aberrations per cell against absorbed dose for 429 MeV/AMU argon ions at the Bragg peak on a logarithmic plot. Aerated and hypoxic conditions within each accumulation period are indicated. Standard errors are shown, and the dashed lines are for guidance only.

Fig. RB-7-6 The frequencies of total aberrations per cell against absorbed dose for 429 MeV/AMU argon ions on the entrance plateau on a logarithmic plot. Aerated and hypoxic conditions within each accumulation period are indicated. Standard errors are shown, and the dashed lines are for guidance only.

hypoxic conditions reflects real differences in stage sensitivity for exposure under aerated or hypoxic conditions or differential mitotic delay. The effect on OER estimates is substantial, however, overall the OER for both peak and plateau irradiation is certainly not less than 2. The OER is estimated as the ratio of doses given under aerated and hypoxic conditions which produce equivalent frequencies of aberrations.

The mean aberration yields over all accumulation periods are plotted in Fig. RB-7-7. Using a least-squares analysis the experimental data were fitted to the equation

$$y = \alpha D + \beta D^2$$

where y is the mean number of aberrations per cell at absorbed dose D , and α and β are constants.

For three of the four experimental regimes a quadratic exponent is indicated. Values for the exponents to the dose-response relation for the individual aberration types are given in Table RB-7-III. For the plateau irradiations, substantial quadratic components to the dose-response relation are indicated in many instances, while it is apparent that exposure of meristems at the two depth-dose positions results in considerable differences in the relative proportion of the different aberration types.

Aberration completeness

The majority of aberration types involve interaction between two breaks of two sister chromatids - isochromatid deletions; of two disparate chromatids - chromatid interchanges; and two sites of one chromatid - chromatid intrachanges. If one (or both) of the two breaks do not reform in their new configuration, then an incomplete aberration results. The frequencies of the four classes of isochromatid deletions and the frequency of incomplete-

Fig. RB-7-7 Frequencies of total aberrations per cell against absorbed dose for all accumulation periods after 429 MeV/AMU argon ion irradiation. The curves depict least squares fits to the relation $y = \alpha D + \beta D^2$, where y is frequency, D is absorbed dose in rad, and α and β are constants, the values of which are shown.

ness of other aberration types are given in Table RB-7-IV. While there were differences in the proportion of incomplete aberrations between doses and over the different accumulation periods, these were not consistent, and hence data summed overall is presented for each treatment regime. It is readily apparent that peak irradiation has resulted in a higher frequency of incomplete aberrations than irradiation on the plateau.

Multiple aberrations

In many cells, particularly some of those irradiated with argon ions at their Bragg peak, highly complex multiple aberrations were noted. In these instances two or more aberrations were localized to a restricted number of chromosomes. Individual chromosomes contained up to five aberrations. Cells from plateau irradiation also contained chromosomes with two aberrations (rarely more), however the multiple aberrations were usually not as complicated as those from the peak. It is highly probable that these complexes are the direct result of the prior passage of highly energetic argon ions through the cell.

Conclusions

If radiation at "the Bragg peak" refers to all of the particles that appear there the following statements can be made:

- 1) Neither on the entrance plateau nor at the Bragg peak does the OER fall to a value near 1.0.
- 2) For equal absorbed doses more cells exhibit damage after plateau than peak irradiation. That is, the RBE is lower at the peak.
- 3) At the plateau there is a pronounced cell cycle sensitivity for aberration production, such that cells closest to mitosis at the time of irradiation (late G_2) are comparatively indifferent to these 100 keV/ μ m

Table RB-7-IV Levels of aberration incompleteness after argon ion irradiation

	Isochromatid deletions						Chromatid interchanges		Chromatid intrachanges II		Triradials	
	SU	NUp	NUd	NUpd	Total no.	% inc.	Total no.	% inc.	Total no.	% inc.	Total no.	% inc.
Peak - aerated	188	91	81	84	444	57.7	345	41.4	101	29.7	86	69.8
Peak - hypoxic	106	28	34	11	179	40.8	125	43.2	32	18.8	21	66.7
Plateau - aerated	347	99	74	38	558	37.8	573	28.6	137	27.0	81	49.4
Plateau - hypoxic	142	41	35	21	239	40.6	220	28.6	43	48.8	42	45.2

SU, sister union : NUp, non-union proximal.: NUd, non-union distal
: NUpd, non-union proximal-distal

particles. This variation is much less but still apparent for Bragg peak irradiation.

4) The great majority of chromosomal aberrations scored are two break aberrations (Fig. RB-7-3). The coefficients of the dose-response relation, $y = \alpha D + \beta D^2$ represent the two breaks being produced by one or two particle tracks respectively. The finding of significant values of β in the plateau region indicates intertrack effects there despite the small number of particle traversals per cell. This may possibly be due to the considerable range of delta rays.

5) Aberrations induced by irradiation at the peak are more likely to be incomplete than those induced on the plateau. This is probably due to energy density and hence more localized damage at the site of the induced lesions preventing completion of aberration formation. However many peak aberrations are similar to those seen after low LET irradiation.

6) Triradials and complex aberrations comprise a higher relative proportion of aberrations induced at the peak than at the plateau. These manifestations of extreme damage doubtless reflect the "overkill" at the peak.

7) The proportion of cells damaged, the dose-response relation and the cell age response indicate significant contributions to the effect by ions other than argon.

These findings point up the necessity for the determination of microdosimetric spectra and quantities.

The results reported here are compatible with other observations (Annual Report 1976) with regard to low RBE, significant OER and some variation in cell age sensitivity.

RB-8 Cytological effects of 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol,
(Ro-07-0582) on hypoxic mammalian cells *in vitro*

Charles R. Geard and Stephanie F. Povlas

The need for chemical radiosensitizers of hypoxic cells, and the properties that they must possess to be effective in a clinical situation can be briefly stated. Such agents need to be non-toxic to normal tissues, to have minimal sensitizing action on oxygenated cells, to penetrate rapidly to non-vascularized regions of a tumour, and to be relatively resistant to metabolic breakdown. Efforts have concentrated on the 5-nitroimidazole, metronidazole (Flagyl) a drug of long standing in human use for treatment of certain forms of amebiasis and giardiasis, particularly infections of Trichomonas vaginalis; and the more efficiently radiosensitizing 2-nitroimidazole, Ro-07-0582. Both compounds are currently under evaluation as radiotherapy adjuncts in humans. Apart from their radiosensitizing abilities these compounds have proven to be selectively cytotoxic to hypoxic cells in vitro (see previous Annual Reports and RB-3). Further, in vitro studies have shown that the cytotoxic action of Ro-07-0582 is greatly enhanced by modest hyperthermia (see section RB-3).

The mode of action of the hypoxic cell cytotoxic drug Ro-07-0582 is not known and this study reports observations on hypoxic Chinese hamster V-79 cells up to 42 hours after incubation with drug. Drug concentration and contact times studied included one which resulted in about 99% cell death as estimated by the colony survival technique.

V-79 Chinese hamster cells were grown at 37.5° in Falcon plastic flasks in Gibco's F10 medium supplemented with 10% fetal calf serum. For assessment of drug effects on aerated cells, drug was added directly to cells growing in exponential phase. Hypoxia was induced in cells by respiration and metabolism of the large number of cells confined in the small volume of medium in air-free sealed ampules as described in previous Annual Reports.

Ro-07-0582 was added to aerated cells growing in log phase to concentrations of 1, 2 and 5 mM and cultures returned to the incubator. Colcemid (10 μ M) was added at 1.5 hr intervals to 6 hr post drug addition. After 1.5 hr colcemid accumulation of mitotic cells, 2 flasks at each concentration were trypsinized, spun down, washed, spun down and made hypotonic with 0.07 M KCl for 7 min at room temperature. Cells were then fixed in cold, fresh 3:1 ethanol, glacial acetic acid, changed twice through fixative, then cell suspensions dropped onto wet slides, 6 slides per flask. Air dried slides were stained with Giemsa and rendered permanent with Permunt.

Cells entered into and were maintained in a hypoxic state and in hypoxia plus 5 mM Ro-07-0582 for 1, 3, 4, 5 and $5\frac{1}{2}$ hr at 36.5°C . (A preliminary experiment with 2 mM Ro-07-0582 at 1 and 4 hr drug contact resulted in little difference for cell survival or chromosome aberrations but in lower mitotic indices up to 6 hr post drug treatment for hypoxia plus drug versus hypoxia alone).

At appropriate intervals ampules from each drug-time regimen were agitated on a vortex mixer, opened, and aliquots of cell suspension plated into Falcon tissue culture flasks containing fresh medium. Cells were then

incubated for 8 days before fixation, staining and counting of cell colonies. By comparison with controls, the fraction of cells surviving each treatment schedule was determined.

To establish cell populations for microscopic study 10^6 cells from each ampule (0.5 ml) were plated into 20 ml of complete medium in Falcon plastic flasks. Cells were then treated as for aerated conditions. Since colcemid (10 μ M) results in blockage of cells at mitosis, cell progression through the cell cycle can be monitored by comparison of the frequencies of mitotic figures between treatments. For cell examination after hypoxic treatments medium was decanted, trypsin added for 2 min. at 37.5°C, then trypsin plus cells added to the medium prior to spinning down and slide preparation as for aerated cells.

The proportion of cells in mitosis (mitotic index) was determined in 2,000 cell samples per treatment-fixation time, while the frequency and spectrum of chromosome aberrations was determined from 50 well-spread metaphases per treatment-fixation time wherever possible, except for the 15 to 42 hr accumulation period where 200 metaphases were examined.

The 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (Ro-07-0582) was donated by Roche Products, Welwyn Garden City, U.K.

Results

Aerated cells

Mitotic indices over four sequential 1.5 hr accumulations for drug treatments and controls are shown in Fig. RB-8-1. The initial comparatively low indices can be ascribed to handling and reduced temperatures before

Fig. RB-8-1. Percentages of cells in mitosis during treatment of aerated Chinese hamster V-79 cells with Ro-07-0582 at varying concentrations.

return to the incubator. It is apparent that concentrations to 5 mM have no effect on cell progression into mitosis up to 6 hr post initiation of drug treatment. There was no indication of increased chromosome aberrations in drug treated cells, overall 97% of metaphases were free of aberrations.

Hypoxic cells

Figure RB-8-2 shows a survival curve for cells exposed to 5 mM Ro-07-0582 for various times at 36.5°C, from 2 separate experiments. These values are expressed relative to the 1 hr treatments, where plating efficiencies were 96 and 87%, and 94 and 81% for the 1 hr hypoxia and 1 hr hypoxia plus drug from the 2 experiments respectively. It is evident that cytotoxicity is strongly contact-time dependent, while prolonged hypoxia alone has a slight deleterious effect.

Mitotic indices for the 4 consecutive 1.5 hr accumulation periods to 6 hr post-treatment are shown in Figure RB-8-3. It is apparent that increasing drug contact time results in fewer cells progressing into mitosis. Since passage through the mitotic block is unlikely over the 1.5 hr accumulation period, cells progressing into mitosis relate to the whole population, therefore hypoxia alone influences subsequent cell progression through the mitotic cycle. Only 14% of cells have moved into mitosis in 6 hr after 1 hr hypoxia compared to about 40% for aerated cells. Increasing time in hypoxia to 5 hr results in 9% of the cell population moving into mitosis. Simultaneous drug treatments result in 60 and 32% of cells moving into mitosis relative to hypoxia alone, for the 1 and 5 hr drug contact times.

The levels of chromosome aberrations in metaphase cells after 5 hr hypoxia or 5 hr hypoxia plus drug were similar and low in each consecutive

Fig. RB-8-2. Proportions of Chinese hamster V-79 cells surviving after prolonged hypoxia or hypoxia plus 5 mM Ro-07-0582.

Fig. RB-8-3. Percentages of cells in mitosis after treatment of hypoxic Chinese hamster V-79 cells with 5 mM Ro-07-0582 for increasing periods of time.

collection period with 92 and 90.5% of cells being aberration free in the total 6 hr collection period. There were 0.08 and 0.10 aberrations per cell in the respective treatments. It should be noted that less than 3% of the total cell population had progressed into mitosis in the 6 hr period after drug treatment, which compares with about 10% for hypoxia alone. Since the survival study indicated that about 10% of cells survived after the 5 hr drug treatment, cells with chromosome aberrations which could result in cell lethality could have been progressing into mitosis at a later time. In the second experiment cells were sequentially accumulated to 15 hr after treatment, then realizing that continued short accumulations would increasingly consist of cells in their second or third mitotic division after treatment, continuous colcemid accumulation was used to 42 hr after treatment. This ensures that second or third division mitotic cells appear as tetraploids or octoploids. For the 15 to 42 hr accumulation period cells in suspension and cells attached were handled separately, instead of being pooled as before. Mitotic indices from the different periods for each treatment are shown in Table RB-8-1. As is also shown in Figure RB-8-3 it is apparent that rendering cells hypoxic results in severe perturbations before anything approaching near normal progression of the cell population through the cell cycle takes place. The last collection period (13.5 to 15 hr) is the first to show a near normal mitotic index expected of aerated cells. Up to 15 hr, 32, 21, 22 and less than 1% of cells from the respective treated populations (Table RB-8-1) have progressed into mitosis. However the extended accumulation period from 15 to 42 hr shows that for all but the 5.5 hr drug treatment the majority of cells from the incident population have progressed into mitosis.

Table RB-8-1 Percentage of mitotic cells in Chinese hamster V-79 cell samples collected after hypoxia or hypoxia plus "0582"

Time after Treatment (hr)	1 hr. Hypoxia	1 hr Hypoxia +5 mM "0582"	5.5 hr Hypoxia	5.5 hr Hypoxia +5mM "0582"
0 - 1.5	2.8	1.3	1.8	< 0.1
1.5 - 3	1.9	1.1	1.9	< 0.1
3 - 4.5	1.9	2.4	2.1	< 0.1
4.5 - 6	2.5	2.1	1.2	< 0.1
6 - 7.5	4.4	3.0	3.4	< 0.1
7.5 - 9	2.5	0.3	2.0	< 0.1
9 - 10.5	2.7	2.4	2.6	< 0.1
10.5 - 12	2.0	1.7	1.9	< 0.1
12 - 13.5	3.9	2.4	3.1	< 0.1
13.5 - 15	7.3	4.3	1.5	< 0.1
15 - 42 Suspended Cells	84.3	70.9	80.5	0.8
15 - 42 Attached Cells	50.5	16.5	53.8	1.0

Cells were observed by phase-contrast prior to each fixation and it was noted that attachment of cells from the prolonged drug treatment was negligible for the first 6 accumulation periods, with a slightly increasing incidence of attachment through the later periods. But whereas the cells were near confluent from the other treatments at 42 hr the prolonged drug treatment still showed few attached cells, as can be seen in Figure RB-8-4. There were also substantial aggregations of cellular material suspended in the medium. When cells were concentrated, fixed, stained and examined microscopically, it was noted that the nuclear morphology of many cells from the drug treatments was altered as can also be seen in Figure RB-8-4. Throughout the 1.5 hr accumulation periods after 5.5 hr, no mitotic figures were found in the cells scored. A low frequency appeared to 42 hr and from these, assessments of chromosome aberrations were made, along with those for all time periods from the 1 hr drug treatment and after 5.5 hr hypoxia. Since the incidence of chromosome aberrations was comparatively low, the data from the individual accumulation periods have been pooled for 0 to 15 hr post treatment, as shown in Table RB-8-2. The background level of cells of higher ploidy appears to be about 2 to 3%, while after the 27 hr colcemid accumulation, the majority of cells have gone through two cell cycles. For other than the 5.5 hr drug treatment the incidence of chromosome aberrations is fairly low, with the majority being isochromatid deletions. The high incidence of symmetric dicentrics in tetraploid cells is compatible with these arising from isochromatid deletions with sister union in the previous cell cycle and indicates that further induced chromosome aberrations in the second cell cycle are negligible.

In relation to the total cell population only 1 to 2% of cells progres-

Fig. RB-8-4.

- (1) and (2) Phase contrast photomicrographs of Chinese hamster V-79 cells 42 hrs. after 5 hr. hypoxia (1) or 5 hr. hypoxia plus 5 mM Ro-07-0582 (2). The "haloed" cells in (1) are rounded mitotic cells while large aggregates of cellular debris are apparent in (2).
- (3) and (4) Nuclear morphology of V-79 cells after 5 hr. hypoxia (3) and 5 hr. hypoxia plus 5 mM Ro-07-0582 (4). A normal metaphase cell is present in both.
- (5) and (6) Chromosomal morphology of V-79 cells after 5 hr. hypoxia (5) and 5 hr. hypoxia plus 5 mM Ro-07-0582 (6). (5) shows a tetraploid cell with a symmetric dicentric chromosome while (6) shows extensive chromosomal alterations as a consequence of drug treatment.

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Table RB-8-2 Properties of cell populations after 10, 1.5 hr and 1, 27 hr colcemid accumulation periods following treatment of Chinese hamster V-79 cells

	1 hr Hypoxia +5 mM " 0582 "		5.5 hr Hypoxia		5.5 hr Hypoxia +5 mM " 0582 "
	Time after treatment (hr)				
	0 - 15	15 - 42	0 - 15	15 - 42	15 - 42
Cells of higher ploidy %	1.5	86.0	2.5	88.7	10.7
Aberration-free cells %	90.0	90.0	92.5	93.0	68.0
Aberrations per cell	0.11	0.13	0.08	0.09	0.61
Isochromatid deletions (% of total aberrations)	62	6	78	14	22
Symmetric dicentrics (% of total aberrations)	0	71	0	50	8

sed into mitosis up to 42 hr after 5.5 hr drug treatment. Of these, 11% were tetraploids indicating that an absolute minority of cells had progressed through two cell cycles. However, of the tetraploid cells, 31% were endoreduplicated whereas no endoreduplicated cells were observed in the considerably more frequent tetraploid cells from the other treatments. The incidence of chromosome aberrations per cell is 6 times higher than that of the other treatments, even though 68% of cells were free of aberrations. Thus cells with aberrations were likely to contain multiple aberrations. (Figure RB-8-4). It should be noted that acromatic lesions were not a feature at any time having an incidence of 0.13 per cell for the prolonged drug treatment. The appearance of symmetric dicentrics in the few tetraploid cells shows that repair had to take place to produce the original sister union isochromatid deletions. This is reinforced by the observation that only 22% of chromatid interchanges from this treatment were incomplete. In all but five instances the aberrations recorded were chromatid-type, i.e., virtually no G1 phase chromosomal aberrations were induced by the drug.

Discussion

The pronounced cytotoxicity of Ro-07-0582 under hypoxic conditions in vitro appears in large part to be mediated via an induced interphase cell death. The proportion of cells progressing through the cell cycle into mitosis declines both with increasing drug concentration and contact time. Following short drug treatments cellular recovery and cycle progression resumes, however a 5.5 hr treatment with 5 mM Ro-07-0582 results in only 1 to 2% of the cell population moving into mitosis up to 42 hr after treatment. This, in conjunction with the finding that about 98% of cells as assessed by the colony survival technique fail to proliferate, infers that the vast majority of cells are dying in interphase. Hence the effects of this drug are cell cycle stage-nonspecific, which is supported by the finding that crudely synchronized hypoxic cells at three putative stages of the cell cycle responded similarly to the drug.

Nevertheless, a minor component of induced cell death can be attributed directly to chromosomal damage, since the level of chromosomal aberrations in cells arriving at metaphase after the 5.5 hr drug treatment was six times that found after hypoxia alone. While the proportion of chromosomal aberrations which do not result in cell death is not known, there can be little question that the maldistribution of genetic material in post-mitotic progeny cells results in cell death.

Interactions with DNA, leading to disruptions of the genetic integrity of the cell, and manifesting as chromosomal aberrations at metaphase follow ionizing radiation treatments and the use of many antineoplastic agents. Thus, for many therapeutic modalities the desired end point, the death of the tumor

cell can be reasonably attributed in large part, to induced genetic inequalities of post-mitotic cells. This contrasts with the presumptive mode of action of this compound.

While the finding of chromosomal aberrations unquestionably indicates some interaction with DNA, DNA damage need not be the direct cause of interphase cell death. If the degenerative processes which lead to hypoxic cell death are dramatically accelerated by disruption of membranes by hypoxia-induced nitroimidazole-free radicals, then the nearly specific cytotoxicity of nitroimidazoles under hypoxic conditions is explained.

Ro-07-0582 appears to be a cell cycle stage non-specific cytotoxic compound which could induce cell death by free-radical disruption of membranes of hypoxic cells. This sensitivity of non-proliferating hypoxic cells thus differs from that usually found, where proliferating cells are more sensitive to anticancer agents. The response of resting, hypoxic malignant cells to this cytostatic drug could conceivably be exploited as an adjunct to chemotherapeutic schedules as well as acting as a sensitizer in radiotherapeutic schedules.

RB-9 Molecular ion beam: Cytogenetic effects of 1.8 MeV single and paired deuterons on Chinese hamster V-79 cells

C.R. Geard, S. Povlas and R.P. Bird

Chromosome aberrations represent directly observable consequences of energy deposition events in cell nuclei. Since different aberration types result from damage to one or two chromosomes with particular spatial relations, controlled energy deposition events can be further related to different aberration types. Some possibilities are presented in Figure RB-9-1. This report presents results from a study of the biological effectiveness of aberration production in Chinese hamster V-79 cells by simultaneous charged particles (deuterons) separated in space by a few hundred nanometers, or by single deuterons.

Culturing techniques for V-79 cells on thin Mylar foils ($6\ \mu$) irradiated under 70 Torr pressure have been reported previously. Cells were irradiated with 60, 150 or 250 rad of paired and single deuterons in three separate experiments and mitoses either continuously accumulated with colcemid or sequentially accumulated at hourly intervals. Mitotic indices after continuous and sequential colcemid accumulations are shown in Figures RB-9-2 and RB-9-3 respectively. While there is a dramatic decline in the frequencies of mitoses after irradiation, in this series of experiments there is no obvious dose-response relationship. Induced delay is considerably greater for the 250 than the 60 rad dose, however, the 150 rad dose shows the least effect.

The substantial delays caused by the irradiations will result in considerable intermixing of cells from different stages of the cell cycle at

Fig. RB-9-1. Possible chromosomal aberrations after irradiation with single or paired particles.

Fig. RB-9-2. Percentages of Chinese hamster V-79 cells in mitosis after irradiation with 60 and 250 rad of single and paired deuterons. Mitoses were continuously accumulated with colcemid.

Fig. RB-9-3. Mitotic figures as a proportion of control after irradiation with single or paired deuterons (3 doses of each). Mitotic cells were colcemid accumulated over 1 hr. periods after treatment.

fixation times later than those covered, thereby invalidating any attempt at assessing cell stage sensitivities.

It should be noted that the experimental regimen required for these charged particle irradiations when chromosomal aberrations are being studied does not allow for pulse labeling with tritiated thymidine or for the use of most synchronisation techniques.

Frequencies of chromosomal aberrations, which were all of the chromatid type, i.e., induced post replication, are shown in Figures RB-9-4 & 5. There were insufficient mitoses to score from the 1 to 2 hr accumulation period after 250 rad. The other fixation times after 250 rad are represented by comparatively few cells in some instances (down to 38) since there was such a drastic effect on cell progression after this high dose. It appears as if the induced delay in cell progression has produced a saturation effect, probably because highly aberrant cells are delayed more or never enter mitosis. That is, the 250 rad dose fixation times do not accurately reflect induced aberration frequencies. Nevertheless, all three doses show a clear cell stage sensitivity effect, with the period closest to mitosis at the time of irradiation (late G2) being most susceptible to radiation.

Overall, irradiation with paired deuterons results in more aberrations than single deuterons, however, there is not a clear cut substantive difference. Separation of the paired particles is shown in Table RB-9-1 along with the mean separation for single particles at the three doses. Surprisingly, reducing the spacing between particles to about 0.2μ does not result in a substantial enhancement of aberration types produced by two breaks (as shown in Figure RB-9-1). As might be expected chromatid deletions which would

Fig. RB-9-4. Frequencies of total aberrations per cell and chromatid interchanges per cell after irradiation with single and paired deuterons. Frequencies are shown for 1 hr. accumulation periods after irradiation.

Fig. RB-9-5. Frequencies of isochromatid deletions per cell and chromatid deletions per cell after irradiation with single and paired deuterons. Frequencies are shown for 1 hr. accumulation periods after irradiation.

Table RB-9-1 Molecular ion experiment - single and paired deuterons

V-79 cells on 6 μ Mylar		(1.8 MeV per deuteron)
<u>Depth in the cell</u>	<u>LET</u>	<u>Mean separation</u>
(μ)	(KeV/ μ)	(μ)
0	32.4	0.092
2	33.3	0.136
4	34.3	0.186
6	35.3	0.242
8	36.6	0.303
10	38.0	0.369

Separation of single particles at given doses

Dose (rad)	Mean separation (μ)
50	3.285
150	1.900
250	1.469

largely result from one event show no difference between single and paired particles. However the aberrations which require interaction between two lesions that are spatially distinct (lesions on sister and non-sister chromatids respectively) whilst being bolstered by paired particle irradiations are not greatly increased. The meaning of this finding is unclear at present since it is difficult to accept that these aberration types, particularly chromatid interchanges, could result from interactions occurring predominantly between lesions separated by less than 0.2 μ .

RB-10. Cell membrane architecture of normal and malignant epithelial cells in culture

C. Borek and C. Pain

Our interest in the surface features of neoplastic cells using scanning electron microscopy has been extended to transformed epithelial cells.

In earlier reports we have shown that fibroblasts transformed in Vitro by x irradiation have lost contact inhibition acquire a variety of surface excrescences which are not present on the normal contact inhibited counterparts. These surface features are not seen in controls which have been irradiated but were not transformed cells. The surface features are composed of microvilli and blebs which appeared on the cells within 8 days after transformation. This was established by isolating a transformed clone originating from a single cell irradiated with 300 rad. The surface features remain as an integral part of the transformed cells as long as the cell line is viable. This could be seen by viewing cell lines transformed by radiation and grown in culture for 8 years. While transformed fibroblasts possess complex permanent surface features throughout the cell cycle normal cells acquire temporary surface excrescences during mitosis. Once a normal cell goes back into interphase the surfaces smooth out and the normal cells joins their neighbor in culture and becomes contact inhibited.

Thus, scanning electronmicroscopy can serve as a tool to distinguish single fibroblasts transformed by radiation from normal counterparts. This is in contrast to other assay methods morphological or biochemical where large populations of cells must be analyzed to differentiate neoplastic cells from normal.

In our present studies we concentrated on transformed and normal

epithelial cells in which criteria of transformation are not as clear cut as in fibroblasts. When searching for reliable criteria from epithelial cells transformed in Vitro it is important to establish the characteristics of epithelial tumor cells which have been induced in Vivo.

We therefore have chosen to study primary cultures and cell lines from differentiated hepatoma and normal lines. A moderately differentiated hepatoma 7777, a cell line H₄-II-E-C₃, primary rat hepatocytes and a line of normal rat livers. In addition we studied a number of rat liver cell lines which have been transformed in Vitro by chemicals and have been shown to produce carcinomas upon injection into syngeneic rats. The cells had been transformed at different times by Aflatoxin B₁, nitrosomethyl urea and 2 amino anthroquinone. Transformation was carried out by adding the chemicals at 1 µg/ml in acetone to growing cultures and passaging the cultures in the presence of the chemical for a period of 8 weeks.

Primary cultures of the hepatoma and normal rat livers were carried out by treating fresh minces of tissue with 1 µg/ml collagenase and by plating the dissociated cells in F12 medium with 5% fetal calf serum directly onto coverslips for scanning EM.

Growth and fixation of cells for SEM. All cells were grown in medium containing the same batch of fetal calf serum. Following trypsinization and removal from the Petri dishes, the cells were counted and seeded on glass coverslips (24 x 60 mm) in 0.2 ml of medium at cell densities of 2×10^6 , 10^5 , and 5×10^4 . All coverslips were placed in Petri dishes and were incubated in a humidified CO₂ incubator until cells attached. Additional medium was supplemented so that the coverslips were completely immersed in fluid. The cells were further incubated for 48 hr after which the cells were observed in their living state by a phase-contrast microscope and

photographed. The medium was then removed from the Petri dishes and the cells were gently washed 3 times with Puck's saline (Grand Island Biological) to remove all traces of serum. Fixation and preparation for SEM were carried out as reported elsewhere. The coverslips were fixed in vials containing 2.5% glutaraldehyde in Sorenson's phosphate buffer (pH 7.3) for 2 hr. Following fixation, they were rinsed in Sorenson's phosphate buffer and dehydrated in a graded series of alcohol and amylacetate. The dehydrated coverslips were dried in a critical-point dryer with liquid CO₂ using the Denton critical-point drying apparatus. The coverslips were then mounted on aluminum stubs with conducting silver paint and coated with gold palladium. All specimens were examined in the JSM-U3 scanning electron microscope with acceleration voltage of 25 kV.

Results

Hepatoma cells

We have found a remarkable similarity between the surface features and mode of growth of the hepatoma cells as compared to the normal. The tumor cells grow as monolayers with close intercellular contacts. The regularity and smoothness of the cells was striking. Cells which were not yet adherent and also cells in mitosis displayed an abundance of microvilli on their surfaces both in the tumor and the control cells.

In the established cell lines individual hepatoma cells in interphase were indistinguishable from those of the normal liver and displayed widely dispersed short microvilli equally distributed over the cell surface. Normal epithelial cells in mitosis were indistinguishable from the dividing tumor cells. At higher cell density when cell-cell contact was established the normal cells formed tight contacts with fine filopodia and the surface of the cells became smooth. In contrast, at these higher densities the

hepatomacells established contact only in focal point, their ability to pile over one another was evident. Fine microvilli persisted on the surface of the cells and appeared to increase in number.

Transformed cells in Vitro

The various liver cell lines transformed in Vitro by chemical carcinogens exhibited patterns of growth which differed slightly from one another, yet in general mimicked the growth of the normal cells. The transformed cells were striking in their flat appearance and they grew in contact inhibited sheets as did the normal.

Few short microvilli were evenly dispersed on the surface of the transformed cells while the microvilli in the normal cells were mostly close to the periphery of the cells. In one of the growing edges of the cell line transformed by 2 amino anthroquinone some areas in which cell orientation was more haphazard could be seen.

In earlier reports we have shown that hepatoma cells which were anaplastic and had lost differentiation had an abundance of surface features which were not present on the control cultures. Here we see that if the hepatoma cells are still differentiated these surface features are absent.

Comparison of nonneoplastic and neoplastic cells growing at different rates requires the consideration of the influence of the cell cycle on surface morphology. In this study of the liver cells, the doubling time of the normal and neoplastic cell is similar thus making the comparison more valid.

We conclude that in the case of epithelial cell transformation where the transformed cells have maintained their differentiated quality more rigorous criteria must be used to ascertain the neoplastic nature of the cells. In contrast to the usefulness of the SEM in establishing the mali-

gnant nature of individual transformed fibroblasts it does not serve in the case of individual differentiated epithelial cells in ascertaining their neoplastic nature.

RB-11 Neoplastic transformation of hamster embryo cells irradiated in
Utero and assayed in Vitro

C. Borek, C. Pain and H. Mason

The induction of neoplastic transformation in vitro by x rays and neutrons has been reported. Using short term cultures of hamster embryo cells we found that transformation by x-rays can be detected with doses as low as 1 rad. The rate of transformation increased with dose and reached a peak of 1% between 150 to 300 rad. This frequency of neoplastic transformation in vitro is higher than the frequency of radiation induced tumors observed after exposing animals to similar doses of radiation.

In an earlier report we demonstrated that transformed cells which are malignant can be obtained from embryos irradiated in utero and assayed in vitro and that the frequency of transformation is lower than that obtained when the irradiations are performed in vitro, and closer to the incidence in animals.

Pregnant Syrian hamster strain LSH (Lakeview) were irradiated at 12 days of gestation with 300 rad of x rays. The embryos were surgically removed within 30 minutes after irradiation. Passaging was carried out as described below. We endeavored to conserve in culture as many of the original cells as was technically possible. The cultures were passaged twice a week and each flask was handled individually. At every passage half the population of the freshly dissociated cells, resuspended in fresh medium, was replated back into the original flask while the rest of the cells were seeded into a new flask containing 5×10^4 feeder cells (Syngenic cells irradiated with 4000 rads). After the third passage cells were replated only into the flasks from which they had been removed. Embryos at 12 days of gestation from non-irradiated hamsters of the same litter served as

controls and were cultured in the manner as described above.

Transformed cells were initially identified by their altered morphology and growth patterns as described previously. An additional criterion of transformation is the ability of cells to grow in low serum concentration, which will not support the growth of normal cells. Consequently cells which appeared to be transformed by their morphology were isolated and grown into mass cultures in medium fortified with either 10% or 1% fetal calf serum. The ability of the cells to proliferate at the lower serum concentration served as an immediate and simple check of their transformed nature.

Within 30 to 40 days (7 to 9 passages) after primary cultures had been established from irradiated embryos, cell transformation was observed in four out of five experiments as reported earlier.

✓ Chromosome analysis carried out at early passages after transformation indicated that the modal number of the transformed cells was close to the diploid, 44. The number of chromosomes per cell in one cell line ranged from 40 to 56; modal chromosome number 46 (in 40% of the cells), and in another the range was 38-60, modal chromosome number 48 (in 30% of the cells). No difference was found between the banding patterns of specific metaphase chromosomes from the transformed cells as compared to the normal.

The transformed cells are agglutinable by plant lectins and grow in agar as reported.

✓ We have investigated some biochemical markers associated with the neoplastic state of the cells; namely the fibrinolytic activity of the transformed cells, the release of macrophage inhibitory factor (MIF), the pattern of cellular lysosomal hydrolases and the ganglioside content of the cells. We have found that the fibrinolytic activity of the transformed

cells was over 20 fold greater than normal; MIF activity was confined exclusively to the transformed cell population. Out of fifteen cellular acid hydrolases which were determined fluorometrically we found that only acid phosphatase appeared to be significantly elevated in the transformed cells (a two fold increase over the normal). While the full range of gangliosides was present in the normal cells the transformed cells showed a complete absence of gangliosides larger than GM₃.

Experiments kindly carried out by Drs. H.S. Kaplan and A. Dèclève using a reported immunofluorescent assay demonstrated that there was no radiation leukemia virus (Rad LV) present in the radiation transformed cell lines.

The critical assay for the malignant nature of transformed cells is their ability to give rise to tumors when injected into appropriate hosts. The cells transformed in utero by radiation and cultured into cell lines in vitro have all given rise to tumors in syngeneic LSH hamsters and also in hamsters of the LHC strain. All animals were two weeks old when injected. The tumors have been characterized as nonmetastasizing malignant invasive sarcomas or carcinomas. They grew progressively and killed the animals. We find that a number of cells as low as 10^2 can induce a tumor 2 cm in size within 2 weeks after injection.

The tumors are transplantable and have been cultured in vitro. The morphological and biochemical properties of the cells cultured from the tumors are similar to those of the original transformed cells.

The characteristic of the in utero transformed cells are presented in Table RB-11-I and in Figures RB-11-1 to 4.

The frequency of transformation in utero is assayed in vitro using the clonal method reported earlier on cell transformation in vitro. The

Table RB-11-1
Characteristics of cells transformed in utero by X-irradiation

	Normal (Secondary culture)	Transformed (4th to 30th passage after isolation)	Tumour (1st to 10th passage) after reculturing
Karyotype	Diploid	Near diploid	Aneuploid
Growth in culture	Monolayers	Multilayers	Multilayers
Saturation density no. cells cm ⁻²			
in 10% serum	7.6 x 10 ⁴	1.8 x 10 ⁵	1.5 x 10 ⁵
in 1% serum	Minimum growth	10 ⁵	10 ⁵
Doubling time			
in 10% serum	16±2 h	14±2 h	16±2 h
in 1% serum	90±5 h	24±2 h	25±2 h
Morphology-scanning electron microscope	Flat and regular surface features except during mitosis	Pleomorphic with complex surface features throughout the cell cycle	Pleomorphic with complex surface features throughout the cell cycle
Agglutinability by con A (20 µg ml ⁻¹) and WGA (50 µg ml ⁻¹)	-	+	+
Plating efficiency			
in 10% serum	2.75±1 %	52.5±5.5%	49.5±6.5%
in 1% serum	0.05±0.05%	5.5±2.5%	6.0±2.5%
Efficiency in 0.33% agar	0	10%	30%
Plasminogen activator	low	high	high
Lysosomal hydrolases:phosphatase	low	high	high
Production of MIF	absent	high	high
Gangliosides	Presence of full range of gangliosides	Absence of gangliosides higher than GM ₃	Absence of gangliosides higher than GM ₃
Presence of radiation leukemia virus			
Tumorigenicity (invasive malignant sarcomas and carcinomas)	none	29/30	Transplantable 12/12
No of cells required to induce a tumor	No tumors produced	10 ²	10 ²

Fig. RB-11-1. Thin-layer chromatogram of gangliosides from normal hamster embryo cells and X-ray transformed LSH 5 cells. Lane 1 gangliosides in control cells, lane 2 ganglioside standards, and lane 3 gangliosides in X-ray transformed cells. Note that in the transformed cells gangliosides higher than GM3 are absent. Analysis was by Drs. P.H. Fishman, R.O. Brady and R.M. Bradley.

Fig. RB-11-2. Section of 3 week old malignant invasive carcinoma growing in a Syrian hamster following s.c. injection of 5×10^5 cells of a cell line (LSH 5) transformed in utero by X-ray irradiation (x 220).

Fig. RB-11-3. Normal liver cell in interphase (1)--Magnification X 5250 Transformed liver cell in interphase (2)---Magnification X 5250. See Proc. Nat. Acad. Sci. 69, 956 (1970).

Fig. RB-11-4. Normal liver cell in mitosis (3)--Magnification X 8750.
Transformed liver cell in mitosis (4)--Magnification X 9000. See Gene
Expression and Carcinogenesis in Cultured Liver Cells, Eds. L.E. Gerschenson
and E. Brad Thompson, pp. 62-93 (1975).

embryos at 12 days of gestation were irradiated in utero with 300 rad of x rays. Irradiations were performed in a parallel opposed setup so that the animals were irradiated simultaneously from above and below with two x ray tubes operated at 180 kV, 30 milliamperes with added filters of 0.28 mm aluminum and 0.28 mm copper. The dose rate in the position occupied by the animals was computed to be 250 rad/min within 30 min after irradiation and the embryos were removed and cultured in vitro on feeder cells (4000 rad irradiated syngeneic cells) as reported previously. In the screening we use morphological criteria to distinguish the transformed cells from the normal. The transformed clones have a random cell-cell orientation compared to the nontransformed counterparts. Microscopic examination of the clones is essential. Using the criteria we formed and reported that the incidence of transformation in utero is at least 10 fold lower than that found in vitro.

More recently we have carried out three large scale self contained experiments each involving 1000 - 1200 flasks in which we have compared the frequency of transformation in utero and in vitro. The embryos were irradiated in utero cultured and cloned in vitro. Animals of the same litter served for the in vitro experiments. Embryos were removed cultured and cloned in vitro on feeder cells and irradiated 24 hours later as described before. Control cells were derived from the cultures used for the irradiation in vitro. The results of these experiments are presented in Table RB-11-2. No transformation was found in the control. We find that the rate of transformation in utero is about 15 times lower than that obtained in the in vitro experiments carried out under the same conditions. It is of interest to note that in parallel experiments in vitro carried out with the chemical carcinogen Benzo(a) Pyrene (BP) we find that after treating the hamster cell with 1 $\mu\text{g/ml}$ BP the rate of transformation in vitro is also

Table RB-11-2

The yield of transformation of embryonic cells irradiated in Vitro or in Utero
with 300 rad. (Average values of 3 experiments)

	<u>Number of clones counted</u>	<u>P.E. or (S.F.)</u>	<u>Number of clones transformed</u>	<u>Percent transformation</u>
Control	58,100	1.27	--	--
<u>In Vitro</u>	29,970	0.57	246	0.8208
<u>In Utero</u>	42,120	0.72	22	0.05223

around 1%.

Several intriguing questions are evoked. Does the host mediate in modulating transformation by radiation? Is there a repair of transforming events before they can be expressed? How significant is the state of cells during irradiation in determining the rate of transformation? It is well known from in vitro studies that cell replication is required for fixation of the transformation. With the in vitro technique, cells are seeded as single cells with ample opportunity to divide. In addition, of course, they are not in contact with one another, and constitute a mixture of cell types from many tissues. In utero the situation is quite different; the embryonic cells are irradiated as tissues where there is cell to cell contact in tissue specific arrangements, and where the rate of cell replication varies with the tissue. Which of these factors, if any, is responsible for the lowered yield of transformed cells characteristic of in utero as opposed to in vitro irradiation. We are presently trying to elucidate in our current experiments.

RB-12 Molecular ion experiment: cell survival results

R. P. Bird

The molecular ion experiment has been described in previous Annual Reports (COO-3243-2, -3, -4, and -5) including detailed descriptions of the concept, equipment, and dosimetry in use and including some early biology results with Chinese hamster V79 cells for both chromosome aberrations and cell survival as end-points. Briefly, molecular ions accelerated at the RARAF accelerator are used to produce pairs of ions to irradiate cultured cells. The paired ions have average (r.m.s.) separations dependent on the thickness of material traversed; by using thin foils of Mylar both as the growth surface for cells and as the vacuum window for the accelerated ions, the average separation can be a fraction of a micrometer.

The majority of irradiations to date have been made with diatomic deuterium and three Mylar thicknesses (4 μm , 6 μm , and 10 μm) to achieve deuteron ion separations of 0.16, 0.21, and 0.31 μm , respectively calculated at a depth of 5 μm in a cell, with an LET of 33 keV/ μm in each case for the individual ions. The killing effectiveness of the paired ions is directly compared with that of single (unpaired) ions produced by using a very thin break-up foil before the cells. The ratio of surviving fractions for irradiation to the same dose (same particle fluence) whether as single ions or paired ions is determined. Alternatively, since there is sufficient capacity in the irradiation fixture, a survival curve for single or paired ions can be obtained in detail. Both modes of obtaining data have been used and are reported here.

The experimental design requires the unusual conditions of irradiating the cells at one-tenth atmospheric pressure. In order to keep the cells from dehydrating as a consequence of pressure reduction, the cells, which are attached and growing on the Mylar

surface and irradiated in a vertical plane, have a thin layer of agar spread over them. After irradiation, the cells are trypsinized from the surface and replated to commercial plastic Petri plates for colony formation. Samples from each trypsinized dish are electronically counted and monitored for cell multiplicity. The effect of the reduced pressure on the radiation sensitivity of the cells is discussed in a separate section (RB-14).

Molecular ion irradiations of the V79 cells have been accomplished primarily with cells at two times in the mitotic cell cycle as defined by hydroxyurea-induced synchrony (1). These two times are the radiation sensitive time immediately after removal of the 1.25 mM hydroxyurea from the growth medium by rinsing and replacement (G_1 -to-S phase transition), and four hours later which is a radiation resistant time (late S phase).

Figures RB-12-1 and -2 show the results of comparing single ion and paired ion irradiations as a function of the dose for the two synchronized times, late S phase and G_1 -to-S-transition, respectively. The survival ratio is the ratio of surviving fractions for single ions relative to paired ions. The increasing survival ratio with dose, especially for late S phase cells, indicates the increased killing effectiveness of the paired ions for mean ion separations as large as a few tenths of a micrometer.

Figures RB-12-3 and -4 show survival curve data obtained for irradiations with single deuteron ions ($LET = 33 \text{ keV}/\mu\text{m}$) and for comparison single helium-3 ions with approximately double the LET value ($LET = 67 \text{ keV}/\mu\text{m}$) to simulate the paired ion irradiation with zero separation. In Figure RB-12-3, the dashed line indicates the late S phase survival curve for paired deuteron ions determined from the survival ratio and the survival curve data for single ions.

Further analysis of the data remains to be done, but clearly two conclusions can be made: the paired ion effect is most prom-

Fig. RB-12-1 (above) and -2(below). Survival ratios measured with different Mylar thicknesses for synchronized cells in late S phase and the G₁-to-S transition, respectively.

Fig. RB-12-4. Survival curves for G₁-to-S transition cells irradiated with ³He ions or single deuterons.

Fig. RB-12-3. Survival curves for late S cells irradiated with ³He ions or single deuterons. The dashed curve is the survival curve for late S cells irradiated with paired deuterons as derived from the survival ratio data (Fig. RB-12-1) and the single deuteron survival curve.

inent at the late S phase but at neither cell cycle time is it as great as the simulated zero separation effect.

In addition to diatomic deuterons, the molecular ion experiment has been extended to trial runs of the effect of triatomic protons with individual ions having an LET value of 33 keV/ μ m and also of diatomic protons whose individual ions are of lower LET value (20 keV/ μ m). For late S phase cells, a survival ratio greater than unity has been measured for both these additional cases as expected.

Future work to be done with the molecular ion experiment will emphasize a different synchrony method with which it is expected that additional cell cycle times can be used with both cell survival and chromosome aberration studies.

Reference

1. Hall, E. J., Gross, W., Dvorak, R. F., Kelllerer, A. M., and Rossi, H. H. "Survival curves and age response functions for Chinese hamster cells exposed to x-rays or high LET alpha-particles." Radiat. Res. 52, 88-98 (1972).

RB-13 Track segment experiments: cell survival data (preliminary)

R. P. Bird

A series of irradiations with charged particle beams in the Track Segment Facility have been done using hydroxyurea-synchronized Chinese hamster V79 cells. The Track Segment Facility was described in the previous Annual Report (COO-3243-5) including a description of equipment and dosimetry procedures and is discussed further elsewhere in this Report (PD-2). The cell synchrony technique is the same as that used for the molecular ion experiment (RB-12) with the simplification that irradiations are performed with a vertical beam so that cell cultures have growth medium left in place without an agar overlay. Two synchronized cell cycle times have been used: the G_1 -to-S transition time (immediately after 1.25 mM hydroxyurea removal) and late S phase (four hours after removal).

The data obtained and reported here are preliminary since some of the survival curves have not been repeated. A more detailed series of irradiations with a different synchrony technique and using the 50 kV x-ray source as a reference radiation will be conducted in the near future. It is planned that in addition to the radiation quality as a variable, other factors such as chemical modifiers will be used.

Figure RB-13-1 and -2 show survival data for various radiation qualities for the two cell cycle times, respectively. Figures RB-13-3 and -4 show a replotting of data in order to estimate survival curves parameters. The replotted survival data clearly are in reasonable agreement with the analytical expression

$$S = \exp[-(\alpha D + \beta D^2)].$$

The survival data obtained for late S cells irradiated with helium ions of 100 keV/ μ m or of about 145 keV/ μ m appear to be of the

Figure RB-13-2. Survival curves for G_1 -to-S transition cells with various charged particle beams as indicated in the Figure.

Figure RB-13-1. Survival curves for late S cells irradiated with various charged particle beams as indicated in the Figure.

Fig. RB-13-3 (upper left). Survival curves for late S cells irradiated with various charged particle beams. For survival curves of the form $S = \exp(-\alpha D - \beta D^2)$, α is the intercept and β is the slope of the line.

Fig. RB-13-4 (lower left). Survival curves for G_1 -to-S transition cells irradiated with various charged particle beams. (See Fig. RB-13-3 caption.)

Fig. RB-13-5 (upper right). The values of α obtained graphically from Figs. RB-13-3 and -4 have been used to derive the inactivation cross sections, σ , plotted here as a function of the LET of the radiation.

single hit form ($\beta=0$). Figure RB-13-5 shows the changing inactivation cross section as a function of LET for the initial slope parameter (α) of the survival curves for the two cell cycle times.

Additional data will be required for detailed analysis of track segment results. However, the survival curve parameter, α , for the late S cells shows a squared dependence on the LET of the radiation used, for the lower part of the LET range, as predicted by the theory of dual radiation action (1).

Reference

1. Kelllerer, A. M. and Rossi, H. H. "The theory of dual radiation action." Current Topics in Radiat. Res. Quarterly 8, 85-158 (1972).

RB-14 The effect of reduced pressure on radiation sensitivity of
synchronized V79 cells

R. P. Bird

The design of the molecular ion experiment requires cells to be irradiated at reduced pressure (RB-12). The cell cultures are routinely irradiated at 70 torr pressure in the Molecular Beam Facility. A brief x-ray study using asynchronous cultures at 70 torr pressure indicated no substantial effect of the reduced pressure on radiation survival. However, with the initiation of the Track Segment Facility where cells are irradiated at atmospheric pressure, the effect of reduced pressure on survival after charged particle irradiations also can be investigated by comparison of results from the Track Segment and Molecular Beam Facilities.

Figure RB-14-1 shows survival curves for two cell cycle times and for irradiations at atmospheric pressure and 70 torr pressure using single deuteron ions of 33 keV/ μ m. The data for late S phase cells clearly coincide for the two pressure regimes but the G₁-to-S transition curves are displaced such that the survival at reduced pressure is greater. Figure RB-14-2 shows survival curves for the G₁-to-S transition cells using single ion irradiation with 20 keV/ μ m particles. Again, the reduced pressure regime resulted in higher survival. In both cases, the effect of the reduced pressure is approximately that of a dose modifying factor of about 1.3 for the G₁-to-S transition cells. The lack of a reduced pressure effect on late S cells using 33 keV/ μ m particle irradiation is consistent with the lack of effect found for asynchronous cells that were x-irradiated since the asynchronous cell survival is largely determined by the radioresistant population (late S cells).

Additional x-irradiation measurements of the reduced pres-

sure effect are being made with hydroxyurea synchronized cells. The initial results for x rays delivered either at atmospheric pressure or after cells were exposed several minutes to reduced pressure (70 torr) are consistent with a dose modification of about 1.3 for G_1 -to-S transition cells.

Figure Captions

Fig. RB-14-1. Survival curves for late S cells and G_1 -to-S transition cells irradiated with 33 keV/ μ m single deuteron ions in either the Track Segment Facility or Molecular Ion Facility.

Fig. RB-14-2. Survival curves for G_1 -to-S transition cells irradiated with 20 keV/ μ m single ions in either the Track Segment Facility or Molecular Ion Facility. The ions used in the Track Segment Facility were deuterons while those used in the Molecular Ion Facility were protons.

DOSE (RADS)
Fig. RB-14-1

DOSE (RADS)
Fig. RB-14-2

TEACHING

Each month of the academic year, three members of the staff contributed a series of radiobiology lectures to medical students taking the general Radiology elective. Lectures were also given to second year medical students in the Human Oncology course. In addition, a laboratory elective in "Cellular and Radiation Biology" was offered for medical students.

During the Fall, a course in radiobiology was given for residents in both therapy and radiodiagnosis; about 40 residents attended from Columbia-Presbyterian and affiliated hospitals. The practice was continued whereby suitable third year residents in radiotherapy spend six months full time in the laboratory to train in research techniques; one resident took advantage of the option during the past year.

Members of the staff participated in graduate teaching in the departments of Pathology and Physiology of Columbia University and also as guest lecturers in other institutions in the city.

INVITED SEMINARS AND PAPERS AT SCIENTIFIC MEETINGS

Dr. R.P. Bird

Radiation Research Society, San Juan, Puerto Rico, May 1977

Dr. Carmia Borek

Cancer Biology seminar, Aspen, Colorado, August 1976

Seminar, Dana Institute for Disease Prevention, Valhalla, N.Y., Oct. 1976

Conference in Neuroscience, Toronto, Canada, November 1976

International Workshop on Cancer Invasion and Metastasis, N.Y., Dec. 1976

International Workshop on Cell-Cell Interaction, LaJolla, Cal., Feb. 1977

Seminar, Salk Institute, La Jolla, California, February 1977

Seminar, UCLA, Los Angeles, Cal., February 1977

ERDA Workshop on Actinide Biology, Seattle, Washington, April 1977

Radiation Research Society, San Juan, Puerto Rico, May 1977

American Association for Cancer Research, New York, May 1977

Dr. C.R. Geard

Seminar, University of Melbourne, Victoria, Australia, January 1977

Seminar, Australian National Univ., Canberra, Australia, January 1977

Seminar, Commonwealth Scientific and Industrial Research Organization -
Genetics Section, Canberra, Australia, January 1977

Radiation Research Society, San Juan, Puerto Rico, May 1977

Mr. L.J. Goodman

Third Symposium on Neutron Dosimetry in Biology and Medicine, Neuherberg/
Munich, Germany, May 1977

Dr. E.J. Hall

Seminar, Mid-Atlantic Neutron Therapy Association, Washington, D.C., July 1976

Dr. E.J. Hall (continued)

Particles and Radiation Therapy II; an International Conference, Sept. 1976

ROTG Hyperthermia Workshop, Buffalo, N.Y., October 1976

Seminar, Colorado State University, October 1976

Seminar, Harvard University Medical School, Boston, Mass., November 1976

Seminar, University of Minnesota, Minneapolis, Minn., November 1976

Symposium: The Treatment of Human Cancer by Neutrons, National Institutes of Radiological Sciences, Chiba, Japan, December 1976

Seminar, Tokyo Medical and Dental University, Japan, December 1976

Seminar, University of Florida, Gainesville, Florida, January 1977

Annual Meeting of the American Society of Therapeutic Technologists, Chicago, Illinois, February 1977

Clinical Oncology: Symposium organized by the Medical College of Virginia, Williamsburg, Virginia, March 1977

Twelfth Annual San Francisco Cancer Symposium, San Francisco, California, March 1977

Seminar, New York Medical College, New York, March 1977

Seminar, Memorial-Sloan Kettering Cancer Center, New York, April 1977

Radiation Research Society, San Juan, Puerto Rico, May 1977

Dr. P.J. Kliuga

Radiation Research Society, San Juan, Puerto Rico, May 1977

Dr. Y-M.P. Lam

ERDA 10th Annual Radiological and Chemical Physics Contractors Meeting, Berkeley, California, December 1976

Dr. R.C. Miller

Radiation Research Society, San Juan, Puerto Rico, May 1977

Dr. H.H. Rossi

Seminar, University of Illinois, Urbana, Illinois, February 1977

Seminar, University of Rochester, Rochester, N.Y., March 1977

Health Physics Society, No. California Chapter, San Francisco, March 1977

Dr. Dusan Srdoc

Seminar, University of Miami, Miami, Florida, May 1977

Radiation Research Society, San Juan, Puerto Rico, May 1977

Seminar, Argonne National Laboratory, Argonne, Illinois, July 1977

Mrs. Laurie Towle

Radiation Research Society, San Juan, Puerto Rico, May 1977

LOW LEVEL RADIATION GROUP

During the year, Drs. Rossi, Hall, Bird, Borek, Geard, Kliauga, Kosiara, Lam, Miller and Srdoc and Mr. Goodman and Mr. Colvett and Mrs. Towle have attended monthly meetings of the Low Level Radiation Group at the Brookhaven National Laboratory. This group, which consists of scientists from the Medical and Biological divisions of B. N. L., as well as representatives from the laboratory, was set up to discuss the feasibility of extending radiobiological studies to lower doses and lower dose-rates. The group serves the dual function of reviewing experiments that have been performed by members in the past, and also advising on the plan of future investigations, and coordinating the efforts of the different laboratories concerned.

OTHER ACTIVITIES

A Bibliography of Publications is attached.

Members of the Laboratory were active on the following:

International Commission on Radiation Units and Measurements (ICRU)

Main Commission

Technical Areas:

Radiobiology (Co-sponsor)

Radiation Physics - Neutrons and Heavy Particles (Sponsor)

Report Committees:

High Energy and Space Radiation Dosimetry (Sponsor)

Fundamental Quantities and Units

Concepts and Principles of Radiation Protection Measurement
(Cooperative effort with the ICRP) (Chairman)

Intercomparison of Neutron Instruments (Sponsor, Member, Consultant)

Microdosimetry (Co-sponsor, Member)

Neutron Dosimetry for Biology and Medicine (Sponsor, Member)

Radiobiological Dosimetry (Co-sponsor)

ICRU/ICRP Liaison Committee

International Commission on Radiological Protection

Task Group on the Influence of Radiobiological Factors such as Protraction of Exposure and LET in the Estimation of Risks of Cancer Induction, for Purposes of Radiation Protection

National Council on Radiation Protection and Measurements (NCRP)

Main Council

Sc. Comm. 1: Basic Radiation Protection Criteria

Sc. Comm. 4: Heavy Particles (Neutrons, Protons and Heavier) (Chairman)

Sc. Comm.13: Safe Handling of Bodies Containing Radioactive Isotopes
(Chairman)

Sc. Comm.15: Radiation Protection in Teaching Institutions

Sc. Comm. 29: Brachytherapy (Consultant)

Sc. Comm. 49: Radiation Protection Guidance for Paramedical Personnel
(Chairman)

Ad Hoc Committee on Comparison of Radiation Protection Philosophies

Mayor's Technical Advisory Committee on Radiation, City of N.Y. (Chairman)

Bureau of Radiation Control, City of New York (Consultant)

Review Committee for Biological and Medical Research, Argonne Universities
Association (Chairman)

Medical Advisory Committee to the Bureau of Radiological Health, State of
New York (Consultant)

Health Advisory Council, Department of Health, State of New York

Nuclear Regulatory Commission (Consultant)

Committee to Review the Use of Ionizing Radiation in the Treatment of Benign
Diseases, National Research Council

Committee on the Biological Effects of Ionizing Radiations (BEIR), National
Research Council

Panel on Reference Nuclear Data, Radiation Research Society

Nominating Committee, Radiation Research Society (Chairman)

Biomedical Panel of the BEVALAC Program Advisory Committee, Lawrence
Berkeley Laboratory

Delegate to the United Nations Scientific Committee on the Effects of
Atomic Radiation

American College of Radiology, Section on Radiation Biology and Pathology

National Board of Medical Examiners: Examination Committee for Radiology

Radiation Study Section, National Institutes of Health

Council: Radiation Research Society

Carcinogenesis Study Section, National Institutes of Health

Third Symposium on Neutron Dosimetry in Biology and Medicine, Neuherberg/
Munich, Germany (Chairman, Session X)

Electronics Stock Committee at Brookhaven National Laboratory

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