ANNUAL REPORT ON RESEARCH PROJECT

H.H. Rossi, Director

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TABLE OF CONTENTS

INTRODUCTION	5-8
STAFF	9-10
RADIOLOGICAL PHYSICS	11
A) Dosimetry	
PD-1 Specialized dosimetry at RARAF S.A. Marino, R.E. Mills and L.J. Goodman	12-22
PD-2 International neutron dosimetry intercomparison L.J. Goodman and R.D. Colvett	23-35
PD-3 Development of a microbeam for radiobiology R.D. Colvett	36-39
PD-4 Computed and measured relative kerma S.A. Marino	40-45
PD-5 Study of a proposed neutron therapy beam S.A. Marino, L.J. Goodman and R.D. Colvett	46-51
B) Microdosimetry	
PM-1 Event distributions from monoenergetic photons R. Dvorak and W. Gross	52-60
PM-2 Event distributions from heavy charged particles W. Gross, R. Rodgers and J. Kitzman	61-63
PM-3 Event distributions from 5.6 GeV neon ions J. Dicello, U. Kraljevic and W. Gross	64-86
PM-4 Event distributions from monoenergetic neutrons R. Rodgers, J. Dicello and W. Gross	87-101
PM-5 Studies for nanometer microdosimetry S. Pszona	102-107

C) The Radiological Research Accelerator Facility (RARAF)

PA-1 Operation and development of the Facility
L.J. Goodman, N. Rohrig, C.M. Turner, S.A. Marino
and R.E. Mills 108-121

PA-2 Development of comprehensive dosimetry apparatus
R.D. Colvett, R.E. Mills, H.H. Rossi and
L.J. Goodman 122-145

BIOPHYSICS 146

BP-1 The molecular ion beam experiment
H.H. Rossi 147-151

BP-2 The mean spacing of protons arising from molecular
ions
W. Gross and A.M. Kellerer 152-159

BP-3 Multiple coulomb scattering of protons
W. Gross and R.D. Colvett 160-169

BP-4 RBE, OER and dose rate studies in plants
A.G. Underbrink 170-200

BP-5 Quantification of radiation induced cancerogenesis
A.M. Kellerer 201-213

BP-6 Comparison of effect rates in samples of time
decreasing size
A.M. Kellerer 214-222

BP-7 Microdosimetry and the dispersion of chromosome
aberrations
A.M. Kellerer 223-244

BP-8 Analysis of Japanese leukemia data
A.M. Kellerer and H.H. Rossi 245-263

BP-9 Theory of dual radiation action
A.M. Kellerer and H.H. Rossi 264-276

RADIOBIOLOGY 277

B-1 Analysis of OER and RBE data from irradiation of
Vicia seedlings with monoenergetic neutrons
A.M. Kellerer and E.J. Hall 278-298

RADIOBIOLOGY (continued)

B-2	OER and RBE studies with ²⁵² californium and radium at low dose-rate. E.J. Hall, L.A. Roizin-Towle and R.D. Colvett	299-310
B-3	Irradiation of mammalian cells with fast neutrons E.J. Hall and J. Novak	311-314
B-4	Split dose experiments with hypoxic cells. E.J. Hall, S. Lehnert and L.A. Roizin-Towle	315-324
B-5	Dose response relationship for <u>in vitro</u> transformation of mammalian cells by x rays Carmia Borek	325-338
B-6	The quinacrine fluorescence and giemsa banding of normal and neoplastic rat liver cells Carmia Borek (In collaboration with D.A. Miller, O.J. Miller and V.G. Dev (Dept. of Human Genetics)	339-340
B-7	Surface alterations in transformed epithelial cells Carmia Borek (In collaboration with Max Burger (Biology Department, Princeton University)	341-343
B-8	The relationship of intracellular cyclic AMP to radio-sensitivity Shirley Lehnert	344-353
B-9	Inhibition of repair of potentially lethal damage in mammalian cells by medium harvested from cells in plateau phase H. Norwint, I. Horowitz and E.J. Hall	354-361
TEACHING		362
INVITED SEMINARS AND PAPERS AT SCIENTIFIC MEETINGS		363-364
OTHER ACTIVITIES		365-367
BIBLIOGRAPHY		368-370

INTRODUCTION

The Radiological Research Laboratory is devoted primarily to inquiries in the general area of radiation biophysics. Successful investigations in this field necessitate an interdisciplinary approach, and the aim of the laboratory has been to bring together biologists and physicists in close collaboration.

The ultimate goal is the identification of basic mechanisms involved in the action of ionizing radiations on biological material. The theory of dual radiation action forms a framework for the activities of the laboratory. However in recognition of the warning by J.J. Thompson that: "A theory should be policy and not a creed," it simply serves as a unifying theme for the diverse efforts of scientists in the laboratories at Columbia and Brookhaven.

The subjects of research which may be considered to be more pragmatic or to have more obvious and immediate practical applications fall into two categories. First, there is a major portion of the work which relates to the effects of small doses of radiation which is a subject of essential concern to the community at the present time, and second, there are experiments with immediate application to radiotherapy.

The past years have seen an expansion of research activities centered around the RARAF facility. A variety of biological systems, ranging from plant spores to mammalian cells in culture and to whole animals, have been irradiated and studied. The plan of these experiments has been monitored, and the priorities assessed, by the Low Dose Panel which has continued to meet monthly during the past year. Attempts have been made to push towards lower doses, and there has been a continual search for biological systems in which the effects of very small doses of radiation may be detected. Most of the irradiations at RARAF have

been with neutrons of a wide range of energies but more complex techniques involving charged particles, singly and in small clusters, are under development.

The quality of the results of the biophysical research has been improved by developments in dosimetry and especially by the introduction of superior methods of statistical analysis.

During the year the body of experimental data collected during the few months that heavy ions were available at the Princeton Particle Accelerator was analyzed. The results will appear shortly as a series of articles in Radiation Research. A number of experiments in radiological physics have been conducted at the Yale HILAC, but as yet the biological research has not been started. Radiobiological investigations of immediate relevance to radiotherapy have been conducted with the high energy neutron beam at the Texas A&M variable energy cyclotron and also at the Naval Research Laboratory cyclotron in Washington.

Due to reductions of the research funds available for this laboratory there has been a serious cutback in the total staff in recent months. The staff of the laboratory reached its peak size in 1971 and 1972, and this was reflected by a large research output and a high rate of publication which because of the usual delays is reaching a maximum at the present time. In more recent months the resources of the laboratory had to be curtailed, especially as far as technical support is concerned. These trends if not promptly reversed will inevitably and seriously affect the performance of the laboratory during the coming year.

It is with sadness and regret that we report the death of Dr. Frank Gonzalez in September 1972. His research group, which had been engaged in study of the radiation genetics of drosophila, has been disbanded. This is a serious loss to our effort.

ACKNOWLEDGEMENTS

A significant portion of the research of this laboratory is carried out at the Brookhaven National Laboratory where a number of staff members are permently located in connection with RARAF and related projects. In addition extensive collaborative research activities continue with many members of the Brookhaven staff. We would like to express our indebttness to Dr. V.P. Bond who has been primarily responsible for these developments. Because of our special interest in radiations of high LET it is often desirable to use accelerators having capabilities exceeding those of the machine at RARAF. Access to the Yale HILAC has been of considerable value to our work, and we are grateful to Drs. Robert Baringer and Barclay Jones for their cooperation in making this machine available. Experiments have also been performed at the Texas A. & M variable energy cyclotron, and we are obliged to Dr. Peter Almond of the MD Anderson Hospital for arranging this opportunity. Experiments have also been started at the Naval Research Laboratory cyclotron in Washington, D.C., and we wish to thank Dr. H. Attix and Dr. Leon August for their help and kindness in making this possible.

Most of the instruments and equipment used in the research described in this report were built in the Machine 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 construction but also in many important features of design.

Some of the work described has been carried out with additional support from other sources including the U.S. Public Health Service. Such additional support is indicated where applicable.

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RADIOLOGICAL PHYSICS

A) Dosimetry

- PD-1 Specialized dosimetry at RARAF
S.A. Marino, R.E. Mills and L.J. Goodman
- PD-2 International neutron dosimetry intercomparison
L.J. Goodman and R.D. Colvett
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R.D. Colvett, R.E. Mills, H.H. Rossi and L.J. Goodman

PD-1 Specialized Dosimetry at RARAF*

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

A. Neutron dosimetry for Drosophila irradiations

The study of the genetic effects of monoenergetic neutrons on Drosophila melanogaster was continued. The apparatus and experimental techniques employed for dosimetry and fly irradiations using 6 MeV neutrons were described in last year's Annual Report (C00-3243-1).

Three additional irradiations were performed. Dosimetry data for the first two investigations were combined with the results of three previous investigations and used for the remaining irradiation. A total of about 7,000 flies divided into 16 groups were given neutron doses ranging from 1680 to 8430 rads. Measurements using a Geiger-Mueller gamma ray dosimeter (described in Annual Report NYO-2740-7) indicated that gamma rays accounted for approximately 5 percent of the total doses.

This project has been discontinued and no further Dros. irradiations are planned.

B. Neutron dosimetry for Tradescantia irradiations

The study of the effects of monoenergetic neutrons on somatic mutations in Tradescantia was continued. The apparatus and

*This investigation was supported in part by Public Health Service Research Grant No. CA-12536 from the National Cancer Institute.

experimental techniques were described in a previous Annual Report (NYO-2740-6). Irradiations were performed using neutron energies of 0.39, 0.43, 0.55, 0.68, 1.0, and 2.3 MeV. Doses ranged from 0.2 to 8.0 rad.

A new investigation has been initiated to study oxygen enhancement ratio (OER) in Tradescantia for various neutron energies. The special gas-flow irradiation fixture designed for this project is shown in Figure PD-1-1 together with a set of plants taped in place. In order to deplete as much oxygen as possible from the Trad., the enclosure is light-tight, as well as gas-tight, since the green cuttings produce oxygen by photosynthesis. The two longer fastening studs also serve as inlet and outlet ports for the gas flow. Two capacitor ionization chambers used for dosimetry during x-irradiations are shown at the left and right sides of the Trad. inflorescences in the Figure. Also visible are the nylon rings which completely surround the specimens to establish tissue equivalent secondary particle equilibrium and backscatter.

The gas flow system, shown in Figure PD-1-2, is mounted on a gas cart and is easily transported to the irradiation area with the plants. The four manifolds fabricated have provisions for measuring gas flow rate, for evacuating the fixture to speed oxygen removal, and for bubbling the gas through water to prevent desiccation of the

PD-1-1 Tradescantia inflorescences in gas-flow fixture used for studies of oxygen enhancement ratio.

PD-1-2 Gas-flow system for oxygen enhancement ratio studies with Tradescantia.

specimens. The manifold shown includes a constant current source used during early studies to generate variable oxygen concentrations in the nitrogen flow. The current was passed through platinum electrodes in contact with a water solution of potassium hydroxide on filter paper in the gas stream, thereby electrolytically decomposing the water. This provided the small, well-regulated amounts of oxygen required to determine the concentration allowable during subsequent studies with maximum attainable anoxia. A commercial, continuous flow analyzer^{*} with a resolution of 1 ppm of oxygen was used to monitor the effluent gas from the Trad. fixture during these early studies.

As shown in Figure PD-1-3, one of the eight Trad. fixtures has a resealable hole in the cover plate to insert the 6 mm diameter spherical multiplication ionization chamber used for neutron dosimetry at the specimen location. The vertically upward beam was chosen for this experiment for ease in support and location of the relatively heavy fixture, and for a slight reduction in the amount of scattered radiation. The support stand is bolted directly onto a flange of the beam tube, and the tritium target protrudes through the opening in the center of the fixture. The latter simply rests in a machined groove in the stand and can be easily removed and replaced.

^{*}Model 80 Oxygen Trace Analyzer, Beckman Instruments, Inc., Fullerton, California.

PD-1-3 Dosimetry apparatus for neutron irradiations of Tradescantia.

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PD-1-4 Dosimetry apparatus for neutron
irradiations of mammalian cells.

the Biology Department of Brookhaven National Laboratory. The apparatus used is the same as that described previously for Tradescantia irradiations, except that the gas-flow equipment is not used.

Osmunda spores on moist filter paper are located in the irradiation fixture in the same position used for Trad., and the various doses required are obtained by successive removals of spores until the maximum dose has been delivered to the last remaining group. Efficient re-use of the equipment for the Trad. experiment is achieved by irradiating spores with neutrons of the same energy as used for Trad., and by utilizing the same dosimetry measurements it is possible to execute both experiments in one day.

Neutron energies of 0.43 and 0.68 MeV have been used for spore irradiations, and doses have ranged from 10 to 200 rad.

E. Neutron dosimetry for mouse irradiations

The study of the effect of monoenergetic neutrons on colony-forming units from mouse bone marrow was continued in collaboration with members of the Medical Department of Brookhaven National Laboratory.

The apparatus and experimental techniques were essentially the same as those used in previous mouse whole-body irradiations and have

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The Intercomparison Committee responsible for organization and management of the Intercomparison consists of

Dr. Randall S. Caswell (Chairman)

Dr. David K. Bewley

Dr. Klaus W. Geiger

Dr. Johan J. Broerse

Mr. Leon J. Goodman

Twelve scientists were selected by the Committee for participation in INDI on the basis of their current activity and competence in neutron dosimetry. Measurements are being performed at RARAF during a series of 2-week sessions in each of which three participants complete their measurements. The RARAF staff provides the required neutron beams and performs the essential dose, spectrum, and fluence monitoring measurements, as well as participating in the absolute dosimetry intercomparison itself. About half of the measurements for this project have been completed.

Quantities intercompared

The quantities being intercompared are tissue kerma in free air, and tissue dose at depth in a water phantom. Gamma ray kerma and gamma ray dose, respectively, are also evaluated for each neutron energy and measurement situation. All measurements are normalized to the response of the RARAF monitor ionization chamber, and kermas and doses will be reported for the ICRU muscle tissue composition specified in ICRU Report 10b (1).

Tissue kerma in free air 30 cm from the source may be measured for the radiation qualities shown in Table PD-2-1. Tissue dose at depths of 5, 10, and 20 cm along the central axis of a 30 cm cubical water phantom may be measured for the two highest energies. The front face of the phantom is 20 cm from the source so that the position 10 cm deep in the phantom corresponds to the 30 cm position in air. See Figure PD-2-1.

Participants select those energies at which they wish to make measurements, and may request nominal kerma rates up to the maxima indicated, except for ^{252}Cf .

Geometry and monitoring

All measurements are performed at the RARAF low-scatter target position T4, shown in Figure PD-2-2, and along the 0° line colinear with the axis of the ion beam as shown in Figure PD-2-1. The fixed alignment telescope shown in the latter figure is used to accurately locate instruments along the beam axis. A second alignment telescope is translatable along a calibrated tooling bench and, together with an integral right angle telescope and wall-mounted reference mirror, serves to accurately establish instrument height and distance with respect to the source.

Two basic radiation monitoring instruments are provided; a new disc-shaped, 2.4 cc air filled tissue equivalent ionization

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Table PD-2-1

Nominal Radiation Qualities and Kerma Rates

Reaction	Neutron energy MeV	Energy spread ± percent	Max. total tissue kerma rate in air 30 cm from source, rad h ⁻¹	Approx. gamma ray tissue kerma rate, percent of total tissue kerma rate
$^3\text{H}(\text{d},\text{n})^4\text{He}$	15.4	4	40	5
$^2\text{H}(\text{d},\text{n})^3\text{He}$	5.5	7	80	5
$^3\text{H}(\text{p},\text{n})^3\text{He}$	2.1	5	20	5
$^3\text{H}(\text{p},\text{n})^3\text{He}$	0.67	20	5	5
fission	^{252}Cf spectrum		6 (~2mg)	40

chamber, described in Section PA-2B, to monitor radiation emitted in the forward direction, and a neutron-insensitive Geiger-Mueller counter (2,3) to monitor gamma radiation (described in Annual Report NYO-2740-8, Section P-11B). Essential details of the ionization chamber, designated the G chamber, are shown in Figure PD-2-3. The 35° conical transmission angle subtended by the chamber cavity should be adequate for all dosimeters used in air or in the phantom. The location of the GM dosimeter should provide reliable monitoring of gamma rays without obstructing the useful beam.

Figure PD-2-4 shows the arrangement used for irradiations with target-generated neutrons. The target is at the center of this view. From left to right are seen the monitor G chamber, a dosimeter support stand with an overhead X-Y-Z positioner, the GM gamma ray monitor, and the lucite phantom box on its support stand. The translatable transit telescope on the tooling bench can be seen in the background.

^{252}Cf measurements are performed in the same location as the other measurements by removing the neutron-producing accelerator target, and placing the pneumatic receiver tube for the ^{252}Cf source at the target position, as shown in Figure PD-2-5.

Supplementary monitoring measurements are made before each set of participant measurements to verify the integrity of the target

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PD-2-5 INDI apparatus arrangement for
 ^{252}Cf irradiations

and the quality of the radiation produced.

Calibration service

A 10 Ci ^{137}Cs gamma ray source calibrated by the National Bureau of Standards is available at RARAF for use by INDI participants to calibrate their dosimeters. This calibration is to be in addition to the calibration routinely used by the participant, and final values based on both calibrations will be reported. It is hoped that this procedure will clarify differences in the absolute values reported which may be due to differences in calibration sources. Further details of the RARAF calibration source are given in Section PA-2F.

RARAF participation

The participation of RARAF includes measurements for all neutron energies and measurements situations. One set of kerma and depth dose measurements has been completed, except for 0.67 MeV. Measurements for this neutron energy will be made in the near future in conjunction with measurements to be made by two invited participants. At the same time the previous kerma measurements will be repeated. A final set of kerma measurements will be made by RARAF after all invited participants have completed their work.

A new tissue equivalent plastic ionization chamber was designed and fabricated for the kerma measurements. It is designated the KT

chamber and is described in Section PA-2B.

Measurements of gamma ray kerma in air and gamma ray dose at depth in the phantom were performed using a Geiger-Mueller dosimeter identical to that mentioned previously for use as a monitor. The low neutron sensitivity of this instrument (from 0.5 to 1.5 percent for the range of energies considered here) should help evaluate neutron sensitivity for aluminum-argon chambers.

Depth dose measurements in the water phantom were performed using a tissue equivalent ionization chamber identical to that used as a transmission monitor (see Figures PA-2-3 and PD-2-3), except that tissue equivalent gas flow was used and the assembly was rendered water tight with silicone rubber seals. A similar chamber with aluminum cavity walls and Teflon insulators was used with flowing argon to supplement the gamma ray depth dose measurements made with the GM dosimeter.

Results of the RARAF measurements for the INDI project will be reported after all measurements have been completed.

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PD-3 DEVELOPMENT OF A MICROBEAM FOR RADIOBIOLOGY

R.D. Colvett

When a biological specimen is irradiated with neutrons, energy is transferred from the radiation field to the sample by a variety of recoil particles with different distributions of energy and LET. The inhomogeneities of the resultant energy deposition are of comparable size to the microstructure of the specimen, so that the observed biological effects proceed from initial physical causes through a distribution of events operating on a distribution of sensitive elements. A major thrust of microdosimetry has been to determine the relevant distributions that link energy deposition with biological effects.

When the source of charged particles is an accelerator or isotopic source, and the specimen under investigation is thin compared to the range of the particles, a simplification of the microdosimetry is achieved because all the incident particles have the same LET. However, a sensitive element may be traversed by a number of particles, and that number, and therefore the deposited energy, is available only as a member of a probability distribution.

In order to perform radiobiological studies with more specific quantities instead of elements of distributions, a microirradiation facility is being developed at RARAF. The apparatus will retain the constant LET feature of track segment irradiations with two important

additions: the site of the irradiation will be known to be within a diameter of about 3 μm , and the number of particles passing through the site will be counted. The specimens, mammalian cells attached to a thin Mylar sheet, will be localized sufficiently to permit split dose studies with individual cells. The score of radiation damage will be the ability of individual cells to form colonies. The major features of the design of the apparatus and of the initial experiments are as follows:

1. The largest component of the irradiation fixture will be a microscope attached to the RARAF vertical beam (1V), and modified so that the transmitted-light illuminator will be replaced with an extension of the charged particle beam transport system terminated with a microaperture.

2. Immediately above the microaperture a thin transmission-type detector will provide a pulse to mark the passage of a particle. The amplitude of each pulse will be recorded. An incorrect pulse height may indicate an erroneous irradiation, e.g., the passage of a scattered particle rather than a direct particle.

3. The dishes for irradiating the cells will be designed to contain compartments for single cells attached to a thin Mylar sheet. If the cells are deposited from a suspension yielding an average of 1 cell per compartment, about 35 percent of the compartments will have

single cells. The cell(s) in each compartment will be irradiated with a known and recorded number of particles, making each cell in effect an individually identifiable test specimen.

4. Each dish of compartments can contain cells subjected to several types of treatment, e.g., traversal by 1, 2, or 3 particles, or controls.

The several technologies required for this work are being developed simultaneously:

A. Beam collimation and control

1. Commercially-obtained microapertures of 2.5 μm diameter in 25 μm thick nickel foil have been tested and appear to be satisfactory for proton energies up to about 1.5 MeV.

2. Each cell irradiation will involve a predetermined number of incident particles. Electrostatic beam deflectors have been fabricated to switch the beam onto the microaperture or onto a beam-dump aperture outside the experiment area. Control logic for these electrodes will switch the beam off the microaperture after each particle is registered by the detector. For irradiations with more than one particle, the particles will be applied individually.

B. Optics

Since the transmitted-light illuminator must be removed from the microscope to allow connection to the beam transport system,

the cells to be irradiated must be located and positioned in reflected light. Instruments utilizing interference phase-contrast techniques developed recently are available from several commercial sources and are adaptable for this work. Specific hardware development will depend on the optical properties of the irradiation fixture, cell dish, and culture medium.

C. Cell holders must meet the competitive requirements of well-localized cells on a thin substrate in a biologically inert and sterile fixture. Prototype holders have been fabricated for studying cell survival and optical properties.

PD-4 Computed and Measured Relative Kerma.

S.A. Marino

Participants in the International Neutron Dosimetry Inter-comparison (INDI) (see Section PD-2) perform measurements using a variety of dosimeters which differ in size. Each participant is required to report various kerma and dose values for a point on the 0° axis. However, kerma, dose, and neutron energy are generally functions of the angle with respect to the 0° direction, so that the response of each instrument will reflect an integration over its volume, rather than a measurement at the required point. RARAF undertook to provide data which may be used by INDI participants to compute correction factors for individual dosimeters by an appropriate process of integration over the size and shape of the instrument.

Calculations were made using an adaptation of computer program ANY (1) to provide relative tissue kerma in air and mean neutron energy at points on an arc separated by small angular increments for nuclear reactions using the neutron-producing targets employed in INDI. Differential charged particle cross sections used by the computer program to calculate neutron yield are spaced every 10° for the $T(p,n)$ reaction, every 5° for the $D(d,n)$ reaction, and

approximately every 7° for the $T(d,n)$ reaction.

The $T(d,n)$ reaction used to produce neutrons with an energy of 15.4 MeV is almost isotropic so that corrections, if any, for chamber size are small. The variation of dose with angle in a water phantom should, therefore, be very small. The $D(d,n)$ reaction is quite anisotropic so that the dose variation with angle in a water phantom is quite large and difficult to calculate accurately.

Measurements were made of the variation of tissue kerma in air in the vicinity of 0° and at 30 cm from the neutron target. In addition, a 30 cm cubical water phantom was placed 20 cm from the target surface with its central axis collinear with the ion beam direction, and measurements were made of the relative variation of tissue dose at depths of 5, 10, and 20 cm along the axis. All measurements were made for 5.5 MeV neutrons produced at 0° by bombarding a deuterium target with 2.55 MeV deuterons, and using a 6 mm diameter spherical tissue equivalent multiplication ionization chamber with a 1.3 cm diameter nylon cap.

Results of the measurements and the calculations are shown in Figure PD-4-1. A change in slope of the calculated curve (5) occurs at about 2.6 cm since the values for the linear interpolation

Figure Legend

PD-4-1 Relative tissue dose in a 30 cm cubical water phantom and relative tissue kerma in air as a function of transverse displacement off the beam axis for 5.5 MeV neutrons. Curves 1 through 4 were measured at the following distances:

1. 40 cm from target, 20 cm deep in water phantom;
2. 30 cm from target, 10 cm deep in water phantom;
3. 25 cm from target, 5 cm deep in water phantom;
4. 30 cm from target, in air;
5. Computer calculation for 30 cm from target, in air.

Fig. PD-4-1

of the differential charged particle cross sections in the computer program change from the interval 0° to 5° to the interval 5° to 10° . The poor agreement between the calculated (5) and measured (4) curves for tissue kerma in air is believed to be due to uncertainties in the charged particle cross sections.

Tissue dose decreases faster with transverse displacement in the phantom (curve 3) than in air (curve 4) at the same axial distance from the target due to attenuation by the phantom. This attenuation becomes greater with increasing displacement since more water is traversed by the neutrons as the distance to the target becomes larger. This effect decreases as the depth in the phantom increases (curves 2 and 1). In addition, for a given displacement, reduction due to the inverse-square law decreases with increasing depth. Neutron scattering in the phantom and hydrogen capture of thermalized neutrons also reduce dose variations as depth in the phantom is increased. Curves 1, 2, and 3 show the expected increased dose reduction with transverse displacement as depth in the water phantom decreases.

Reference

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Calculations of parameters of dosimetric significance in
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PD-5 Study of a Proposed Neutron Therapy Beam.

S.A. Marino, L.J. Goodman, and R.D. Colvett

It has been suggested by Cranberg (1) that a low-energy accelerator could be used with the reaction ${}^7\text{Li}({}^2\text{H},n)$ to produce a neutron spectrum of sufficiently high energy and yield to be considered for radiotherapy. The Q of 15 MeV for neutron production to the ground state of ${}^8\text{Be}$ is particularly attractive, and if deuteron energy is kept below 2.1 MeV (the energy of the third excited state of ${}^8\text{Be}$), neutron energies in the reaction to ${}^8\text{Be}$ will all be in the range of 4 to 16 MeV. Indeed, earlier work by Foster and Glasgow (2) utilized the continuous spectrum produced by this reaction to measure total cross sections over a broad range of neutron energies. However, as noted by Cranberg, a competing reaction produces lower energy neutrons via ${}^7\text{Li}({}^2\text{H},{}^5\text{He}){}^4\text{He}$ in which ${}^5\text{He}$ decays to ${}^4\text{He}$ producing low energy neutrons, but the relative yields are not known. Furthermore, the presence of ${}^6\text{Li}$ in the target material will provide yet another source of low energy neutrons.

Nevertheless, a study was undertaken to evaluate the possible use of lithium as a target for deuterons to produce a practical neutron therapy beam. Two 10 mg/cm^2 thick targets of unanalyzed commercial grade lithium metal (probably depleted of ${}^6\text{Li}$) were

prepared by the Instrumentation Division of Brookhaven National Laboratory. This thickness exceeds the range of the most energetic deuterons used in this study so that the targets were "thick". They were checked for surface contamination and purity by measuring the threshold energy and yield with the reaction ${}^7\text{Li}({}^1\text{H},n){}^7\text{Be}$ and found to be satisfactory. Deuteron beams of 1.1, 2.0, and 2.5 MeV were used to bombard one of the targets, and various measurements were made at angles of 20° and 100° with respect to the ion beam direction, without a neutron beam collimator. The lowest energy was obtained by utilizing the break-up in the target of a beam of triatomic deuterons with an energy of 3.3 MeV.

Spectroscopy for neutrons with energies greater than about 0.5 MeV was performed using the organic liquid scintillator spectrometry system described in previous Annual Reports (NYO-2740-8 and C00-3243-1). Similar results were obtained for all three deuteron energies used. Two neutron peaks of comparable size were observed in the unfolded spectrum at about 14 and 4 MeV. However, a prominent third peak was observed between 1 and 2 MeV, which was several times larger than those at the higher energies. The number mean neutron energy was at approximately 4 MeV, and the kerma mean was at about 9 MeV.

Further measurements using the beam with an effective deuteron energy of 1.1 MeV were made to evaluate yield, to study the build-up to complete secondary particle equilibrium (important for skin-sparing in the therapy situation), and to estimate the depth dose profile in a tissue-like phantom.

Yield measurements were performed using the disc-shaped ionization chamber described in a previous Annual Report (NYO-2740-1, Section P-15). They indicated a total tissue kerma in air of approximately 10 rad/h per uA of beam current at 10 cm from the target and at an angle of 20°, and 5 rad/h-uA at 100°. The value at 20° is comparable to that obtained with the reaction ${}^3\text{H}({}^2\text{H},n){}^4\text{He}$ using 0.6 MeV deuterons to bombard a thick titanium tritide target. It would also be comparable to the minimum therapy dose rate of 10 rad/m at 125 cm proposed by Brennan (3) if beam currents as high as about 10 mA could be used.

The Geiger-Mueller dosimeter described in a previous Annual Report (NYO-2740-8, Section P-11B) was used to measure gamma ray doses in air, which ranged from 11 to 18 percent.

The disc-shaped chamber was also used to study the buildup to secondary particle equilibrium by adding external discs of tissue equivalent plastic to one thin face of the chamber. An analysis of

these measurements showed that although the 2 mm tissue thickness required for equilibrium was comparable to that obtained earlier for the therapy beam used at Hammersmith Hospital (4), the initial rise in dose with increasing thickness was higher; 99 percent of the equilibrium value was reached at 1.2 mm, and 90 percent at only 0.2 mm. This was probably due to the large component of low energy neutrons. Attempts to improve skin sparing by modifying the spectrum with a 5 cm thick paraffin filter, with or without a backing of 1.2 mm of aluminum, were unsuccessful.

Depth doses were measured at the 20° angle using the 6 mm diameter spherical tissue equivalent multiplication chamber described in previous Annual Reports (NYO-2740-3 and -4). A 1.3 mm diameter nylon cap was used over the cavity end to establish secondary proton equilibrium for the measurements made in air. The ratio of chamber response at a given depth in a 30 cm cubical water phantom to the response in air at the same location was determined for several depths to obtain a depth-dose profile unaffected by inverse-square attenuation. This ratio was found to be 0.5 at a depth of only 6.4 cm. and at a depth of 10 cm it was reduced to 0.41. Even without the further decreases that would result from including inverse-square attenuation and renormalization to the front surface

of the phantom, these values are considerably lower than Brennan's criterion (3) of 10 cm as the minimum acceptable depth for 50 percent of the front surface dose.

A semi-logarithmic plot of the water-to-air dose ratios indicated two attenuation rates, suggesting the presence of two major neutron energy groups, as observed in the spectrometry.

Based principally on the rapid decrease in dose at increasing phantom depths, it was concluded that the proposed reaction is unsuitable for producing fast neutrons useful for radiotherapy.

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PM-1 Event Distributions from Monoenergetic Photons

R. Dvorak and W. Gross

Event distributions have been measured for two photon energies, 11.9 keV and 25.3 keV, at six simulated cavity diameters ranging between .25 and 8 μm . The data have been analyzed to determine several quantities characterizing the microdosimetric distributions.

The fluorescent x-ray generator and the one-inch diameter wall-less counter described previously (1,2) were used as source and detector in this series of measurements. The spectral output of the fluorescent x-ray generator was verified using the silicon detector, low energy x-ray spectrometer also described earlier (2).

Fluorescent x rays of 11.92 keV were generated in an ammonium bromide powder target. The powder, loosely packed into an aluminum holder 3.2 mm thick and having entrance and exit windows of 1.65 mg/cm^2 Mylar, was suspended in the 30 kVcp excitation x-ray beam.

The fluorescent photons, a mixture of bromine K_α and K_β photons superimposed on a scattered photon background, were then passed through a selenium powder filter. This filter was prepared by dispersing 4 g of selenium powder in 6 cc of styrene casting monomer, the mixture being polymerized in an 8.5 cm diameter petri dish. The mold was subsequently broken away, leaving a filter with selenium density approximately 90 mg/cm^2 and a thickness of about 1 mm.

To determine the photon spectrum irradiating the counter, the generator output was examined, using the silicon spectrometer, through an interposed TE plastic filter of thickness equivalent to the wall of the counter container. Corrections were made for the energy response of the spectrometer and the resultant spectrum is shown in Fig. PM-1-1A. Analysis shows that 92% of the photons are within the peak region, 11.3 to 12.5 keV, and the mean photon energy is 12.14 keV.

Event spectra were taken at simulated cavity diameters of 0.25, 0.53, 1, 2, 4, and 7.97 μm . Fig. PM-1-2 shows the microdosimetric distributions $y_d(y)$ plotted as a function of the logarithm of y . The dose and frequency mean values $\bar{y}_D$ and $\bar{y}_F$ as well as the variance are listed in Table PM-1-1.

Fluorescent x rays of 25.27 keV were generated in a tin foil target of thickness 175 mg/cm^2 . The fluorescent photons were then passed through a 63 mg/cm^2 silver foil filter to attenuate the K_β and scattered photons. The photon spectrum shown in Fig. PM-1-1B is, as before, the distribution irradiating the counter interior. Analysis shows that 84% of the photons are in the peak region 24.9 to 26.0 keV, and that the mean photon energy is 23.8 keV.

Event spectra were taken at simulated cavity diameters of 0.25, 0.53, 1, 2, 4, and 8 μm . Fig. PM-1-3 shows the derived microdosimetric distributions $y_d(y)$ again plotted as a function of the

Fig.PM-1-1A

Fig.PM-1-1B

Fig. PM-1-1 Photon spectra within the counter wall

Fig. PM-1-2 The microdosimetric distribution $yd(y)$ plotted as a function of the logarithm of y for 11.9 keV photons with diameter as a parameter. The diameter ranges from 0.25 to 8 μ m (25 to 800 μ g/cm²).

TABLE PM-1-1

MICRODOSIMETRIC AVERAGES FOR MONOENERGETIC PHOTONS

Cavity Diameter μm (nominal)	11.92 keV photons			25.27 keV photons		
	$\bar{y}_D$ (keV/ μm)	$\bar{y}_F$ (keV/ μm)	Rel. Var.	$\bar{y}_D$ (keV/ μm)	$\bar{y}_F$ (keV/ μm)	Rel. Var.
0.25	8.50	3.91	1.17	6.21	2.20	1.82
0.50	6.67	3.39	.96	4.97	2.02	1.46
1.0	5.28	2.94	.79	4.13	1.91	1.15
2.0	4.14	2.48	.67	3.47	1.82	.91
4.0	2.95	1.85	.60	2.82	1.59	.77
8.0	1.88	1.28	.47	2.25	1.33	.69

Microdosimetric averages $\bar{y}_D$, $\bar{y}_F$, and the variance for 11.92 keV and 25.27 keV photons with simulated cavity diameters between .25 microns and 8 microns.

Fig. PM-1-3 The microdosimetric distribution $y \, d(y)$ plotted as a function of the logarithm of y for 25.3 keV photons and for diameters ranging from 0.25 to 8 μm (25 to 800 $\mu\text{g}/\text{cm}^2$).

logarithm of y . The dose and frequency mean values $\bar{y}_D$ and $\bar{y}_F$ as well as the variance are listed in Table PM-1-1. The value of $\bar{y}_F$ was calculated assuming the event frequency was constant below the lowest reachable experimental point. The area below this point is of the order of two percent of the total dose.

For both energies, the distribution $y_d(y)$ shifts to lower values of y as the simulated diameter is increased. At these energies, the predominant interaction for energy transfer is the production of a photoelectron having essentially the same energy as the incident photon. The initial, almost monoenergetic electrons, are produced throughout the cavity and walls so that within the counter itself there is a spread of electron energies making up the "slowing down" spectrum. With a diameter that is small compared to the electron range, the distribution in lineal energy is determined by energy loss fluctuations, path length variations, and the spread in LET of the electrons passing through the counter. As the size increases, the mean energy per event increases and consequently the energy loss fluctuations decrease. Thus, the spread of the distribution decreases as does the relative variance (see Table PM-1-1). The limit at very large diameters, i.e., large compared to the electron ranges, is a single line of fixed energy equal to the energy of the photon. For this case, the spread of the distribution would depend on avalanche statistics and the y value for the peak would be inversely

proportional to the simulated diameter. For the sizes used here this limit is not reached, although for the lower energy radiation the peak is beginning to appear at the largest size used.

The $y_d(y)$ distribution falls more rapidly at high lineal energies as the size increases. These largest y events are produced by electrons which enter the counter with energy just approximately equal to that required to cross the diameter. For two simulated sizes which differ by a factor of two, the energies required to just cross differ by less than a factor of two. For such events, the lineal energy (being the quotient of deposited energy and mean diameter) is lower at the larger size.

The difference in the spectra at the two photon energies is ascribable to the differences in slowing down spectra of the electrons. This spectrum is wider at the higher energy since it extends to higher electron energies. Because the LET of electrons of low energy changes appreciably with energy, the microdosimetric distributions are also wider. This increase in width with increasing energy can be discerned from the relative variance values given in the Table.

The frequency and dose mean values of lineal energy given in Table PM-1-1 also demonstrate the behavior discussed above. The dose mean changes more rapidly with simulated size than does the frequency mean because these changes occur primarily at high lineal energies and $\bar{y}_D$ is particularly sensitive to such changes. For the smaller sizes,

a change of photon energy, however, affects $\bar{y}_F$ more than $\bar{y}_D$ because the distribution is altered more at low lineal energies by a photon energy change, i.e. the low lineal energy content is greater for higher energy photons.

Braby and Ellett (3) obtained microdosimetric data for x-ray spectra at several tube potentials. While our data are still too sparse to do a careful comparison to their results, it seems apparent at this time that a difference in $\bar{y}_D$ will exist when this comparison is made. Using a 250 kV therapy tube at 65 kVp (HVL = 1.9 mm Al) they obtained 5.14 keV/ μ m for $\bar{y}_D$ in a .92 μ m sphere. The values in this work for a 1 μ m sphere are 5.28 at 11.9 keV and 4.13 at 25.3 keV. Considering the inherent filtration at a 250 kV tube, the x rays from this tube should have little spectral content below 25 keV and thus $\bar{y}_D$ would be expected to be even less than 4 keV/ μ m rather than over 5. A more definite comparison will be made when more data are available.

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PM-2 Event Distribution from Heavy Charged Particles.

W. Gross, R. Rodgers and J. Kitzman

The possible use of a heavy ion beam scattered from a gold foil to determine the microdosimetric distributions for the 10 MeV/amu ions at the Yale HILAC was described last year (1). The objective of this study is to obtain the event spectra as a function of the distance between the beam and the center of the counter. In this manner the relative contributions from "direct" and "indirect" events can be assayed separately.

During the past year equipment to accomplish this study was developed, tested and perfected to the point where it could be installed in a reasonable period of time with the required accuracy in alignment. It was found necessary to perform the alignment with the scattered beam collimator evacuated to overcome the difficulties produced by the motion of the equipment when vacuum was applied.

The first data obtained showed an unacceptably high background rate for spectra from indirect events (i.e. those produced when the beam passed outside the counter), which are obtained at a higher gas gain than are direct event spectra. The source of this background seemed to be from the main beam tube and direct beam collimator

upstream from the gold scattering foil as well as from the Faraday cup downstream from the foil. A shadow shield was build to protect the chamber from these unwanted radiation sources. The shield consists of 2 inches of lead both inside and outside the tube housing the scattered beam collimator. A small hole in the lead inside the collimator allows passage of the beam.

After installation of this shield an experiment to study the background was performed using He ions. In order to secure a suitable counting rate for direct events, it was necessary to use a peak direct beam current irradiating the foil of a few tenths of a microampere. During the beam spill time of 2 msec this produced a few events in the counter, i.e. the primary beam was about 10 times more intense than the useable scattered beam. When the counter was moved out of the beam to measure the indirect, lower energy events, the background was found to be more intense than the expected signal by a ratio of about 10^4 . Thus, even though a coincidence system is employed, random coincidences and chamber overload prevent the measurement of the indirect event spectra. It was also found that the source of this background was much more general than had previously been suspected, i.e. it seemed to arise from the entire room (wall scattering, etc.).

If these microdosimetric data are to be obtained using the

present method, the entire large tank housing the chamber will have to be shielded. This is a massive job which will require a great deal of installation time. Other methods involving variation of the beam size while measuring in the direct beam to obtain integral spectra are also being considered. This latter procedure would obtain the data with somewhat reduced accuracy but would fit better with the biological studies which are currently being planned.

Reference

1. W. Gross, R. Rodgers and J. Kitzman, Annual Report on Research Project C00-3243-1, July 1, 1972, page 74.

J.F. Dicello, U. Kraljevic and W. Gross

The biophysical properties of the 3.9-GeV nitrogen ion beam accelerated in the Princeton Particle Accelerator have been described in a number of reports from this laboratory (1-5). Dosimetry for a beam of 5.7-GeV neon ions was presented previously by Gross and Colvett (6). The beam intensity obtained with neon ions was too low to permit biological experiments but it was possible to obtain microdosimetric data. Measurements were made at several depths in a phantom, with particular emphasis on positions just upstream from the Bragg peak. These results are presented together with calculations of biological effectiveness and oxygen enhancement ratios.

Conventional 2.5 cm walled counters, described for the nitrogen ion measurements (2), were used for this experiment. The gas flow system, energy calibration, and data handling techniques also were described. Data acquisition was modified somewhat in order to reduce background corrections. The pulse height analyzer was gated to be on only for a period shortly before to shortly after the beam spill time (1 msec, at a rate of 20 pps).

The phantom was constructed of Lucite and filled with water. The absorber upstream from the measuring equipment consisted of a cylindrical Lucite tank (16-mm diameter) filled

with water, and whose thickness could be varied by remote control. The water path could be set to an accuracy of about 0.1 mm. This portion of the phantom is described elsewhere (6). Downstream from the equipment a nonadjustable, square cross section (30-cm side) water-filled Lucite tank was used. The 11-cm thickness of this portion of the phantom was greater than the range of the neon ions.

Depth dose curves for beam of 5.7-GeV neon ions and 3.9-GeV nitrogen ions (2) are shown in Fig. PM-3-1. Arrows, numbered 1 through 10, designate the positions where microdosimetric data were obtained for the neon ion beam. The microdosimetric pulse height spectra are qualitatively the same as those obtained for nitrogen ions (2). However, the nitrogen data are for a wall-less counter and therefore show a delta-ray component in the entrance plateau region which was not observed here (Fig. PM-3-2, curve a, Position 1). Apart from this aspect, the quantitative differences can be understood merely by noting the difference in stopping power between nitrogen and neon ions. Slightly upstream from the Bragg peak (curve b, position 7) the distribution is distorted at both the low and high energy ends from the rounded triangular spectrum expected on the basis of the path length distribution. The high energy tail is the result of straggling, while the low energy rise results both from straggling and the production upstream in the phantom of ions lighter than neon. Only this latter component appears in curve c, position 9,

Figure PM-3-1 Dose relative to entrance dose for 5.7-GeV neon ions and for 3.9-GeV nitrogen ions (1) as a function of depth in the phantom. The numbered marks on the neon curve indicate the positions at which microdosimetric data were obtained.

Fig. PM-3-1 -67-

taken beyond the range of the neon ions. At the Bragg peak, the distribution is very much wider and the peak is extremely broad. Note that the ordinate scales for curves a, b, and c are arbitrary and different, and have been chosen simply to demonstrate the salient features.

The fractional dose per unit logarithmic interval of lineal energy is plotted against the logarithm of lineal energy ($y d(y)$ vs. $\log y$) for all ten positions in Fig. PM-3-3. At depths much less than the Bragg peak the distributions are sharp and similar, and reflect the almost uniform LET of the ions crossing the counter. The curves shift upward in lineal energy as the Bragg peak is approached and widen considerably in the immediate vicinity of the peak. In this region the ion energy is much lower, the LET changes significantly with energy and, therefore, straggling introduces a very noticeable broadening. In addition a low lineal energy component appears which, at positions less than 0.6 g/cm^2 from the Bragg peak, contains additional small peaks. These effects are undoubtedly produced by ions of lower atomic number which are formed by interactions upstream from the point of observation. Such ions can have ranges very large compared to that of the neon ions and thus are present in increasing numbers at increasing depth. Beyond the peak the curves in Fig. PM-3-3 are entirely attributable to such ions, and they show a broad distribution to low lineal

Figs. PM-3-3a and PM-3-3b

Dose per unit logarithmic interval of lineal energy versus the logarithm of lineal energy at various depths in the phantom. Numbers on the curve refer to the positions in Fig. PM-3-1 and in Table PM-3-1. The curves are plotted in two groups for purpose of clarity.

Fig. PM-3-3a
- 71 -

Fig. PM-3-3b
-72-

Table PM-3-I

Mean Values of Lineal Energy

Fig. 1	Position		Mean Value		
	Relative to peak g/cm^2	Frequency $\bar{y}_F$ $\text{keV}/\mu\text{m}$	Dose $\bar{y}_D$ $\text{keV}/\mu\text{m}$	Relative variance $\frac{\bar{y}_D}{\bar{y}_F} - 1$	
1	-7.84	42	50	0.19	
2	-4.00	50	59	0.18	
3	-1.01	85*(86)+	104*(108)+	0.22*(0.26)+	
4	-0.52	79	147	0.86	
5	-0.30	117	196	0.68	
6	-0.15	170	320	0.88	
7	-0.05	260	640	1.46	
8	0	280	680	1.43	
9	+0.05	85*(96)+	183*(310)+	1.15*(2.23)+	
10	+7.78	4.0	14.7	2.67	

*Based on incomplete spectrum

+Extrapolated values

energies.

The values of the frequency mean, $\bar{y}_F$, and the dose mean, $\bar{y}_D$, as well as the relative variance have been calculated for each of the ten positions indicated on Fig. PM-3-1 and the results are listed in Table PM-3-I. Because of the need to extrapolate the integrand to zero lineal energy in the denominator of $\bar{y}_F$, the values of the frequency mean are less certain than those of $\bar{y}_D$. The other integrands need only be interpolated to zero because they approach zero with decreasing y . It was here assumed that below the lowest experimental point the frequency decreases linearly to the origin. The mean values have been plotted in Fig. PM-3-4 in order to demonstrate their sharp variation near the Bragg peak. For example, the dose mean decreases by a factor of more than two as the depth is decreased by 150 mg/cm^2 from the peak.

The objective of these microdosimetric measurements was to secure data principally in the region just upstream from the Bragg peak. At depths greater than 1 g/cm^2 upstream from the peak (positions 1, 2 and 3) and just downstream from the peak (position 9) the data were acquired in one pulse height spectrum. At positions 3 and 9 the data obtained did not quite include all the significant events at high lineal energies. The extent of this deficiency can be seen in the curves for these two positions shown in Fig. PM-3-3. The integrations performed to obtain the mean lineal energies given without brackets in

Fig. PM-3-4
-75 -

Dose mean, $\bar{Y}_D$, (closed circles and
frequency mean, $\bar{Y}_F$, (open circles)
lineal energies as a function of
depth in the phantom measured from
the Bragg peak.

Table PM-1-I were terminated at the highest lineal energy point for which experimental data were obtained. Thus, a portion of the distribution was ignored for each of these values which affects the dose mean value primarily, but which has little effect on the frequency mean. To determine the extent of this deficiency the curves of Fig. PM-3-3 were extrapolated to zero at high lineal energies with the shape of the curve for the adjacent position as a guide. The integrals were then recalculated using the extrapolated points and the results are shown in parentheses in Table PM-1-I.

The extrapolation of the curve for position 3 results in only minor changes, but that for the curve at position 9 results in a change by almost a factor of two in the value of $\bar{y}_D$. However, the effect of this operation on the theoretical biological quantities (to be discussed below) is not significant.

The values of $\bar{y}_F$ and, therefore, the corresponding relative variances do not vary in a smooth fashion between positions 3 and 5. The $\bar{y}_F$ value at position 4 appears somewhat low compared to positions 3 and 5. The presence of low lineal energy components, which can be observed in Fig. PM-3-3 for this position, contributes sufficiently to produce this low value and these components are not evidenced at position 3. At position 5 the contributions to the distribution at high lineal energy have increased so the significance of this lower portion is reduced.

Kellerer (8,9) has shown that the relative variance in microdosimetric distributions can be approximated by a sum of terms which includes the relative variance of the chord length distribution (0.125 for a sphere), of the energy straggling distribution and of the LET distribution of the ionizing particles. At position 1 and 2 (furthest upstream) the experimental values for the relative variance are not inconsistent with the assumption that the spread in LET is small, and the values are merely the sum of 0.125 for the chord length distribution and a small relative variance from energy straggling. In the upstream region energy straggling is small and the LET of the high energy ions changes only slightly with ion energy, confirming this conclusion. With a small spread in LET, it is valid to compare a calculated stopping power value (LET_{∞}) at these two positions with the experimental $\bar{y}_F$. The calculated values for LET_{∞} were obtained by using the range and stopping power data provided by the ICRU for protons (10) and by assuming the stopping power in the momentum region above 150 MeV/amu is proportional to the square of the atomic number of the ion. The best estimate of the thickness of material upstream from the counter (1 g/cm^2) was included to obtain 40 keV/ μm at position 1 (249 MeV/amu) and 53 keV/ μm at position 2 (163 MeV/amu). These values are to be compared to 42 and 50 keV/ μm for $\bar{y}_F$, respectively. This agreement is considered excellent in view of the uncertainty in $\bar{y}_F$.

The quantity $(1-D(y))$ is the fraction of the absorbed dose delivered at lineal energies exceeding the specified value of y . In Fig. PM-3-5 are plotted the values of $(1-D(y))$ vs. y for the most upstream position (#1), 0.15 g/cm^2 upstream from the Bragg peak (#6), at the Bragg peak (#8), and at a position well downstream from the peak (#10). Also on the figure are the data for similar positions for nitrogen ions (2) (the curve corresponding to 0.15 g/cm^2 from the peak is actually for 0.12 g/cm^2 from the peak and the most downstream position is closer to the peak). In all but the most downstream cases the neon ions deliver more dose at a higher lineal energy than do the nitrogen ions. In the entrance region, however, 97 percent of the dose from the neon ions is from events of less than $100 \text{ keV}/\mu\text{m}$ and the dose mean is just $50 \text{ keV}/\mu\text{m}$. At the peak both ions deliver the preponderance of their dose well above $100 \text{ keV}/\mu\text{m}$. Notice, however, that over 45 percent of the neon dose is delivered at y values greater than $600 \text{ keV}/\mu\text{m}$. In the region just upstream from the peak the difference between the two ions may be of biological significance particularly with regard to RBE and OER in that the neon ions deliver 93 percent of their dose above $150 \text{ keV}/\mu\text{m}$ while the nitrogen ions deliver less than 20 percent of the dose in this region.

Curves d (beyond the peak) show the nitrogen ions to have a higher lineal energy content than do the neon ions. However, the curves are not strictly comparable because the neon data are for a greater depth from the peak than are the nitrogen. The

Fig. PM-3-5 $(1-D(y))$, fraction of dose exceeding a given lineal energy, versus the logarithm of lineal energy for neon (solid lines) and nitrogen ions (dashed lines) (2). Phantom depths are a) well upstream from Bragg peak, b) about 0.15 g/cm^2 from peak, c) peak, and d) well downstream from peak.

Fig. PM-3-5
- 80 -

curves are presented to demonstrate their qualitative similarities and to show the changes which occur beyond the peak.

Owing to the low beam intensity no biological data were obtained during the course of these neon ion measurements. However, Kellerer and Rossi (11) were able to obtain some correlation between microdosimetric data and biological effects for nitrogen ions. By use of the same approach, a quantity termed "effective lineal energy" and symbolized y_A^* can be calculated from the microdosimetric distribution. It is analogous to $\bar{y}_D$ except for the fact that it takes into account the saturation of biological effects which has been found to occur for high LET radiation (9). For exponential survival curves this quantity is directly proportional to the theoretical RBE. Calculated values of y_A^* for the neon ions are shown in Fig. PM-3-6 as a function of depth in the phantom as measured from the Bragg peak. The saturation lineal energy required for the calculation was chosen to be 125 keV/ μm which is that value derived by Kellerer and Rossi (11) from the data of Barendsen (12) and Todd (13). Also shown in Fig. PM-3-6 are the y_A^* values for nitrogen ions (11).

Notice that for the neon ions the value of y_A^* (and therefore the theoretical RBE) reaches a maximum considerably upstream from the Bragg peak (0.5 g/cm^2), sharply drops to a minimum, and rises again beyond the peak. This minimum is created by the large "wastage" of energy in the immediate vicinity of the Bragg peak.

Fig. PM-3-6 Effective lineal energy Y_A^* , versus depth in phantom measured from the Bragg peak for neon and nitrogen (8) ions.

No such decrease is noted in the nitrogen ion curve. Of course, such a dip would also be expected merely from a consideration of the LET of the ions and the biological data for high LET particles.

By the same technique used for the calculation of y_A^* , a quantity y_H^* proportional to the effect probability per unit dose in the absence of oxygen can be calculated. This permits a theoretical determination of the OER and values of this OER are shown in Fig. PM-3-7, again plotted as a function of depth measured from the Bragg peak. Nitrogen ion data are also shown in the figure (11). The neon ion calculation shows a larger region over which the OER is low as compared to nitrogen. For ions heavier than neon this region would widen rapidly with increasing atomic number. The calculations used here to predict the OER are questionable in that the calculated OER values for nitrogen ions were found to be too low, probably because they involve a parameter obtained for data for ions of quite different radial profiles (11). Proper analysis may require information on the pattern of energy deposition in much smaller sites than the 2 μm used in these experiments. However, the calculated neon ion OER values obtained here are useful as a comparison to the nitrogen ion values. Unlike the OER, the RBE has been shown to depend on energy concentration in sites of the order of a micrometer in size, (14) and therefore the y_A^* values can be considered reliable for the prediction of biological effectiveness.

Fig. PM-3-7 Theoretical OER versus depth in phantom measured from the Bragg peak for neon and nitrogen (8) ions.

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PM-4 Event Distribution from Monoenergetic Neutrons

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A program has been under way for some time to determine the microdosimetric distributions for the entire range of monoenergetic neutrons currently employed at RARAF, and has already been described in part (1,2). Complete data have been obtained with a wall-less counter for monoenergetic neutron beams ranging from 0.22 to 15 MeV, and for simulated counter diameters ranging from 0.5 to 8 μm ; in addition data has been collected for the 60 and 80 keV neutron spectra used in some biological experiments. The complete tabulation of energy and sizes is given in Table PM-4-1. In addition, for the same monoenergetic range, at a few selected energies, data were obtained with a walled counter simultaneously with the wall-less counter in order to assess the magnitude of "wall-effects", particularly the delta ray effect (3).

The final analysis of this considerable body of data is not yet completed. Preliminary results below demonstrate some of the salient features and functional dependencies but final numerical values will undoubtedly be somewhat different from those presented here.

In our prior report (2) the general behavior of the microdosimetric spectra with neutron energy was described. Fig. PM-4-1 shows the

Table PM-4-1

Tabulation of Neutron Microdosimetry Data

Neutron Energy (MeV)	Simulated Sphere Diameter (μm)				
	0.5	1	2	4	8
.06 spectrum	X	X	X		
.08 "	X	X	X		
0.22	X	X	X		
0.34	X	X	X	X	
0.43	X	X	X	X	
0.55	X	X	X	X	
1.0	X	X	X		
1.8	X	X	X		
2.2		X	X		
2.8	X	X	X		
4.5	X	X	X		
6.0		X	X		
15.0	x	X	X	X	X

Dose per logarithmic interval of lineal energy vs. the logarithm of the lineal energy for a number of neutron energies in MeV as noted on the figure.

Fig. PM-4-1

entire pattern of neutron energy dependence over the range from 0.22 to 15 MeV. In this figure is plotted the dose per unit logarithmic interval of lineal energy against the logarithm of the lineal energy ($y d(y)$ vs $\ln y$). Beyond those features previously noted these more complete data show the following:

1. For neutron energies 1.02 MeV and below the entire distribution is essentially determined by the recoil protons. Even at higher neutron energies, the pattern below y values of 150 keV/ μ m reflects the recoil proton energy distribution which, of course, decreases in energy with decreasing neutron energy. Because the LET increases as the proton energy decreases, the peak in the region between 10 and 100 keV/ μ m shifts to higher values of lineal energy. The 0.22 MeV peak is at a lower lineal energy than that for 0.55 MeV, i.e. a reversal of the pattern. At 0.22 MeV about half the protons are formed with energies below that of the Bragg peak (100 keV) and the slowing down spectrum is largely composed of protons below this energy. This reversal is a result of the fall of LET with proton energy below the Bragg peak.

2. The alpha peak resolved on the 15 MeV curve at about 300 keV/ μ m is present at 5.8 and 2.8 MeV but not clearly separated from the heavier ion peaks (carbon and oxygen recoils). These recoils, however, are themselves not resolved even on the 15 MeV curve.

The behavior of the lineal energy distributions with the simulated size of the sphere can be seen in Figs. PM-4-2 and PM-4-3 for 0.43 and 15 MeV neutrons respectively. The dependence of the 0.43 MeV distributions on cavity size can be understood in terms of the energy distribution of the protons traversing the counter. A significant number of these protons have a range which is comparable to 1 μm and therefore there is a sizeable contribution from "insiders" (4) at the larger sizes. For insiders, i.e. protons which arise and terminate inside the cavity, the energy deposited is constant so that these shift left, or to a lower value of lineal energy as the cavity size increases. Also, protons in the neighborhood of the Bragg peak (100 keV) have a mean LET which decreases as the track length over which this LET is measured increases. Such protons contribute significantly to these distributions also causing the spectra to shift to the left as the cavity size increases.

The behavior of the width of these curves with increasing size is interesting when contrasted with that for monoenergetic photons (see Section PM-1). In the case of photons the distributions widen rapidly as the size of the cavity decreases because of the increase in energy loss fluctuations. With 0.43 MeV neutrons the effect of size on the distribution widths is small because energy loss fluctuations do not play a significant role in broadening these distributions. The dominant

Dose per logarithmic interval of lineal energy vs. the logarithm of the lineal energy for 0.43 MeV neutrons at several simulated sizes. The simulated diameters in μm are given in the figure.

$y \text{ (keV/}\mu\text{m)}$

Fig. PM-4-2

Fig.PM-4-3 Dose per logarithmic interval of lineal energy vs the logarithm of the lineal energy for 15 MeV neutrons at several simulated sizes. The simulated diameters in μm are given on the figure.

factor is the spread in the LET of recoil protons.

At 15 MeV, the components of the distribution that are heavier than proton i.e. those with y above 150 keV/ μm , show a behavior similar to that of the protons at 0.43 MeV. The shift to the left of this part of the spectrum with increasing size is first observed for carbon and oxygen recoils and then for alphas for the reasons given above. It is interesting to note, however, that the bimodal proton distribution seen at 1 μm (Fig. PM-4-1) and at 1/2 μm (Fig. PM-4-3) is completely erased at 4 μm . The upper peak at the smaller sizes is formed by protons near the Bragg peak whose range is only slightly over 1 μm . Thus at 4 μm most protons of this energy are insiders and blend into the lower y events at large cavity sizes.

Kellerer (3,5) has described and predicted the magnitude of the distortion due to event simultaneity in gas filled cavities surrounded by solid walls as compared to similar volumes in a homogeneous material. Three classes of distortions are described but only one, the delta-ray effects, is of importance for neutron fields of 15 MeV or lower in energy. The magnitude of this distortion increases with increase of neutron energy and with decrease of the size simulated by the cavity. As an example of the magnitude of this process Kellerer predicts that less than 10 per cent of the absorbed dose will be delivered by indirect

events in a 1 μm cavity irradiated by 15 MeV neutrons (5). Indirect events are those registered separately by a homogeneous (wall-less) counter while being registered simultaneously with direct events in a biphasic (walled) counter.

The separation of the dose into indirect and direct components in the wall-less counter used in these neutron experiments is not feasible. These indirect events are low energy events and are masked by the small gamma-ray contamination of the neutron field. While this gamma contribution is small in terms of absorbed dose it is far from negligible in terms of number of events in the low energy or lineal energy region.

While Kellerer has not predicted the decrease in the number of events measured for neutron fields with a conventional walled counter, he has given information as to the fraction of indirect events for protons as a function of their energy with cavity size as a parameter (3). For protons above 5 MeV in a 1 μm cavity this fraction is almost independent of proton energy and is about 15 percent.

The fraction of indirect events can be assessed experimentally from the frequency mean values of the lineal energy $\bar{y}_F$, as measured in the walled and wall-less counters. The frequency mean is defined as

$$\bar{y}_F = \frac{\int_0^{\infty} y n(y) dy}{\int_0^{\infty} n(y) dy}$$

where $n(y) dy$ is the number of events in the interval y to $y + dy$.

It is easily shown that, because the number of direct events is the same in the walled and wall-less counter exposed to the same dose, the fraction of indirect events, F_I , is

$$F_I = 1 - \frac{(\bar{y}_F)_{WL}}{(\bar{y}_F)_W}$$

where the subscripts WL and W refer to the walled and wall-less counters, respectively.

While the determination of F_I by this means is straight forward in principle, in practice it is hampered by several factors of which two are particularly serious. These are first, the need to extrapolate the number or frequency distribution to zero in order to calculate $\bar{y}_F$ and second, the presence of gamma ray contamination of the neutron field. The frequency spectrum is, in all cases, increasing in magnitude as the lineal energy decreases to the lowest values measured. The area between zero and the lowest lineal energy point for which the distribution is measured can contribute significantly to the total area (the denominator in the expression for $\bar{y}_F$). The low lineal energy events arise from gamma rays in both counters and from indirect events

in the case of the wall-less counter. Thus, the shape of the frequency curve for the walled counter may be different from that of the wall-less at low lineal energies. If the shapes were identical then a reasonable extrapolation applied equally to both distributions would produce an accurate ratio of frequency mean lineal energy.

The counters were located in the irradiation facility in a manner made as symmetrical as possible. However, there were slight differences between the two with regard to the room walls. Because these walls are a source of gamma ray contamination it is uncertain that the gamma ray fields at the counters were identical. In addition the physical dimensions of the counter assemblies are significantly different which again may be a source of asymmetry. From a dose viewpoint any differences were insignificant, but nevertheless these differences may be important to the frequency distribution.

One additional factor makes the calculation of F_I uncertain. The wall-less counter volume is determined by the electric field in the neighborhood of the spherical grid used to delimit this volume. Neglecting the slight blurring of the boundary of this volume resulting from electron diffusion which is relatively unimportant here, it is uncertain as to whether this boundary occurs at the inside of the spherical grid, the outside of the grid, some surface between, or at some different location

because the shape of the electric field is not well known. In addition, the position of this boundary is undoubtedly a function of the potentials applied to the counter. Fortunately, these boundary uncertainties are small and, therefore, do not affect microdosimetric measurements made with the counter in any important fashion. They are important, however, when the magnitude of a small wall distortion is considered.

The effect of these difficulties can be seen in Table PM-4-11 where the $\bar{y}_F$ values for the walled and wall-less counters are given for several neutron energies and for simulated sizes ranging from 1/2 to 2 μm . The values were calculated assuming the frequency distributions decreased linearly to zero with lineal energy below the lowest experimental point. Thus, they may be considered as upper limits of the true values. In the last column the ratio of indirect to total events, F_I , is given for each case. These were calculated from the $\bar{y}_F$ ratios as indicated above. Notice that in every case except at 15 MeV this quantity is negative. For truly identical conditions a negative value is not possible. The reason for this error in the $\bar{y}_F$ ratios can be discerned from a comparison of the pulse height spectra of the walled and wall-less counters. Below 600 eV the walled counter shows a far greater number of events than does the wall-less.

Table PM-4-11

Frequency Mean Lineal Energies and Calculated Fraction of Indirect Event

E_n (MeV)	Nominal Size (μm)	$\bar{Y}_F$ (keV/ μm)		F_I $1 - \frac{(\bar{Y}_F)_{WL}}{(\bar{Y}_F)_W}$
		Walled	Wall-less	
0.2	1	15.2	17.4	-0.14
0.55	1	28.8	34.8	-0.21
0.55	2	22.2	23.9	-0.08
2.2	1	5.6	7.1	-0.27
15	1/2	9.2	7.6	+0.17
15	1	6.4	5.3	+0.15

The room geometry was such that a somewhat greater gamma-ray contribution might be expected in the walled counter which along with other equally minor differences could be responsible for these events. However, to date this has not been firmly established.

Despite these obvious difficulties the data do show that, at 15 MeV, there is a significant difference as compared to the other energies and it is in the direction expected. The magnitude of this difference is uncertain, and it will be necessary to examine the gamma-ray and extrapolation problems more carefully before any final results can be obtained.

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PM-5 Studies for Nanometer Microdosimetry

S. Pszona

There is considerable interest in obtaining the pattern of energy deposition in small volumes with dimension of nanometers. An attempt has been made to construct a device for studying this problem.

The scheme is illustrated in Fig. PM-5-1. The positive ions, produced in a volume above an orifice F, move in a constant electric field toward that orifice. Some of these ions pass through the orifice F and are then accelerated and detected by a "channeltron." The differential pumping through the orifice, using a high-speed pump, maintains the low gas pressure required for the channeltron.

The number of pulses registered at the output of a channeltron, N_s , can be expressed as:

$$N_s = \sum \eta_{iF} \eta_{is} n_i \quad (1)$$

where η_{iF} - is the efficiency of collection of type i ions through an orifice F

η_{is} - is the efficiency of a channeltron for detecting type i ions

n_i - is the number of type i ions created in the volume defined by the field over an orifice F.

Fig. PM-5-1 Schematic presentation of an idea of a "track ion counter."

The expected values for η_{iF} and η_{iS} have been discussed in many publications (1,2,3) which deal with the operative characteristics of channeltrons. The following conclusions can be inferred:

a) the detection efficiency of ions reaches a plateau and, within the accuracy of reported measurements, is independent of the mass of ions above 3 keV.

b) the absolute value of detection efficiency of ions depends on many factors which are usually not well determined and which may vary by a factor of 2 for different types of channeltrons.

While (a) is a favorable feature of channeltrons, (b) requires a special method for evaluation of the detection efficiency of ions by a channeltron.

The efficiency of collection of type i ions through an orifice F, η_{iF} , has been evaluated following Huxley and Bennett (4) and Cochran and Forester (5). The efficiency with which type i ions reach the surface of an orifice, R_i , can be expressed as:

$$R_i = 1 - \left\{ 1 + \left(\frac{b}{h} \right)^2 \right\}^{-1/2} \exp \left(\frac{W_i}{2D_i} \left\{ \left(1 + \left(\frac{b}{h} \right)^2 \right)^{1/2} - 1 \right\} \right) \quad (2)$$

where: W_i - ion drift velocity along electric field direction

D_i - coefficient of diffusion of type i ions

$2b$ - diameter of an orifice

h - distance between an orifice and ion source

The R values for H_2 and N_2 ions have been calculated, according to formula (2), as a function of $\frac{W}{D}$ and for a given volume $\frac{b}{h}$ and are plotted in Fig. PM-5-2. It is seen from Fig. PM-5-2 that R attains a value close to unity when an appropriate electric field is applied. For H_2 and N_2 , $E \geq 10$ V/cm, and $\frac{b}{h} = 0.1$, R is not lower than 0.96. The values for W and D have been taken from the data of Saporoschenko and von Engel (6,7,8). The value for η_{iF} approaches 1 when the thickness of a metal sheet in which the orifice is located is properly chosen. Thus, it seems possible to construct a device in which η_{iF} and η_{is} will be constant and independent of the type of ion. This is very promising for situations where a tissue equivalent gas mixture is to be used.

A "track ion counter" has been constructed based on these considerations. The main effort at present is directed toward the design of an efficient ion source for estimating overall efficiency $\eta = \eta_{iF} \eta_{is}$. A method for estimating the absolute value of the number of ions emitted by a source has been devised.

Efficiency of ion collection through an orifice F versus $\frac{W}{D}$ and field strength E for different values of $\frac{b}{h}$. Dashed curve - data for N_2 . Solid curve - data for H_2 .

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PA-1 OPERATION AND DEVELOPMENT OF THE FACILITY

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Introduction

The Van de Graaff accelerator is at present being fully used during its scheduled one shift per day, five days per week. High priority projects are frequently accommodated at short notice by overtime shifts, but advance scheduling of two to three months is still required for accelerator use involving blocks of time of several days. All new beam lines are now fully operational and in use.

The total accelerator time used has increased over the past five years to the present saturation level shown in Table PA-1-1. During 1972 the accelerator was idle during only 4 percent of the scheduled time. For the first four months of 1973, overtime use of the facility caused the total time used to exceed the scheduled time. This overtime was needed principally for the International Neutron Dosimetry Intercomparison (INDI), and is reflected in the high usage listed for radiological physics in 1973. 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.

Table PA-1-1

<u>Application</u>	<u>Percent of annual total time scheduled</u>				
	1969	1970	1971	1972	1973 [*]
Radibiology	7	7	17	10	14
Dosimetry for radiobiology	9	11	14	8	15
Radiological physics	17	28	22	28	47
Van de Graaff development	23	25	31	42	15
Target development	6	10	2	5	2
<u>Miscellaneous projects</u>	--	--	--	<u>3</u>	<u>6</u>
Total time used	62	81	86	96	102
Idle time	38	19	14	4	1

* January through April only

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

1. Genetic effects of monoenergetic neutrons on Drosophila.
2. Somatic mutations in Tradescantia produced by monoenergetic neutrons (see Section BP-4).
3. Effect of monoenergetic neutrons on the survival of spores of Osmunda regalis.
4. Studies of RBE as a function of neutron energy using Chinese hamster cells in tissue culture.
5. Induction of mammary gland neoplasms in rats irradiated with monoenergetic neutrons.
6. Survival of colony-forming units in mouse bone marrow irradiated with monoenergetic neutrons.
7. Instrument development and dosimetry studies made in connection with the above radiobiology investigations. See Section PA-2 and PD-1.
8. Production and monitoring of reproducible neutron beams for use in INDI. See Section PD-2.
9. Microdosimetric measurement with neutrons, using walled and wall-less tissue equivalent proportional counters. (See Section PM-4).
10. Dosimetry studies for the production of a dual ion beam useful for irradiation of cells in tissue culture. (See Section PD-3).

11. Dosimetry studies for the production of a charged particle microbeam useful for irradiation of individual cell nuclei in tissue culture. (See Section PD-4).

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

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

Facility development

Beam lines for the four new target positions have been completed including all auxiliary services. An overall view of the RARAF Van de Graaff accelerator, and the new beam lines is shown in Figure PA-1-1. Figure PA-1-2 shows the termination in the radiological physics laboratory of the new beam line used for charged particle studies.

All controls for operation of the accelerator and beam transport systems have been relocated into the new console shown in Figure PA-1-3. The safety door to the machine area has been relocated so that the old console is now part of the machine area.

Efforts concentrated on refining the beam handling equipment for target position T4, and installing in this area the additional equipment required for INDI. This included a system of two transit telescopes

Fig. PA-1-1 Beam lines in the RARAF T area. The 3.7 m high elevated line is at the right, and the 4 MV Van de Graaff accelerator is visible in the background.

Fig. PA-1-2 The termination of beam line 5
in the RARAF radiological physics
laboratory.

PA-1-3 Control, dosimetry, and safety console of the RARAF Van de Graaff accelerator.

and a reference mirror for accurate positioning of dosimeters, a liquid phantom and support stand, a precision long counter, a pneumatically-controlled 2.5 mg ^{252}Cf source, and a 10 Ci ^{137}Cs gamma ray source calibrated by the National Bureau of Standards. (See Sections PD-2 and PA-2 for details).

An interesting effect was observed with triatomic hydrogen beams. Triatomic molecules break up into separate atoms when they hit residual gas in the beam pipes, since their binding energy (~ 1 eV) is negligible compared to the beam energy ($\sim 10^6$ eV). The monatomic component of the beam focuses differently than the triatomic component, and so does not reach the target. Upon removal of the beam stop, target current is initially high and then drops as pressure in the beam pipe increases. At equilibrium, the beam current is reduced by 10 to 15 percent. For some experiments this effect can be a problem and alternatives for additional pumping nearer the targets are being considered.

Significant misalignment of the vertical beam line 1V had required the beam to be tightly focused to avoid hitting the target holder. This line was disassembled and carefully reassembled to eliminate the problem. Target current at 1V is now limited by heat dissipation problems in the targets, by the ion source, or by the experiment.

Components for the beam sampler mentioned in the previous Annual Report (COO-3243-1) have been fabricated. Low intensity beams will be periodically deflected electrostatically into a Faraday cup for a short period of time. Currents larger than a preset maximum will cause the main beam-stop to be inserted. The system will also be useful as a fast beam-stop if the beam is well focused at the Faraday cup.

Van de Graaff accelerator

Energy regulation system

The generator voltmeter-corona feedback energy regulation system works very well except at voltages below about 2 MeV. At these low voltages the electron drift velocity from the corona head to the terminal is reduced, thereby introducing an effective phase shift which causes the system to oscillate if the gain is too high.

A slit regulation system has been developed as an alternate input to the corona feedback system. Beam position at a set of slits after a bending magnet depends on beam energy. An error signal derived from the logarithm of the ratio of the currents picked up on the left and right slits drives the corona current to regulate beam energy. This system is essential for the INDI measurements using the elevated 45° beam line since without slit

regulation beam current can fluctuate 20 to 30 percent, but with this regulation current is stable within 1 percent or less.

Herkolite internal shield rings

Some shield rings described in last year's Annual Report (COO-3243-1) have been installed near the terminal where the problem with voltage breakdown has been greatest. Only limited voltage-testing of the accelerator has been possible, but operation at 4.2 M V with moderate beam currents has been accomplished. Irradiations using 4.2 MeV triatomic protons are planned as an alternate to the use of 1.4 MeV monatomic protons. The advantages will be increased energy stability and higher dose rates due to higher effective beam currents.

Maximizing charge transport capability of the belt

The modifications mentioned in last year's Annual Report (COO-3243-1) have been completed and the belt can now carry 1.4 mA of current, but the tension on the belt must be increased to keep it from slipping.

Neutron-producing targets

Tritium and deuterium neutron-producing targets used for radiobiology have been replaced periodically when their yield

became unacceptably low. In addition, new deuterium and tritium targets were acquired this year for exclusive use during the International Neutron Dosimetry Intercomparison.

Targets are tested by comparing measurements of neutron dose rate per unit of incident ion beam current to calculated values. A total of twenty-one tritium targets, ranging in titanium substrate thickness from 0.5 to 3.0 mg/cm², and three deuterium targets, ranging in titanium substrate thickness from 5.4 to 6.4 mg/cm², have been produced for RARAF by the Instrumentation Division of Brookhaven National Laboratory. Of the sixteen tritium targets tested to date, only six have had tritium-to-titanium atomic ratios greater than 1.0, the minimum loading ratio considered acceptable. Loading ratios for the unacceptable tritium targets ranged from 0.1 to 0.8, while those for acceptable targets ranged from 1.1 to 1.7. All three deuterium targets were tested and found to be acceptable having loading ratios from 1.3 to 1.5.

The large number of poor tritium targets received in the past may have been caused by the use of too little tritium in the loading process in order to minimize the amount of tritium which is unavoidably discarded. The amount of tritium used during loading has now been increased and all four new targets tested, comprising two different loading runs, were found to be acceptable.

In addition to the deuterium and tritium targets, two lithium metal targets approximately 10 mg/cm^2 thick were also prepared this past year for the ${}^7\text{Li(d,n)}$ therapy beam experiment described in Section PD-5.

Radiation safety

The principal radiation safety work consisted of a series of stray radiation surveys and incorporation into the RARAF safety system of provisions for use of the ${}^{252}\text{Cf}$ and ${}^{137}\text{Cs}$ sources. This work was done for INDI which utilizes these sources and also requires production of high neutron dose rates at the roofless, low scatter target position T4. This location is at an elevated position which results in significant air scattering of radiation into unshielded and uncontrolled areas of the facility.

The stray radiation surveys were performed by members of the Health Physics Division of Brookhaven National Laboratory in consultation with the RARAF staff. The measurements yielded a set of dose equivalent isodose diagrams, and indicated a maximum of about 24 mrem/h outside of the shielded area during 15 MeV neutron production using the reaction ${}^3\text{H(d,n)}{}^4\text{He}$. This high level existed in a normally unoccupied area, whereas maximum levels were typically less than 15 mrem/h in occupied areas. Furthermore, the INDI work requires only occasional use of neutron beams at their maximum

intensities, and even then the duty cycle will probably be about 50 percent. Certain restrictions and warning signs have been introduced for the duration of the INDI project. The following procedures for INDI irradiations were instituted in accordance with recommendations of the health physicists:

- a. The outside roof above the unshielded experimental area has been restricted by locking all access doors.
- b. During operations producing high dose equivalent rates, signs defining the 2.5 mrem/h line are placed in and around the facility.
- c. All individuals working within the RARAF building have been directed to wear personnel monitoring devices.

The controls for operation of the ^{137}Cs and ^{252}Cf sources have been interlocked with the existing RARAF safety system to assure that they are used only if the irradiation area has been locked and appropriate warning alarms have been sounded prior to exposure of the source. All emergency stop buttons, inspection procedures, and required door interlocks remain active and function for these isotopic sources as they do for accelerator-produced hazards. The pneumatic transfer system installed for the ^{252}Cf source utilizes an air tank instrumented to return the source capsule to its shielded position, or to prevent transfer out of the shield, if air pressure falls below a preset minimum, or if the interlock system is violated. Sections PA-2-E and F give further details.

Future plans

All radiobiology projects listed earlier in this report will be continued, except for the Drosophila studies. In addition, it is expected that charged particle irradiations of mammalian cells in tissue culture will begin using a dual ion beam and possibly a microbeam.

Development of the accelerator, beam transport system, and dosimetry instrumentation will emphasize the novel arrangements required to produce the special charged particle beams useful for mammalian cell irradiations. The microbeam experiment especially will entail use of a sophisticated assembly of apparatus for delivering known numbers of protons into individual cell nuclei.

Other planned radiological physics work includes developing a low energy neutron spectrometer and completing all measurements for the neutron dosimetry intercomparison. It is expected that improvements in neutron-producing targets will increase the maximum dose rates attainable for radiobiology.

PA-2 DEVELOPMENT OF COMPREHENSIVE DOSIMETRY APPARATUS

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

a) Miniature electrometer amplifiers

The development at RARAF of miniature solid-state electrometers has been described in previous Annual Reports (NYO-2740-7 and NYO-2740-8). Section PA-2 of last year's report (COO-3243-1) included a circuit diagram. The control module has been modified to increase reliability and to reduce maintenance.

Four new electrometers have been assembled, two with 10^{10} ohms effective feedback resistance and one each with 10^9 and 10^{11} ohms. Additional construction of one each of the four decade values from 10^9 to 10^{12} ohms is planned.

Some experimental work was done to study the effect of radiation on the electrometer, measured as output voltage for a given dose rate or dose. Although both neutrons and x-rays were used, only the latter yielded significant effects due to the much higher exposure rates available. The results reported below were obtained using 250 kVp x-rays at exposure rates of about 1.5 kR/h.

Using lead bricks to collimate the x-ray beam, it was possible to selectively expose specific portions of the electrometer. Radiation response of the intentionally unfilled air volume above the input contact was generally about 1 pA. This was a factor of five less than that originating in the epoxy-potted region of the input MOS-FET's when

only this location was irradiated. These currents corresponded to the input of positive charge with a magnitude roughly .01 percent of the response of a 1 liter ionization chamber at atmospheric pressure. Small permanent changes in the quiescent output signal were also observed subsequent to the exposures.

These effects can be rationalized as follows. X-rays interacting with material in the region of the input semiconductor gate transfer sufficient energy to some electrons to eject them from their immediate surroundings. The excess of electrons removed from the MOS-FET gate, and from all conductors electrically continuous with it, produces a positive input current. This is a unipolar, temporary parasitic effect which may be separated from ionization chamber currents by averaging results obtained using positive and negative collecting potentials.

Similarly, an excess of electrons ejected from the SiO_2 gate insulator of a MOS-FET leaves a residual positive charge which dissipates very slowly because of the high resistivity of the insulator. Experiments with a single MOS-FET indicated time constants typically of the order of months. The FET's used are P-channel enhancement mode devices for which a positive insulator charge will act to deplete the channel region. The shift in the transistor characteristic results in only a small shift in the electrometer zero reference since equal amounts of radiation will have nearly equal effects on the two FET's of the differential input. Any difference will be long-lived.

b) New ionization chambers

Q chamber

To facilitate dose monitoring for radiobiology irradiations, or during various absolute dose measurements, new ionization chambers, designated Q chambers, have been designed and fabricated. These instruments are significantly smaller than the 1-liter P chambers used routinely, and will permit monitoring closer to neutron-producing targets at more representative locations with respect to biological specimens or absolute dosimeters.

Figure PA-2-1 shows construction details. The basic structure can be used with either a large or small cavity volume, and with either a thin or thick chamber wall. The thin-walled electrodes provide secondary proton equilibrium for neutrons with energies up to about 10 MeV, whereas the thicker walls are adequate up to about 18 MeV. The collector electrode is guarded, and gas-flow connections are provided. A screw-thread connection assures rigid attachment of the chamber stem to RARAF electrometer amplifiers. The aluminum cap surrounding the outer electrode serves as a reference for precise positioning, protects against inadvertent abuse, shields the high voltage electrode from external contact, and encloses part of the gas-flow path.

Three stem structures are available and can be used with a

Q CHAMBER

PA-2-1 Q chamber structural details

variety of electrodes, including small and large size aluminum electrodes for use with argon to monitor gamma ray doses. Figure PA-2-2 shows two complete assemblies, a partially disassembled unit, and some of the interchangeable electrodes. A RARAF miniature electrometer amplifier with its cable is shown connected to one of the chambers and a similar amplifier is shown disconnected from another chamber.

The variety of available size and wall configurations and their cavity volumes are given in Table PA-2-1.

Assembly 5 is presently being used to monitor neutron irradiations of hamster cells in tissue culture as shown in Figure PD-1-1.

G chamber

A second new tissue equivalent ionization chamber design was used to fabricate three units, designated G chambers, for use as a transmission monitor and also as an absolute dosimeter during the International Neutron Dosimetry Intercomparison (INDI). See Section PD-2. The disc-shaped cavity of this parallel plate chamber has a sensitive volume of 2.4 cc. A complete assembly is shown in Figure PA-2-3, together with a partially disassembled chamber to show the collector and accelerating potential electrode discs, and their aluminum spring contacts. The central electrode discs and their polyethylene insulators were molded together to the surrounding guard electrode. The tissue equivalent

PA-2-2 Q chambers with interchangeable components

Table PA-2-1

<u>Wall material</u>	<u>Size</u>	<u>Wall thickness, cm</u>	<u>Volume, cm³</u>
1. Tissue equivalent plastic	small	.056	.69
2. Tissue equivalent plastic	small	.30	.16
3. Aluminum	small	.15	.45
4. Tissue equivalent plastic	large	.061	7.9
5. Tissue equivalent plastic	large	.38	3.4
6. Aluminum	large	.14	7.2

PA-2-3 G chambers

plastic body was molded in one piece and contains passages for gas flow as well as for the connections to the electrodes.

The aluminum connector block has provisions for applying collecting potential and tissue equivalent gas flow. RARAF FET electrometers are also shown in the Figure to illustrate the direct screw-thread attachment provided. Further dimensional details are shown in Figure PD-2-3.

A similar chamber with aluminum walls and Teflon insulators was constructed for use with argon to evaluate gamma ray doses in mixed neutron and gamma ray fields.

K chamber

A special ionization chamber, designated the KT chamber and shown in Figure PA-2-4, was designed and built utilizing very light construction to minimize attenuation and scatter for the determination of tissue kerma in air during RARAF's participation in INDI. The 15 cm long stem supporting the 2.2 cc sensitive volume terminates in a connector block with provisions for applying collecting potential and tissue equivalent gas flow, and provides a rigid screw-thread connection to RARAF FET electrometers, as shown in Figure PA-2-5. The 0.23 mm thick wall is adequate to provide secondary proton equilibrium for neutron energies up to about 4 MeV. A set of 6 tissue equivalent plastic caps with wall thickness from 0.28 to 4.6 mm is used to

KT CHAMBER

PA-2-4 KT chamber cavity detail

PA-2-5 K chambers and equilibrium caps

establish equilibrium for higher energies and to evaluate wall attenuation.

A similar chamber, designated the KA chamber, is also shown in Figure PA-2-5. It has aluminum cavity walls and Kel-F insulators adjacent to the gas cavity. Its 1.4 cc volume is surrounded by a 1.27 mm thick wall, and a similar set of equilibrium caps made of aluminum are available.

c) Neutron spectrometry

The organic liquid scintillator spectrometry system described in previous Annual Reports (NYO-2740-8, COO-3243-1) is used routinely to verify neutron energy spectra for most irradiations with neutron energies above 1 MeV. A small anomaly in its energy calibration has been eliminated. The unfolding program has been modified to be more compatible with the hardware and operating system at the Brookhaven National Laboratory Central Scientific Computing Facility (CSCF). Set-up, calibration, and spectral data collection requires less than two hours, with the unfolded spectrum available on a next-day basis. The delay between data and analysis is due to an awkward system for handling punched paper tape output from the analyzer. This time-consuming step will be eliminated when a telephone link between RARAF and CSCF is completed.

An auxiliary spectrum monitor, called the Block, has been

developed and is used during INDI irradiations to provide a continuous check of the quality constancy of the radiation field. It is a 20 cm high stack of 30 cm square Lucite plates containing three 6 mm diameter boron-lined proportional counter tubes at nominal depths of 1, 4, and 11 cm. The assembly is located 1.4 m directly below the T-4 target under the steel grating floor. For each tube there is a local plug-in card with amplifier-discrimination-line driver circuits. Output pulses from these cards are sent via the RARAF signal cabling system to scalers in the console area. The prototype for this Block employs a single boron-lined tube, and it is used in the cave for neutron irradiations with beams 1H or 1V.

The Block is calibrated by recording the response of each tube during spectrometry measurements with the scintillator system at very low dose rates. During subsequent irradiations at high dose rates, the tube responses are recorded and their ratios are compared to those obtained during spectrometry to verify that no significant spectral changes have occurred. Although the Block response does not yield a quantitative evaluation of spectral changes, it will provide a sensitive indication when some change worthy of further investigation has taken place. The relative sensitivities of the tubes are checked periodically using a small ^{252}Cf source in a standard position in the Block.

A hydrogen-filled proportional counter for neutron spectrometry below 1 MeV has been acquired and its operating characteristics are being studied.

d) Precision long counter

Precision long counter (PLC) H-3 with BF_3 counter tube UC-469 was borrowed from Battelle Northwest Laboratories for a period of one year for use in the International Neutron Dosimetry Intercomparison (INDI). This is one of the original PLC's studied by De Pangher (1) and was acquired as a complete unit except for the internal neutron calibration source and a pulse preamplifier. A doubly-encapsulated, specially fabricated 50 mCi $^{241}\text{AmO}_2$ -Be source was purchased rather than a Pu-Be source to eliminate uncertainties due to emission growth. The preamplifier was constructed at RARAF to yield a 4.7 μs resolving time. Acceptable pulse resolution of less than 15 percent (FWHM) for the $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ main peak was obtained.

The counter was carefully transported to the low-scatter room of the Radiation Physics Department, National Bureau of Standards, for absolute calibration using the four neutron sources listed in Table PA-2-2.

Total absolute emission rate for each source was determined by NBS using the manganese sulfate bath technique, and adequate measure-

Table PA-2-2

<u>Source</u>	<u>Mean neutron energy, MeV</u>	<u>Total emission[*], 10⁶ neutrons/s</u>	<u>Asymmetry ratio, $\epsilon(90^\circ)$</u>
²³⁸ PuO ₂ -Li ₂ O	1.4	0.573 <u>±</u> 1.5%	0.95
²⁴¹ Am-Be	4.4	15.88 <u>±</u> 1.5%	1.01
²⁵² Cf (SR-Cf-132)	2.2	132.6 <u>±</u> 1.4%	1.04
²²⁶ Ra-γ-Be (NBS I)	0.2	1.251 <u>±</u> 1.0%	1.00

* Values shown are for September, 1972

ments were made by a member of the RARAF staff to determine the asymmetry ratios and to evaluate the scatter contribution to the PLC response for each source. The mean value of the tube sensitivity for the four sources was found to be 3.44 counts per neutron per cm^2 with a standard deviation of 1 percent. This agrees well with the typical value of 3.6 given by De Pangher.

Finally, relative counter efficiencies for the mean energies of the four sources used were compared to the relative efficiencies given by De Pangher and were found to agree within 1 percent.

The results of this study were to establish the absolute value of the sensitivity of BF_3 tube UC-469 and to verify, at least for the mean energies of the sources utilized, that PLC H-3 conforms to the relative efficiencies given by De Pangher. Results of the calibration were consistent both internally and with the earlier original studies.

The PLC was carefully returned to RARAF where its use during INDI should be a valuable adjunct to the other monitoring measurements.

REFERENCE

1. J. De Pangher and L.L. Nichols, A Precision Long Counter for Measuring Fast Neutron Flux Density. Battelle Memorial Institute, Pacific Northwest Laboratory Report BNWL-260 (1966)

e) ^{252}Cf irradiation source

A 2 mg ^{252}Cf neutron source was loaned to RARAF for one year by the USAEC Savannah River Operations Office for the INDI project (see Section PD-2). At 30 cm from the source the nominal tissue kerma rates in air for neutrons and gamma rays are 4 and 2 rads/h, respectively. The source is used at the low-scatter target position T4. A pneumatic system transfers the source from its storage shield to a precision receiver (Figure PA-2-6) mounted at the target position (Figure PD-2-2). The source furnished by the Savannah River Laboratory is doubly encapsulated in a standard SR-Cf-100 series container. The ^{252}Cf is contained in an inner weld-sealed capsule of platinum alloyed with 10 percent by weight of rhodium, surrounded by a weld-sealed zircaloy-2 outer capsule. This assembly was then enclosed in a special pneumatic transfer capsule made of magnetic stainless steel 17-4 PH which was also sealed by welding.

f) ^{137}Cs calibration source

Figure PA-2-7 shows the ^{137}Cs calibration source stand located in a corner of the RARAF T area. The 10 Ci source produces a nominal tissue kerma rate of 2.5 rad/h at 1 m and was calibrated with an uncertainty of ± 2 percent by the U.S. National Bureau of Standards. The Figure also shows the instrument support and wall brackets, and the warning sign

^{252}Cf SOURCE AND RECEIVER

PA-2-6 ^{252}Cf source in precision pneumatic receiver

PA-2-7 10 Ci ^{137}Cs RARAF calibration
source

and beacon at the top. The instrument support bracket is used for positioning dosimeters accurately at distances less than 1 m to eliminate small distance errors which could be introduced by flexing of the grating floor if a free-standing dosimeter support were used instead.

Several portal configurations are available. Either of two lead collimators with conical angles of 15° or 30° may be used, and lead attenuators may be placed over either collimator to reduce the beam intensity by nominal factors of 2, 4, or 10, or any combination of these factors.

This reliably calibrated source is being used for the INDI project (see Section PD-2) as well as for absolute dosimeter calibrations performed for various radiobiology irradiations.

g) Computer installation

A small digital computer^{*} has been added to RARAF's instrumentation. It will be used as a pulse height analyzer with provisions for single, dual independent, or dual coincident multi-channel analysis. It will also be used as a data handler and processor and for several experiments under construction it will provide various on-line control functions.

^{*} Model PDP-8e, Digital Equipment Corporation, Maynard, Massachusetts

The processing hardware includes an 8 K core memory, a 32 K disc memory, and a high speed paper tape punch and reader. In addition to the pulse height equipment, data acquisition hardware includes a clock with several external control features, and several general-purpose input/output channels. The Brookhaven National Laboratory Instrumentation Division has constructed a prototype scaler that will insert directly into the computer main-frame. There is provision for at least 10 of these counting channels in the system.

Communication between the operator and the computer system is through an electronic keyboard terminal** that provides both text and graphic information on an oscillograph screen. A Teletype unit serves as a printer and remote terminal.

The programming system for on-line data collection and handling is the 8 K FOCAL system supplied by the computer manufacturer, with modifications linking the standard software to specific RARAF hardware. A fundamental advantage of this programming system is its provision for modifying the data-handling programs while data are being collected.

** Model 4010-1, Tektronix, Inc., Beaverton, Oregon

The calculating, plotting, and recording functions are, from the operator's viewpoint, independent of the data input facilities. The FOCAL software includes many "hooks" for inserting user-defined functions; these are readily connected to specific RARAF hardware through program sections written in the machine assembly language.

h) Beam monitoring and control instrumentation

In the past, monitoring of ion beam current incident upon a neutron-producing target has required a variety of instruments. First, an electrometer converted target current to a proportional voltage. Current integration to record target usage then required a voltage-to-frequency converter followed by a scaler. Timing of irradiations required additional instrumentation, including another scaler. Switching the electrometer range changed the voltage-to-frequency scale factor, thereby increasing the possibility of misinterpreting the integrated count.

A single instrument, housed in a double-width NIM module, has been constructed to accomplish all of these functions. This beam monitor includes an electrometer with manual range switching, a voltage-to-frequency converter, and two electromechanical counters for totalizing target charge and beam-on time. A frequency divider switched simultaneously with the electrometer range switch maintains a constant scale factor for the charge scaler. The use of electro-

mechanical counters rather than electronic types simplified design and eliminated the possibility of data loss in the event of a power failure. AC actuated counters were chosen to conserve DC NIM bin power, and electrical noise was minimized by using solid state relays to operate the counters.

The three output signals provided are a voltage proportional to input current, for use with analog panel meters; an undivided pulse train with frequency proportional to input current, for auxiliary monitoring or normalization; and a gate signal indicating the presence of a minimum input current, for controlling auxiliary counters.

A similar instrument, also in a double-width NIM module, has been constructed to actuate insertion of the accelerator main beam-stop at the end of a pre-set count. The input may be any pulse train, such as that derived from converted beam current or ionization chamber current, or timing signals. A frequency divider selects the range setting. The pre-set counter is of the mechanical count-down type, and switching of AC currents for counter coil, reset coil, and beam-stop relays is by means of all solid state relays. Start, stop, and reset logic are controlled via pushbuttons, and LED lamps indicate status. A toggle switch can disable the counter to use the instrument as a frequency divider.

Single-width frequency divider modules for general use have

also been constructed employing the same electronic logic and utilizing a seven-segment LED display for range setting indication.

BIOPHYSICS

- BP-1 The molecular ion beam experiment
 H.H. Rossi

- BP-2 The mean spacing of protons arising from molecular ions
 W. Gross and A.M. Kellerer

- BP-3 Multiple coulomb scattering of protons
 W. Gross and R.D. Colvett

- BP-4 RBE, OER and dose rate studies in plants
 A.G. Underbrink

- BP-5 Quantification of radiation induced cancerogenesis
 A.M. Kellerer

- BP-6 Comparison of effect rates in samples of time
 decreasing size
 A.M. Kellerer

- BP-7 Microdosimetry and the dispersion of chromosome aberrations
 A.M. Kellerer

- BP-8 Analysis of Japanese leukemia data
 A.M. Kellerer and H.H. Rossi

- BP-9 Theory of dual radiation action
 A.M. Kellerer and H.H. Rossi

BP-1 The Molecular Ion Beam Experiment

H.H. Rossi

According to the theory of dual radiation action (1,2), somatic radiation effects on higher organisms are determined by the square of the energy concentration in sites within the cell nucleus that have dimensions comparable to the nuclear diameter, i.e. a few micrometers. The quadratic dependence is believed to be due to the requirement that pairs of affected loci must interact within the site in order to cause manifest cellular injury. The loci are believed to be much smaller than the sites and quite possibly to have dimensions of the order of nanometers. Under these conditions LET can influence biological action at two levels:

A) Over micrometers because different energy concentrations within such distances lead to the impairment of different numbers of interacting loci;

B) At the nanometer level where different energy concentrations may result in different efficiencies of locus impairment.

If the theoretical formulae are corrected for saturation of specific energy at the micrometer level it is possible to account at least in part for the dependence of RBE on LET (2) on the basis of (A), but recent research which is still largely unpublished suggests the presence of another factor which quite possibly relates to (B), the energy concentration at the locus level.

In this connection it is important to bear in mind that the diameter of the DNA helix is about 1 nanometer; that the typical range of the great majority of delta rays produced by charged particles is of the same order of magnitude; and that the distance traveled by a charged particle between successive primary ionizations becomes comparable to the delta-ray range when the LET of the particle exceeds a few tens of keV/ μm . Thus the probability becomes appreciable that the DNA chain be traversed by more than one delta particle, and it is at these LET values that RBE increases markedly. It should also be remembered that the diffusion lengths of radiochemical products (e.g. active radicals) are of the order of a few nanometers. All of these considerations indicate the likelihood that energy concentration on the nanometer scale is an important aspect of radiobiological action.

We are about to test postulates of the theory of dual radiation action as they apply to phenomena at the micrometer level by utilization of a micro-beam which is now under development at the RARAF accelerator (see section PD-3). Although the microbeam research has top priority and is expected to result in significant findings, its inherent limitation to micrometer dimensions precludes any possibilities of exploring effects operating at the nanometer level.

In order to supplement the microbeam research, a different mode of attack is under development in which a considerably higher spatial resolution is obtained with a somewhat simpler arrangement in which collimation is unnecessary.

Because of basic characteristics of the ion sources employed in van de Graaff generators, the beam emerging from the machine consists not only of protons but also of singly ionized hydrogen molecules (H_2^+) and other species (e.g. H_3^+). The yield of the various multi-atomic beams is quite comparable to that of protons and the absorbed dose rates attainable are ample for radiobiological experiments. The protons are easily separated by magnetic analysis and the multi-atomic beams are ordinarily dissipated in regions remote from the target. We propose to use these beams in order to perform irradiations of cells by charged particles with minimal separation. In the following the case of H_2^+ will be considered; analogous considerations apply to the other species which may well be investigated also.

The H_2^+ particle consists of two protons that are maintained at a separation of the order of a tenth of a nanometer by a single shared electron. Once the particle has encountered a few atoms of matter the electron is stripped off and the two protons proceed independently in what can be considered a random walk about their initial trajectory. The phenomenon responsible for this transverse motion is multiple Coulomb scattering, a well-known process for which there is good theoretical information and some experimental information. Initial calculations have indicated that if individual mammalian cells are placed on mylar foils of minimal thickness the two protons will traverse the nucleus at separations

of a few nanometers. The basic theoretical treatment of this problem is outlined below in Section BP-2 and the experimental data required as input for these calculations are reported in Section BP-3.

It may be expected that the rather ill-defined delta-ray "haloes" of the two protons will largely overlap. As more matter is interposed^{*} a substantial separation of the delta-ray haloes should be achievable. One may also investigate the case that is tantamount to complete coincidence of the delta-ray haloes by employing single protons having twice the LET of either of the two protons originating from the accelerated molecule.

It should be noted that in addition to a high degree of spatial resolution this approach will also permit a high degree of temporal resolution since the two particles should traverse the nucleus essentially simultaneously. Certainly a time separation considerably less than 10^{-13} seconds should be involved. The cells might be supported on a mylar window of the order of 10 microns in thickness with consequent appreciable separation of the two simultaneous protons and the results can be compared with those obtained by equal numbers of single protons of the same energy.

With the preliminary theoretical and experimental physics studies largely completed, a special irradiation apparatus for these experiments has been designed and is now under construction.

^{*}The particle energy is raised to obtain equal LET after penetration of the scattering material.

REFERENCES

1. Kellerer, A.M. and Rossi, H.H. "RBE and the primary mechanism of radiation action." Rad. Res. 47, 15-34, 1971.
2. Kellerer, A.M. and Rossi, H.H. "The theory of dual radiation action." Current Topics in Radiation Research Quarterly 8, 85-158, 1972.

BP-2 The Mean Spacing of Protons Arising from Molecular Ions

W. Gross and A.M. Kellerer

Consider a narrow beam of particles travelling in the z direction and incident on a foil lying in the x,y plane whose thickness (z direction) is t . Let $\vec{v}$ be the normalized velocity vector and assume that the lateral velocity components are small compared to the axial velocity so that

$$\vec{v} = (v_x, v_y, 1)$$

Thus, v_x and v_y measure the projected angle of a particle in the x and y directions respectively. Examining changes in the x direction only, each collision leads to a small change in v_x which we designate as Δv_x , and each of these changes is independent of the others, both as to occurrence and magnitude of change. This is therefore a compound Poisson process for which the variance is $\sigma^2 = N \overline{\Delta v_x^2}$ where N is the mean number of collisions and thereby proportional to the thickness, t . After a large number of collisions the distribution is Gaussian with a frequency proportional to $\exp(-v_x^2/2\sigma^2)$. The distribution in the y direction is of the same form, therefore the differential distribution in v_x and v_y is

$$\begin{aligned} f(v_x, v_y) &\propto \exp(-v_x^2/2\sigma^2) \exp(-v_y^2/2\sigma^2) \\ &\propto \exp[-(v_x^2 + v_y^2)/2\sigma^2] \end{aligned}$$

From this it follows that at distance $L \gg t$ from the foil the intensity distributions lateral to the beam axis

$$c(R) = c_0 e^{-R^2/2\sigma_1^2}$$

where $\sigma_1 = L\sigma$ and R is the distance from the point of observation to the beam axis. The quantity σ_1 is the one determined experimentally (See Section PD-1). Further, the fraction of the particles inside this distance is

$$F(R) = \frac{\int_0^R c(r) r dr}{\int_0^\infty c(r) r dr}$$

or in terms of the scattering angle θ , the fraction of particles inside a cone of half angle θ is

$$\begin{aligned} F(\theta) &= \frac{\int_0^\theta e^{-\phi^2/2\sigma^2} \phi d\phi}{\int_0^\infty e^{-\phi^2/2\sigma^2} \phi d\phi} \\ &= \frac{1}{\sigma^2} \int_0^\theta e^{-\phi^2/2\sigma^2} \phi d\phi \\ &= 1 - \exp(-\theta^2/2\sigma^2) \end{aligned}$$

The density, θ , is given by

$$f(\theta) = \frac{dF(\theta)}{d\theta} = \frac{\theta}{\sigma^2} \exp(-\theta^2/2\sigma^2)$$

from which we obtain the mean and mean square scattering angle as

$$\overline{\theta} = \sqrt{\frac{\pi}{2}} \sigma$$

$$\overline{\theta^2} = 2\sigma^2$$

The mean square scattering angle is the quantity usually calculated to describe the scattering process (1).

The lateral displacement in traversing the foil can be calculated as follows: In Fig. 1 is shown a schematic diagram of the first two collisions in the foil. At point τ_1 the projected angle in the x,z plane already designated as v_x undergoes a change $\Delta v_{x,1}$. If no further collisions occurred, a lateral displacement $\Delta x_1 = \tau_1 \Delta v_{x,1}$ would result. The second collision occurring at point τ_2 changes v_x by an amount $\Delta v_{x,2}$ and contributes to the lateral displacement an amount $\Delta x_2 = \tau_2 \Delta v_{x,2}$. This process continues as the particle traverses the foil with later collisions becoming less important owing to the decrease in τ . As described above these collisions are independent, so the displacements are independent. The net displacement is the sum of the individual displacements, i.e.

$$x = \sum \Delta x_i = \sum \Delta v_{x,i} \tau_i$$

and because this is a compound Poisson process

$$\overline{x^2} = N \overline{(\Delta v_x \cdot \tau)^2}$$

Fig. 8P-2-1

The random variables τ and Δv_x are independent of each other and therefore

$$\overline{x^2} = N \overline{\Delta v_x^2} \overline{\tau^2}$$

Now

$$\overline{\tau^2} = \int_0^t \tau^2 d\tau / \int_0^t d\tau = t^2/3$$

and as pointed out above

$$N \overline{\Delta v_x^2} = \sigma^2$$

Therefore

$$\overline{x^2} = \frac{\sigma^2 t^2}{3}$$

By an argument analogous to that applied to the derivation of the distribution in θ one finds that if r is the lateral displacement the density in r is

$$g(r) = \frac{r}{b^2} \exp(-r^2/2b^2)$$

where

$$b^2 = \frac{\sigma^2 t^2}{3}$$

and

$$\bar{r} = \sqrt{\frac{\pi}{2}} b = \sqrt{\frac{\pi}{6}} \sigma t$$

$$\overline{r^2} = 2b^2 = \frac{2\sigma^2 t^2}{3}$$

BP-2-2

This distribution is shown in Fig. 2 with the mean and root mean square values indicated on the figure.

Finally, if a molecular hydrogen ion is incident of the foil, the two protons will split apart and travel independently. The variance of the angle between them and of their relative distance is therefore double the variance of θ and v . On emerging, the distributions in the angle between them, ψ , and their relative lateral displacements, s , are accordingly:

$$f_2(\psi) = \frac{\psi}{\alpha^2} \exp(-\psi^2/2\alpha^2)$$

where $\alpha^2 = \sigma^2$ and

$$g_2(s) = \frac{s}{\beta^2} \exp(-s^2/2\beta^2)$$

where $\beta^2 = \frac{2\sigma^2 t^2}{3}$

The mean and mean square values are

$$\bar{\psi} = \sqrt{\frac{\pi}{2}} \alpha = \sqrt{\pi} \sigma$$

$$\overline{\psi^2} = 2\alpha^2 = 4\sigma^2$$

Fig. BP-2-2

$$\overline{s} = \sqrt{\frac{\pi}{2}} \beta = \sqrt{\frac{\pi}{3}} \sigma t$$

$$\overline{s^2} = 4 \frac{\sigma^2 t^2}{3}$$

BP-3 Multiple Coulomb Scattering of Protons.

W. Gross and R.D. Colvett

A project under consideration which involves irradiation of cells attached to a thin mylar foil with a H_2^+ ion beam is described elsewhere in this report (Sections BP-1, BP-2). As is described there the distribution of the separations of two protons traversing cells can be determined from the mean square scattering angle for protons in the Mylar foil on which they are placed. Theoretical calculation of this quantity value from standard formulae is possible (1) but is uncertain and therefore a direct measurement has been made.

The apparatus used is shown schematically in Fig. BP-3-1. A low intensity proton beam is collimated by means of a tantalum diaphragm (D_1) to pass through the center of the scattering foil (F). The hole in this diaphragm has a large diameter to thickness ratio (0.33 mm diameter to .051 mm thickness) in order to reduce the effects of edge scatter from the hole.

The scattered beam then traverses a 46 cm length to surface barrier, solid-state detectors covered by diaphragms. One detector (monitor-SSM) is fixed in position and supported from the distant wall of the container but off the axis of the proton beam. The hole in the monitor diaphragm (D_2) is large (3 mm) and serves only

Fig. BP-3-1

Schematic view of the scattering intensity
measuring system

to insure that all protons striking this detector lie within its sensitive area.

The other detector (measuring-SSI) is used to investigate the intensity profile of the scattered beam. It is mounted from a plate which can be moved perpendicularly to beam axis in either of two directions which are at right angles to one another. The tantalum diaphragm collimator (D_3) on this detector has a small hole but one which has large diameter to thickness ratio (0.51 mm diameter to .051 mm thickness) again to minimize edge scatter.

The container is evacuated to a pressure below 1 m Torr to avoid significant gas scatter. Motion of the measuring solid-state detector is provided by external screws and the position of the detector is obtained from two dial indicator gauges each having a range of 3 inches with .001 inch minor divisions.

Each of the solid-state detectors is connected to its own charge sensitive preamplifier which is followed by a conventional amplifier and single channel pulse height analyzer. The bias for the detectors is from a common source of +100 volts. When the beam was properly aligned the pulse height spectra were narrow and therefore each analyzer was used in an integral mode with the discriminator set sufficiently below the peak to insure freedom from equipmental drift.

The experimental procedure was as follows: The output of the

monitor detector pulse height analyzer was counted by a preset scaler which gated another scaler counting the pulses from the analyzer connected to the moving detector. This latter detector was positioned at the center of scattered beam (maximum count). Thereafter the motion was confined to one direction. The ratio of the count rate of the moving detector to the count rate monitor detector was then determined as a function of position at either side of the center out to a point where this ratio was less than one percent of the center ratio. The data were acquired in a series of steps sweeping back and forth across the center to avoid the effects of any drift in the beam position. (No such drift was detected however.)

The data were then fit by means of a computer to a form

$$C(x) = C_0 e^{-(x-\bar{x})^2/2\sigma^2} \quad (1)$$

where $C(x)$ is the count rate relative to the monitor count rate at position x and C_0 , $\bar{x}$ and σ are constants representing an intensity factor, the center position and the root mean square width of the distribution respectively. The values of these three constants were determined by successive approximation, using the lowest sum of square of the differences between measured and calculated values as an indication of best fit. These approximations were carried to below .03 percent in the values of the constants. Only data where

Fig. BP-3-2
Scattered intensity vs lateral distance

the count ratio was greater than 20 percent of the maximum were included in this determination.

Figure BP-3-2 shows a plot of the actual data as points and a solid line which represent Eq. (1) using the values obtained as outlined above. This plot is for a 1.2 MeV proton beam incident on an 0.55 mg/cm^2 Mylar foil but can be considered typical of the results obtained. The calculated curve fits the experimental data to within the statistical accuracy at all positions where the ratio of the count rate of the moving detector to that of the monitor is greater than 20 percent of the peak value. Where this ratio is less than 10 percent, the calculated values are significantly below the measured points. This divergence between experimental and calculated values at positions well away from the center is to be expected, as in this region the contributions from single scattering of the incident protons outweigh those from multiple scattering.

The root mean square scattering width, σ , (or in terms of angle - the root mean square scattering angle) is expected to be directly proportional to the square root of the foil thickness and inversely proportional to the proton energy. It should also be directly proportional to the square root of a quantity $Z(Z + 1)/A$ of the scatterer where Z is its atomic and A its mass number (1). In this experiment, where the energy loss in the foils was not large, it

is appropriate to assume that the applicable proton energy is the average of the incident energy and the energy of the protons emerging from the foil.

In Table BP-3-I are listed the incident and mean energies, the foil thicknesses and $\frac{Z(Z+1)}{A}$ factors for the several measurements made in this experiment. The mean energy values were determined from the range-energy data for protons in Mylar and aluminum of Northcliffe and Schilling (2). Also given in the Table are the root mean square width values, σ , and in the last column times the mean energy divided by the square root of the product of the thickness and $\frac{Z(Z+1)}{A}$. The values in this column should be constant if the functional behavior of scattering with proton energy, foil thickness and material is as stated above. For Mylar the total variation for all energies and thicknesses is less than 6 percent and the variation is less than one percent for the two cases with aluminum. However, there is a 13 percent difference in the mean value for Mylar as compared to that for aluminum indicating that the dependence on scattering material is not exactly $Z(Z+1)/A$ but somewhat more slowly varying.

The value of the root mean square width can be calculated from theory (1,3). When these calculations were performed, however, a large discrepancy was found between theory and experiment for each of the several theoretical expressions available. The discrepancy among

TABLE BP-3-1
Experimental Scattering Data

Proton Initial	Energy Average	Foil material thickness		$\frac{Z(Z+1)}{A}$	root* mean square	$\frac{\sigma \bar{E}}{\sqrt{\frac{t Z(Z+1)}{A}}}$
(MeV)	(MeV)		(mg/cm^2)		σ_{cm}	
1.2	1.13	Mylar	0.551	3.76	0.673	0.528
2.0	1.96	"	0.551	"	0.408	0.556
2.0	1.68	"	3.57	"	1.146	0.525
4.0	3.92	"	1.696	"	0.350	0.543
4.0	3.83	"	3.57	"	0.530	0.554
2.0	1.95	Aluminum	0.952	6.74	0.622	0.479
4.0	3.94	"	1.725	"	0.416	0.481

the theoretical expression was even larger, however, i.e. the experimental results at least for Mylar were bracketed by the theoretical values. Thus, little credence can be given to the absolute values calculated from scattering theory but the functional behavior with energy and thickness appears appropriate. This situation is satisfactory for the planned biological experiments in that the experimental data supply the root mean square width values for a particular set of foil thicknesses and energies and the known functional behavior permits calculation for other conditions.

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BP-4 RBE, OER and Dose Rate Studies in Plants.*

A.G. Underbrink (In collaboration with A.H. Sparrow and
C.H. Nauman)

A. Relative biological effectiveness (RBE) as a function of neutron energy for pink mutations in Tradescantia stamen hairs. In a series of separate experiments, cuttings of Tradescantia with young inflorescences were irradiated with twelve neutron energies ranging from 0.065-MeV to 13.4-MeV (8) (Table BP-4-1). The stamen hairs were scored for pink mutations from days 11 through 15 after irradiation, since these days encompass the peak mutation frequency (9,17) (Fig. BP-4-1). Dose-response curves were constructed for pink somatic mutations for each neutron energy using the average mutation frequencies found during the 5-day scoring period (9) (Figs. BP-4-2 and Figs. BP-4-3).

The dose-response curves were linear for all neutron energies tested. With x rays the response was linear at low doses (below about 5 rads); from 5 rads to about 100 rads, the data can be fitted to a steeper slope (9). In all cases RBE increases with decreasing dose (Table BP-4-1), but RBE becomes maximum and constant at and below doses of 5 rads of x rays (Figs. BP-4-2 and BP-4-3, arrows). Maximum

*This investigation was supported in part by Public Health Service Research Grant No. CA-12536 from the National Cancer Institute.

Table BP-4-1. Maximum RBE values for pink mutant events in Tradescantia stamen hairs following irradiation with neutrons of various energies and 250-kVp x rays

Neutron energy (MeV)	Pink events per hair* (minus control)	Dose (rads)		RBE _{max}
		X ray	Neutron	
0.065	0.003	5.0	0.34	14.7
	0.01	12.0	1.10	10.9
	0.03	27.0	3.30	8.2
0.110	0.003	5.0	0.23	21.7
	0.01	12.0	0.76	15.8
	0.03	27.0	2.30	11.7
0.220	0.003	5.0	0.19	26.3
	0.01	12.0	0.64	18.8
	0.03	27.0	1.90	14.2
0.340	0.003	5.0	0.15	33.3
	0.01	12.0	0.54	22.2
	0.03	27.0	1.60	16.9
0.395	0.003	5.0	0.115	43.5
	0.01	12.0	0.38	31.6
	0.03	27.0	1.15	23.5
0.430	0.003	5.0	0.105	47.6
	0.01	12.0	0.37	32.4
	0.03	27.0	1.10	24.6
0.552	0.003	5.0	0.15	33.3
	0.01	12.0	0.49	24.5
	0.03	27.0	1.45	18.6
0.680	0.003	5.0	0.16	31.3
	0.01	12.0	0.54	22.2
	0.03	27.0	1.65	16.4
1.02	0.003	5.0	0.27	18.5
	0.01	12.0	0.90	13.3
	0.03	27.0	2.70	10.0
2.05	0.003	5.0	0.33	15.2
	0.01	12.0	1.10	10.9
	0.03	27.0	3.40	7.9
6.0	0.003	5.0	0.37	13.5
	0.01	12.0	1.20	10.0
	0.03	27.0	3.70	7.3
13.4	0.003	5.0	0.48	10.4
	0.01	12.0	1.60	7.5
	0.03	27.0	4.80	5.6

* Values averaged from total mutant events divided by total stamen hairs scored from days 11-15 postirradiation.

Fig. BP-4-1 Plot of percent hairs with pink and colorless events vs. days post-irradiation. Stamen hairs are routinely scored for mutations from day 11 through 15 after irradiation when mutation frequency is highest.

Fig.BP-4-2 Plots of pink mutant events per hair (minus control) vs. dose for 250-kVp x rays and neutrons ranging in energy from 0.065-MeV to 0.43-MeV. Arrows indicate region on the dose-response curves where RBE is maximum .

Fig.BP-4-3 Plots of pink mutant events per hair (minus control) vs. dose for 250-kVp x rays and neutrons ranging in energy from 0.552-MeV to 13.40-MeV. Arrows indicate region on the dose-response curves where RBE is maximum.

RBE is about 14.7 for 0.065-MeV neutrons, increases to a high of 47.6 for neutrons of 0.43-MeV, and then decreases with further increase in neutron energy to a low of about 10.4 at 13.4-MeV.

RBE in this system never reaches 1 at high doses because the mutation frequency reaches a maximum (region of saturation) and then declines with further increases in dose. The region of mutation saturation is illustrated in Fig. BP-4-4. Pink mutation saturation occurs between 10-15 rads of 0.43-MeV neutrons, and 100-150 rads of x rays. In this case, minimum RBE is about 10 to 15. Continuing analysis of these data suggests that the decline in mutation frequency is, at most, only partially related to a killing effect at these doses. The data suggest that the decline in mutation frequency may be due to an excess deposition of energy in the site or the chromosome bearing the pink gene. This suggestion is borne out by the fact that the decline in mutation frequency is very pronounced as early as day 7 after irradiation when no killing (hair stunting) is in evidence. The saturation at this time occurs at the same dose levels, but the maximum mutation frequency is much lower.

Figure BP-4-5 is a graph of maximum RBE, derived from Figs. BP-4-2 and BP-4-3 determined at a mutant event frequency of 0.003 mutant events per hair corresponding to 5 rads of x rays, plotted against neutron energy. The rise in RBE with energy is rapid and reaches a

Fig.BP-4-4 0.43-MeV neutron and x-ray dose-response curves for pink mutant events in stamen hairs. Open symbols represent region of saturation where mutation frequency has reached its maximum and is beginning to decline. Dose rate for x rays was 30 rads/min. (From ref. 9)

Fig. BP-4-5

Plot of maximum RBE vs. neutron energy.

peak at 0.43-MeV, followed by a sharp drop downward to about 1-MeV where the RBE is 18.5. This sharp decline in RBE is followed by a slow decline in RBE with further increases in energy to about 10.4- and 13.4-MeV.

In other systems where RBE as a function of neutron energy has been investigated (1,4), the sharp peak in RBE at 0.43-MeV has not been found. There is a possibility that part of the pronounced peak could be due to an oxygen resonance which occurs at this energy. However, if this is the case, another positive resonance effect at 1-MeV and a negative resonance at 2.3-MeV should probably also be observed. Experiments were repeated at several energies, but changes in expected mutation frequency due to these resonances, were not observed.

There is as yet no explanation for the sharp peak in RBE at 0.43-MeV. However, experiments are planned using thinner neutron-producing targets to narrow the spread of neutron energy in this region. Such targets will allow the determination of RBE within much narrower energy ranges around 0.43-MeV.

B. Oxygen enhancement ratios (OER) for somatic mutations in *Tradescantia stamen hairs*: preliminary results. This year, experiments were initiated to investigate changes in OER which might occur as a function of neutron energy using the *Tradescantia* stamen hair system (15).

In the literature, certain reports indicate a variation of OER with neutron energy while others do not. Of particular interest was a report by Dennis and Boot who also used the pink mutation in Tradescantia stamen hairs (2) and found a rather pronounced dependence of OER on neutron energy. They reported a high OER at 0.43-MeV which was inconsistent with the rest of their data (Fig. BP-4-6); this apparently anomalous result was thought to be due to a possible resonance effect.

For the OER experiments, a light- and air-tight fixture, capable of withstanding vacuum, and suitable for use with monoenergetic neutrons as well as x rays was designed by Mr. L.J. Goodman at RARAF. This fixture is described in the section of this report dealing with RARAF. The inflorescences are taped in place within the aluminum fixture, which is then bolted together and connected to a gas flow system. The gases, either breathing air or nitrogen are first bubbled through water before passing into the fixture to prevent dessication of the plants during the experimental procedure.

The experimental procedure is as follows: The fixture containing the cuttings is subjected to a vacuum (at least 25.5 inches of Hg), brought back to atmospheric pressure and flushed with the appropriate gas. The vacuum removes air which might otherwise remain trapped in

Fig. BP-4-6

Plot of oxygen enhancement ratios (OER) vs. NEUTRON ENERGY (MeV) for human cells, Vicia faba and Tradescantia. Our OER data point for Tradescantia (open square) at 0.43-MeV definitely does not support the OER obtained at this energy by Dennis and Boot (2). Their high OER value was attributed to an oxygen resonance effect.

the bracts of the inflorescences. This procedure is repeated three times and then either air or nitrogen is allowed to flow through the fixture for about an hour before irradiation. The oxygen concentration in the effluent gas is monitored with a Beckman oxygen gas analyzer.

During the initial experiments, the pink mutant event frequency per hair was determined after allowing the nitrogen to flow through the system for varying periods of time up to 4 hours. As seen in Fig. BP-4-7 the reduced mutation frequency remains stable after 20 minutes of gassing up to 4 hours. Oxygen concentration measured in the effluent gas drops below 20 ppm in about 1 hour.

In these experiments it was decided to determine x-ray mutation frequencies simultaneously with each neutron experiment. For practical considerations, this means that at least three separate experiments, each requiring about 700 cuttings must be done before an OER value at each neutron energy can be determined. Results obtained thus far are shown in Figs. BP-4-8 and BP-4-9.

Figure BP-4-8 is a plot of pink mutant events per hair (minus control) vs. dose of 0.43-MeV neutrons and x rays for irradiation in air or nitrogen. The points on these x-ray curves were determined separately from those shown in Fig. BP-4-4. However, we assume that the steep slope of the x-ray curve in air changes and assumes a +1 slope at and below 5 rads. The x-ray curve in nitrogen is parallel

Fig. BP-4-7 Plot of pink mutant events per hair (minus control) and ppm O_2 vs. time in minutes of nitrogen flow. X-ray dose was 50 rads.

Fig. BP-4-8 Plot of pink mutant events per hair (minus control) for 0.43-MeV neutrons and 250-kVcp x rays irradiated under air and nitrogen atmospheres. The slopes of the neutron lines are +1. The x-ray curves have a steeper slope. The low dose region of the x ray curve for inflorescences irradiated in a nitrogen atmosphere has not been completed (horizontal line). Two experiments were done at 2.5 rads of x rays (arrow) and the results suggest an OER of 1 in this region.

Fig. BP-4-8
- 184 -

PINK MUTANT EVENTS / HAIR (-CONTROL)

FIGURE 8

Fig. BP-4-9 Plot of pink mutant events per hair (minus control) for 0.68-MeV neutrons and 250-KVcp x rays. The irradiations were done in both air and nitrogen atmospheres. The slopes of the neutron lines are +1. The x-ray curves have a steeper slope.

to the curve in air. The OER is about 3.3. As yet, the dose at which the nitrogen curve changes slope is not known because the low dose part of the dose-response curve in nitrogen has not been completed.

However, we have done two experiments at an x-ray dose of 2.5 rads (Fig. BP-4-8, arrow) and both experiments suggest an OER of 1 in this low dose region. We are repeating experiments at this dose and some at slightly higher doses, because if OER, in fact, proves to be 1 on the linear part of the x-ray curve, then we must also determine the shape that the x-ray curve assumes under nitrogen at these low doses in order to bring the OER of over 3 down to 1.

Both the air and nitrogen lines for 0.43-MeV neutrons have slopes of +1 and OER is about 1.4.

Figure BP-4-9 is essentially the same as Fig. BP-4-8 except the neutron energy is 0.68-MeV. Again the neutron lines have slopes of +1 and the OER is about 1.5.

Thus far, we have only completed experiments with 0.43- and 0.68-MeV neutrons. However, these two OER values are shown (Fig. BP-4-6) in perspective to other work in which OER was investigated as a function of neutron energy. Figure BP-4-6 is a plot of OER values against neutron energy. Some 1966 data for human cells (1)

do not show much of an OER dependence on neutron energy over a limited range of neutron energies. Data for Vicia faba (4) show changes in OER with neutron energy. The greatest changes, however, are those reported for Tradescantia (2). Our two data points (Fig. BP-4-6, open squares) seem consistent with the majority of the data at these energies. The important exception, however, occurs at 0.43-MeV. Our data point clearly does not support the OER of 2.1 reported by Dennis and Boot (2) for Tradescantia pink mutations at this energy.

C. Effects of dose rate on somatic mutation induction in Tradescantia stamen hairs. Both x rays and gamma rays have been used to investigate the effect of variation of dose rate on the induction of pink mutant cells in stamen hairs of Tradescantia clone 02 (5). X rays were delivered at dose rates ranging from 0.3 to 500 rads/min; ^{137}Cs gamma rays were used at dose rates from 0.0036 to 30 rads/min (a factor of more than 80,000).

For inflorescences exposed to a dose of 80 rads of x rays (Fig. BP-4-10) there was a progressive increase in the number of mutations per stamen hair as the dose rate was increased from 0.3 to 100 rads/min. At 300 rads/min the mutation yield was not increased further, and at 500 rads/min the effect was slightly less

Fig. BP-4-10 Plot of pink mutant events per hair vs. dose rate. The total dose was 80 rads. The data points describing a separate line (lower left) were derived from Tradescantia held in the cold during treatment so that the 80 rads were given during a single mitotic cycle.

and approximately equal to that at 30 rads/min. Thus, with dose rate (as well as dose) a region of mutation saturation is reached followed by a decline in mutation frequency. With 80 rads of gamma rays (Fig. BP-4-10) the mutation yield increased progressively over the entire dose rate range studied.

The three data points describing a separate line to the left of the main curve in Fig. BP-4-10 represent data from inflorescences which were maintained at 8°C for a time sufficient to equilibrate them preceding irradiation and throughout the time required for irradiation. This treatment holds them within one mitotic cycle. The mutation frequency is higher than when the same dose is spread over several mitotic cycles.

BP-4-

In Fig. 11 dose rate is expressed on the abscissa both in terms of exposure time and mitotic cycles irradiated. The three data points from Fig. BP-4-10 (those held in the cold) fall with the others on the main line. The number of pink mutant events per hair is linearly dependent on the log of the number of mitotic cycles irradiated. At high dose rates for which the exposure time is a small fraction of the mitotic cycle time, the mutation frequency also reaches saturation. Analysis of all these data suggests that it is not the unit number of mitotic cycles irradiated that is important, but rather it is the

Fig. BP-4-11 Plot of pink mutant events per hair vs. number of mitotic cycles irradiated (and exposure time in minutes).

fractional part of the mitotic cycle irradiated that seems particularly relevant.

Figure BP-4-12 shows dose-response curves determined at two dose rates, 5 and 0.5 rads/min as compared to a dose-response curve at 30 rads/min (see Fig. BP-4-4). The mutational response for these three dose rates begins to saturate and decline at approximately the same region (100-150 rads). At 0.5 rads/min the slope of the curve approaches +1 on a double logarithmic plot. This may approximate the dose rate at which one may expect a linear relation between mutation frequency per hair and dose. These data are being analyzed further.

D. Radiation-induced pollen abortion in several commelinaceous taxa: its relation to chromosomal parameters. Throughout the year additional species were irradiated to determine the effects of x or gamma rays and 0.43-MeV neutrons on pollen abortion as measured by cotton blue staining (20). To date, 15 members of four genera (Floscopa, Gibasis, Tradescantia and Tripogandra) of the family Commelinaceae have been studied. These species ranged in ploidy level from 2x to 12x. Only a few of these, however, were irradiated with neutrons.

For each species, the maximum percentage of aborted grains was

Fig. BP-4-12 Plot of pink mutant events per hair (minus control) vs. dose for three different dose rates. At 0.5 rads/min, the response is nearly linear on a log-log presentation.

determined over a postirradiation period corresponding to an entire period of microsporogenesis.

Dose-response curves were constructed for each species and these curves were found to vary in slope. From these dose-response curves, RBE values were determined for two species of Tradescantia (2x), and for a diploid and tetraploid species of Gibasis. These RBE values were taken along various regions of the dose-response curves (specifically at 10, 20 and 50% pollen abortion) and are given in Table BP-4-2. These do not represent maximum RBE values but only the highest RBE within the dose range investigated. In all cases RBE increases with decreasing dose.

Correlations were sought between 50% pollen abortion and interphase chromosome volume (ICV) and nuclear volume (NV). No correlations were found using NV. However, for those species irradiated with x or gamma rays an inverse relationship (-1 slope) was obtained between dose to produce 50% pollen abortion and ICV. The same relation appears to hold for neutrons, but since only four species were available this relationship is still inconclusive.

The difference in RBE values among the species studied may be influenced by the differences in their ICV, for it has been shown that RBE in some cases (6,7) but not all (3) tends to increase with

Table BP-4-2 RBE values determined for two species of
Tradescantia and Gibasis with pollen abortion
as an end point

% Pollen abortion	Neutron dose (rads)	X-ray dose (rads)	RBE
<u>Gibasis geniculata</u> (2x) ICV = 41.9			
10	3.2	30	9.4
20	7.8	51	6.5
50	24.5	105	4.3
<u>Gibasis karwinskyana</u> (4x) ICV = 45.9			
10	1.3	30	23.1
20	4.0	51	12.8
50	17.0	105	6.2
<u>Tradescantia subacaulis</u> (2x) ICV = 55.5			
10	0.67	9.5	14.3
20	2.0	25.5	12.8
50	8.8	88.0	10.0
<u>Tradescantia paludosa</u> (No. 2465) (2x) ICV=75.5			
10	0.67	13	19.4
20	1.8	28	15.6
50	6.5	76	11.7

increasing chromosome volume. Although at present few data are available, a plot was made between RBE and ICV (Fig. BP-4-13). In this case, there is a definite trend at 50% pollen abortion between increasing RBE and increasing ICV. It would be of prime interest to determine whether ICV should be considered an important factor influencing RBE in several species, and whether a predictable relationship between ICV and RBE exists.

E. RBE experiments with haploid fern spores of *Osmunda regalis*. Preliminary experiments to determine RBE for survival following x and neutron irradiation in the single-celled haploid fern spore system were initiated by A.H. Sparrow. With slight modifications necessary for germinating fern spores, these experiments were done using the same aluminum fixtures developed for the *Tradescantia* experiments.

Preliminary results suggest a probable x-ray D_0 of 350 rads. Survival curves for 0.43-MeV and 0.68-MeV neutrons were typical in that no shoulders were present. D_0 values of 22 and 43 rads for 0.43-MeV neutrons were found in separate experiments. D_0 was 43 rads for 0.68-MeV neutrons. The reason for the discrepancy in D_0 values for 0.43-MeV neutrons is not known but could be related to aging the spores during preirradiation culturing procedures. Further experiments to clarify this problem are in progress.

Fig. BP-4-13 Plot of RBE at 50% pollen abortion vs. interphase chromosome volume (ICV). The data suggest that larger ICV values may be found in plants with larger chromosomes.

RBE values calculated from these data are 8.1 and 15.9 for the two different 0.43-MeV neutron experiments, and 8.1 for the 0.68-MeV neutrons.

Experiments are planned which will extend RBE determination to the frequency of biochemical mutants.

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BP-5 Quantification of Radiation-Induced Cancerogenesis

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In view of the ongoing studies by Shellabarger and co-workers on the radiation induction of mammary tumors in rats, and also in view of the fact that these studies will have to be compared to earlier results of other laboratories it has been deemed necessary to reconsider the mathematical formalism applicable to such studies. Quantitative evaluation of the dependence of cancerogenesis on radiation dose and radiation quality follows the same principles as that of other radiation effects on the cellular or intercellular level. However, it poses special problems due to various complexities, such as the time distribution of incidence in an irradiated sample or the progressive reduction of its size during the observation period, and due to the fact that one must usually rely on a small number of cases. Proper choice of the mathematical representation of experimental data and suitable statistical procedures are therefore critical. In the following a method for the quantification of the experimental data, for the establishment of dose-effect relations, and the determination of dose-RBE relations will be discussed.

a) Tumor rate, integrated tumor rate, and cumulative incidence

Assume that a group of individuals is exposed to a radiation dose

and that the incidence of a certain type of tumor is scored throughout an observational period after irradiation. If throughout the observation period no irradiated individuals are removed from the sample except due to the incidence of tumors, one may merely score the fraction of elements of the sample which show the effect at a given time, t , after irradiation. This fraction can then be considered as the cumulative incidence in the observed group. The cumulative incidence assumes values between 0 and 1; it is equal to the probability that an individual randomly selected from the population has at least one tumor at time t . In certain cases, such as in the ongoing studies of the induction of mammary tumors in the Sprague Dawley rat (see also (1,2,3)), multiple independent tumors in the same individual can be scored. The mean number of neoplasms per individual can then exceed the value 1. It will be shown in the following that a related quantity can be defined and is equally useful in the case where only one neoplasm per individual can arise.

In general the experimental situation is complicated by the fact that the irradiated samples are reduced during the progress of the observation either because of deaths, not necessarily related to cancer, or because of loss of some of the members of the group due to other causes. The number of tumors divided by the initial sample size has then no direct meaning. One must instead use actuarial

methods. Rosenblatt et al (4) have considered this problem and have offered a scheme to obtain the cumulative incidence. The approach outlined in the following is formally somewhat different; it will therefore be useful to compare the two schemes.

Rosenblatt et al derive the cumulative tumor incidence by a process of repeated multiplication of the probabilities for non-incidence of the effect. Assume that n tumors appear in the sample at the times t_i ($i = 1, \dots, n$), and that the size of the observed group (i.e. the number of tumor free animals) as a function of time t is $N(t)$. The estimate of the cumulative incidence at the time t is then:

$$I(t) = 1 - \prod_{i=1}^n \left(1 - \frac{1}{N(t_i)} \right) \quad (1)$$

where the product is to be taken over all incidence times, t_i , not later than t . It is assumed in this case that no multiple tumors are scored, and that each animal is removed from the sample upon the appearance of a tumor. In practice formula (1) is modified due to the fact that the observation is not continuous but occurs at distinct intervals; several tumors in the group may then be found at the same time. If N_i is the sample size at the i -th observation after irradiation, and if n_i is the number of tumors found at this time, one uses according to Rosenblatt et al the following estimate of $I(t)$:

$$I(t) = 1 - \prod_{i=1}^n \left(1 - \frac{n_i}{N_i}\right) \quad (2)$$

where N_i is the mean number of individuals at risk in the interval between observation $i-1$ and observation i :

$$N_i = \frac{N_{i-1} + N_i + n_i}{2} \quad (3)$$

Eqs. (1) to (3) correspond to the formula given by Rosenblatt et al; different symbols are used in the present context in order to facilitate comparison with the formalism employed in the following.

Before further consideration of the evaluation of experimental data it will be useful to define some of the underlying quantities without regard to their statistical fluctuation. The following relations are therefore valid either for statistical mean values or for values resulting in the limit of very large samples. Let $N(t)$ be the sample size as a function of time, i.e. the number of animals under observation at time t , and let $n(t)$ be the mean number of tumors which appear up to time t . It will be left open whether individuals are removed from the group after appearance of a tumor or whether multiple tumors can be scored; however, it will be assumed that no secondary (e.g. metastatic) tumors are scored.

The number of tumors per time interval divided by sample size is the tumor rate:

$$r(t) = \frac{1}{N(t)} \frac{dn(t)}{dt} \quad (4)$$

Accordingly the time integral of the tumor rate is:

$$R(t) = \int_{\tau=0}^t \frac{1}{N(\tau)} \frac{dn(\tau)}{d\tau} d\tau = \int_{n(0)=0}^{n(t)} \frac{1}{N(\tau)} dn(\tau) \quad (5)$$

In the following this quantity will tentatively be called integral tumor frequency. If multiple tumors can be scored, $R(t)$ is equal to the mean number of tumors per individual. If the first tumor in a specimen eliminates that specimen from the observed sample, $R(t)$ is devoid of concrete meaning. It is however, as will be seen, a useful concept even in those cases, particularly if one studies the time dependence of the tumor rate.

$R(t)$ is the time integral of the tumor rate. Since the tumors are assumed to be statistically independent one deals with a Poisson process. The probability to be tumorfree at time t is therefore $\exp(-R(t))$ for those individuals which are not removed from the observed group for causes unrelated to the tumor induction. Accordingly the cumulative incidence is:

$$I(t) = 1 - e^{-R(t)} \quad (6)$$

After the relevant quantities have been defined one may return to the

problem of estimating these quantities from the observations in finite samples.

According to Eq. (5) one has the following estimate for the integral tumor frequency:

$$R(t) = \sum_{t_i \leq t} 1/N(t_i) \quad (7)$$

where t_i are the appearance times of tumors. With this value Eq. (6) gives the estimated value of the cumulative incidence $I(t)$. In those cases where there are only a finite number of inspections one must use the approximation:

$$R(t) = \sum_i 2n_i/(N_{i-1} + N_i) \quad (8)$$

where, as defined earlier, N_i is the number of specimens which continue to remain in the sample at inspection i , and n_i is the number of tumors which have newly appeared at this inspection.

Eq. (8) together with Eq. (6) take the place of Eqs. (2) and (3). It can be shown that the resulting values of $I(t)$ are nearly the same in both cases. Expanding the expression $\ln(1 - I(t))$ with $I(t)$ defined according to Eqs. (2) and (3) into powers of $2n_i/(N_{i-1} + N_i)$, one finds that the expression differs from the quantity $R(t)$ in Eq. (8) only by terms of the third and higher order (this is the justification for the particular form of N_i in Eq. (3)).

The modified formulation has the advantage that it is simpler; more importantly, and in contrast to the earlier treatment, it permits the correct determination of the standard deviation.

According to Eq. (5) one has:

$$d R(t) = \frac{dn(t)}{N(t)} \quad (9)$$

Since the variance, σ^2 , of the sum of independent random variables is the sum of the variances of these random variables, one need only determine the variance of $dR(t)$ and integrate the resulting value in order to obtain the variance of $R(t)$ ^{*}. For random events which follow a Poisson distribution the mean is equal to the variance, therefore one can set the variance of $dn(t)$ equal to $dn(t)$.

Accordingly the variance of $dR(t)$ can be equated with $\frac{dn(t)}{N^2(t)}$

and by integration one obtains the variance of $R(t)$:

$$\sigma_R^2(t) = \int_{n=0}^{n(t)} \frac{dn(\tau)}{N^2(\tau)} \quad (10)$$

In the case of a finite sample and a finite number of inspections one obtains therefore the estimate of the variance:

* Underlining is used here to distinguish the random variable from its mean value or the estimate of this mean value.

$$\sigma_R^2 = \sum_i 4n_i / (N_{i-1} + N_i)^2 \quad (11)$$

An expression essentially equivalent to Eq. (11) has been given by Rosenblatt et al for the variance of $I(t)$. One should note that this is incorrect except for values of $I(t)$ which are so small that $I(t)$ can be equated with the integral tumor frequency $R(t)$ (see Eq.(6)). Otherwise one obtains standard errors which are too large (the ranges of the standard error given by Rosenblatt et al for the curves with highest incidence frequencies are too large by nearly a factor of 2). It is therefore important to note that one deals with the variance of $R(t)$, i.e. of the quantity $\ln(1-I(t))$, and not with the variance of the cumulative tumor incidence $I(t)$.

For small absolute incidences knowledge of the variance is of limited value because the distribution of R is unknown and cannot be assumed to be normal. Statistical procedures which apply in this case and which are generally applicable to the comparison of the incidence in two observed groups are described in section BP-6.

b) Time dependence of the tumor rate

Due to the limited number of observed tumors it is usually not possible to find a precise analytical description of the time dependence of the tumor rate and of its dependence on radiation dose or radiation quality. The problem has, however, important practical as well as theoretical implications (see for example (5,6)) and it is

therefore of interest at least to compare the data to the simplest theoretical possibilities which involve as few free parameters as possible. Although no experimental results are discussed in the present context it will be useful to consider in the following three simple alternatives.

The first question one may ask is whether with irradiation the spontaneous tumor rate, $r_0(t)$, is merely increased by a constant factor, i.e.:

$$r(D;t) = f(D) \cdot r_0(t) \quad (12)$$

where $f(D)$ is some function of the absorbed dose, with $f(0) = 1$. The alternative is that the relation is of a more complicated form which involves also a change of the time dependence of the tumor rate.

For a finite sample the integrated tumor frequency R can more easily be estimated than its derivative r , since the graphic representation of the experimental data which corresponds to this quantity (see Eq. (8)) is less distorted by statistical fluctuations than the histogram which corresponds to $r(t)$. If $R_0(t)$ is the integral of the function $r_0(t)$, i.e. the integral tumor frequency for non-irradiated animals, one has:

$$R(D;t) = f(D) \cdot R_0(t) \quad (13)$$

One can therefore plot the logarithm of R versus time after irradiation in order to test whether the tumor rate increases with increasing dose by a factor which is dependent or independent of time. In the latter case one should find that the curves for different doses are merely shifted vertically. The shape of the dose-effect relation is then independent of the observation period. If, on the other hand, the resulting curves are of the same shape but are horizontally shifted with respect to each other, one can conclude that irradiation has the effect of an "age increment," a possibility which would be expressed by an equation of the form:

$$R(D;t) = R_0(t + \Delta t(D)) \quad (14)$$

where the age increment $\Delta t(D)$ depends on the dose. This particular possibility has been considered in the interpretation of lens opacification data (7).

Finally, irradiation could have the effect of accelerating the increase of the spontaneous tumor incidence with age:

$$R(D;t) = R_0(f(D) \cdot t) \quad (15)$$

by a dose dependent factor $f(D)$. In this latter case one would observe a horizontal shift of the curves of R versus t in a double logarithmic representation. One should note that similar criteria do not apply to the cumulative incidence, $I(t)$, and that therefore it is more

useful to consider the integral tumor frequency $R(t)$.

One should note that the three simple cases expressed by Eqs. (13) to (15) are not mutually exclusive. If the tumor rate is proportional to a power of t , then both the conditions (13) and (15) can be fulfilled. If, on the other hand, the tumor rate increases exponentially with time t , then both the conditions (13) and (14) can be met.

A preliminary analysis of the data obtained by Shellabarger on Sprague Dawley rats exposed to x rays, γ rays, and monoenergetic neutrons indicates that the results are compatible with a linear increase of the tumor rate with time t after irradiation:

$$\begin{aligned} r(t) &= f(D) \cdot c \cdot t \\ R(t) &= \frac{f(D)}{2} \cdot c \cdot t^2 \end{aligned} \tag{16}$$

where $c \cdot t$ is the tumor rate for the controls.

It is of interest to compare the approach which is based on the analysis of $R(t)$ or $r(t)$ with the analysis chosen by Rosenblatt et al who fit $I(t)$ to a curve which contains several free parameters. In contrast to this approach only one single free parameter is involved if one can assume that tumor rate increases linearly with time.

The observation that the time dependence of tumor rates and integral tumor frequencies can be considered to be the same for different doses and different radiation qualities, makes it possible

to define RBE without ambiguity (this is analogous to the case of the lens opacification studies). In an earlier analysis of the dose dependence of the induction of mammary tumors (8), the mean number of tumors per animal (i.e. the integral tumor frequency $R(t)$) has been derived for $t=11$ months. The observation that the form of the time dependence of $R(t)$ remains unchanged for different doses implies that the choice of t is irrelevant for the shape of the dose-effect curves; different choice of t merely leads to a constant factor in R .

Precise determination of the dose-effect relations and their confidence ranges will have to be based on the continuing observations in the present experimental study. The observed values of the integral tumor frequency after 10 months together with their standard deviations support the earlier observations that even at very small neutron doses the integral tumor frequency increases with a power of D significantly less than 1. The data obtained within 10 months after neutron irradiation have been compared to the earlier data obtained with x rays and γ rays and a dose-RBE relation has been found which is in agreement with the relation obtained in various other systems (9). The lowest neutron dose of approximately 0.1 rad leads to a significantly increased tumor rate, and the RBE at this dose relative to γ rays appears to be larger than 100.

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BP-6 COMPARISON OF EFFECT RATES IN SAMPLES OF TIME DECREASING SIZE

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1. Statement of the problem

For the non-parametric determination of RBE or of other modifying factors effect levels in pairs of irradiated samples have to be compared. Depending on the nature of the experimental system different statistical tests, such as the Mann-Whitney rank order test, the Fisher exact probability test, or the χ^2 -test can be applied; the most common situations have been considered in a recent paper (Kellerer and Brenot, 1973). However, one case which is frequently encountered can not be readily treated by established statistical methods. This is the case where event frequencies are observed in groups which are subject to the loss of elements due to causes unrelated to the observed effect. One deals with this situation in radiation cancerogenesis (see section BP-5), but it arises also, for example, in follow-up studies of medical patients subject to alternative treatments. In order to simplify the explanations, one specific criterion, namely tumor induction, will be considered in the following; but all the arguments are applicable to the general case.

Assume that tumors can appear during an observation period in two groups of animals; assume further that, due to death or other causes

unrelated to tumor incidence, animals can be lost from the two groups. It is postulated that the incidences of tumors in the animals of a group are statistically independent. However, loss of animals from the group need not be statistically independent; animals may, for example, be lost simultaneously due to an infection. Neither will it be postulated that the statistical loss rate is the same in both observed groups.

Two different cases can be distinguished. In case A the animal which has developed a tumor remains in the sample*. In case B the animal is removed from the sample once it develops a tumor. The statistical procedures described in the following will be applicable to case A. In case B they are not strictly valid; it is, however, an open question whether in practice they are not equally applicable in this case.

2. Statistical tests

Assume that n tumors occur in group 1 and m tumors in group 2. Assume further that these $n+m = k$ tumors appear at the times t_i ($i = 1, \dots, k$). For simplicity it is assumed that no two tumors appear at the same time. Let N_i and M_i be the number of animals in group 1

* In the study of mammary tumors of the Sprague-Dawley Rat newly discovered tumors are removed and the animal is returned to the sample (See Shellabarger, 1968).

and 2 at time t_i . In the following, methods will be considered for examination of the null-hypothesis, that the tumor rate and its time dependence during the observation period are statistically equal in both groups.

2.1 Large absolute incidences

In Section BP-5 the integral tumor frequency (i.e. in case A the mean number of tumors per animal) has been defined as the time integral of the tumor rate, and the variance σ^2 of the integral tumor rate has been equated with the time integral of the tumor rate divided by the sample size. Under the null-hypothesis one has therefore the following estimate of the integral tumor frequency:

$$\bar{I} = \sum_{i=1}^k \frac{1}{N_i + M_i} \quad (1)$$

and the following estimates for the variance in groups 1 and 2:

$$\sigma_1^2 = \sum_{i=1}^k \frac{1}{(N_i + M_i)N_i} ; \quad \sigma_2^2 = \sum_{i=1}^k \frac{1}{(N_i + M_i)M_i} \quad (2)$$

The observed integral tumor frequencies in groups 1 and 2 are:

$$I_1 = \sum_i \frac{1}{N_i} ; \quad I_2 = \sum_i \frac{1}{M_i} \quad (3)$$

where in each term the summation includes only those cases which occur in the particular group.

If all the values N_i and M_i and also k are sufficiently large, one can apply the χ^2 -test and compare the following value of χ^2 with the critical value for one degree of freedom (3.84 for the 95% significance level):

$$\chi^2 = \frac{(I_1 - I)^2}{\sigma_1^2} + \frac{(I_2 - I)^2}{\sigma_2^2} \quad (4)$$

2.2 Small samples

There is no obvious way in which such tests as the Fisher exact probability test or the rank order tests can be modified so that they apply to the case where elements from the observed groups are lost. For this reason an alternative procedure has been derived; it will be seen that this procedure is also useful in the case of large samples.

Consider all the instances, t_i , where a tumor appears in one of the two groups. If the null-hypothesis is valid, then the relative frequencies with which the tumors appear in the one or the other group should reflect the relative size of the groups at the times of occurrence. The probability that at instance t_i the tumor should occur in group 1 is $p_i = N_i / (N_i + M_i)$, the probability for its occurrence in group 2 is $q_i = M_i / (N_i + M_i)$. One can apply this consideration to all

instances t_i ; due to the fact that the occurrences in successive events are independent^{*}, one obtains the probabilities for any particular distribution of the k tumors to the two groups by multiplication of the corresponding probabilities p_i and q_i . The probability P_v to obtain exactly v tumors in group 1 is the sum of all products of v factors p_i and $k-v$ factors q_i ($i = 1, \dots, k$). The probabilities P_v are therefore, in close analogy to the binomial distribution, defined by the identity:

$$\sum_{v=0}^k P_v x^v = \prod_{i=1}^k (p_i x + q_i) \quad (5)$$

In other words, $\prod_{i=1}^k (p_i x + q_i)$ is the moment generating function of the distribution P_v of the number v of tumors which should be in group 1 if the null-hypothesis is valid.

Comparison of the observed number, n , of tumors in group 1 with the distribution P_v permits the decision whether the tumor rates in the two groups are significantly different. For this purpose one must evaluate the product in Eq.(5). The test can not, as with other tests, be based on precalculated tabulated functions. The reason is that too

^{*}It is at this point that the considerations do not strictly apply to case B. In this case the occurrence of tumors in successive instances is still statistically independent; however, the values N_{i+1} and M_{i+1} are influenced by the fact whether the i -th tumor occurs in group 1 or 2.

many parameters, namely all the values N_i and M_i are involved. Computer evaluation is, however, straightforward. Moreover one can use the normal approximation if n and m are sufficiently large. The moments of P_v can be calculated directly from the moment generating function; $g(x) = \prod_{i=1}^k (p_i x + q_i)$. One obtains:

$$v = g'(1) = \sum_{i=1}^k p_i \quad (6)$$

$$\begin{aligned} \sigma_v^2 &= g''(1) + g'(1) - g'(1)^2 \\ &= \sum_{i \neq s} p_i p_s + \sum p_i - (\sum p_i)^2 \\ &= \sum_{i=1}^k (p_i - p_i^2) \end{aligned} \quad (7)$$

2.3 Temporal ranking

The test described in the previous section can readily be modified to account for statistics other than the total number of tumors in a group. One can weight the tumors according to their incidence time with factors s_i ; the moment generating function then has the form $\prod_{i=1}^k (p_i x^{s_i} + q_i)$. One possible choice would be to set s_i equal to $T-t_i$, where T is the expected end of the life span. The statistic one deals with may then be considered as the number of years lost due to the effect.

Another similar and somewhat simpler possibility is to rank the tumors according to the temporal order of their appearance, and use the resulting rank sum in one group as statistic; this is then analogous to the procedure in the Mann-Whitney or the Wilcoxon test. Assume that the index i is counted backwards so that t_1 is the time of appearance of the last tumor which is assigned the rank 1, and t_k is the time of appearance of the first tumor which is assigned the rank k . The distribution P_r of the rank sum r is then determined by the identity:

$$\sum_{r=0}^R P_r x^r = \prod_{i=1}^k (p_i x^i + q_i) , \quad R = \frac{k(k+1)}{2} \quad (8)$$

The calculation is complicated by the fact that the statistic r covers a wide range of possible values, namely the range 0 to R . Otherwise the procedure is the same as the one described in the preceding section. The moments are again directly obtained from the generating function, and one finds in analogy to Eqs.(6) and (7):

$$\bar{r} = \sum_{i=1}^k i P_i , \quad \sigma_r^2 = \sum_{i=1}^k i^2 (p_i - p_i^2) \quad (9)$$

It remains to examine the possibility that the observations are performed in discrete time intervals, and that accordingly $\ell > 1$ tumors can appear at the same time, t_i . In case A this leads to no

complications, it merely means that some of the t_i have the same value. In case B however one cannot proceed in this way because the values N_i and M_i change with the appearance of each tumor. This cannot be neglected unless ℓ is small as compared to N_i and M_i . The term $(p_i x + q_i)^\ell$ or its modification $(p_i x^i + q_i)^\ell$ is then to be substituted by a polynomial which results from Fisher's exact probability test (Fisher, 1925). The polynomial is

$$(\pi_\ell x^\ell + \pi_{\ell-1} x^{\ell-1} + \dots + \pi_0) \quad \text{or} \quad (\pi_\ell x^{i\ell} + \pi_{\ell-1} x^{i(\ell-1)} + \dots + \pi_0),$$

where the π_k are the exact probabilities that k tumors out of the ℓ tumors appearing at time t_i are in group 1.

A computer program has been written which applies these tests to the tumor studies presently performed in Brookhaven by Shellabarger. The tests which are based on incidences and on rank sums have been found to lead to roughly the same results. This indicates that irradiation produces a general increase of tumor rates throughout the observational period and not merely a shift towards earlier appearance times. The experiments are still in the early stages in which no extensive loss of animals occurs; as the experiments proceed mortalities will be more important and application of the multiplicative tests which were described here will then be decisive in the determination of RBE.

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BP-7 MICRODOSIMETRY AND THE DISPERSION OF CHROMOSOME ABERRATIONS

A.M. Kellerer

Microdosimetric functions have commonly been applied to the study of dose-effect relations, i.e. of the distributions of doses necessary to produce certain cellular effects. They are equally relevant to the study of the distribution of effect levels produced by a given dose in a population of cells. This latter aspect has found little attention in the past, it is, however, not without interest. The distribution of chromosome aberrations in irradiated cells is an example where it is of importance. In the following no precise quantitative evaluation will be attempted. But it will be pointed out that due to the increased statistical fluctuations of energy deposition one may expect increased relative variance of the distributions with densely ionizing radiation. This is analogous to the case of dose-effect relations where one also finds curves of large relative variance when the LET of the radiation is increased. It is in agreement with the experimental finding that underdispersion of dicentrics is more expressed for γ -irradiation in Tradescantia microspores than for fast neutron irradiation (Bhatt et al (1968)), and the recent observation (Chmelevsky et al (1972)) that dicentrics in γ -irradiated lymphocytes are underdispersed while overdispersion occurs when the same cells are exposed to a mixed field of

neutrons and gamma rays. The increment in dispersion due to the statistics of energy deposition will be considered both for one-break and two-break aberrations.

Random distribution and underdispersion

It will be useful to review some of the established relations before radiation quality as an additional factor will be considered. If eukaryotic cells are exposed to ionizing radiation in G1- or early S-phase chromosome breaks - or, in a more general interpretation (Revell (1959)), local instabilities of the chromosome structure - are produced. Usually the lesions reconstitute and the linear order of the chromosome is preserved; if, however, two lesions from different breaks unite for an asymmetric exchange, dicentrics or centric rings occur. If, as can be assumed (Lea (1946)), only a minor fraction of all breaks are involved in exchanges, one should obtain a Poisson distribution of the number of exchanges in individual cells. If η is the mean number of exchanges in a cell at a given dose, the fraction of cells with v aberrations will be:

$$P_v = e^{-\eta} \frac{\eta^v}{v!} \quad (1)$$

while the mean, $\bar{v}$, is equal to η , one has the following relation for the mean of the square, $\overline{v^2}$, i.e. the second moment of the Poisson distribution:

$$\overline{v^2} = \eta^2 + \eta \quad (2)$$

The relative dispersion^{*} is defined as the ratio of the dispersion (variance), σ^2 , and the mean of the distribution. From Eq.(2) one finds that for the Poisson distribution the dispersion is equal to the mean:

$$\sigma^2 = \overline{(v-\eta)^2} = \overline{v^2} - \eta^2 = \eta \quad (3)$$

and therefore the relative dispersion has the value 1:

$$\frac{\sigma^2}{\eta} = 1 \quad (4)$$

The derivation of this well-known relation will, in modified form, be applied to the more complicated cases. In the following, a relative dispersion of the value 1 will be called normal, while values below and above 1 will be referred to as underdispersion and overdispersion respectively.

Various factors can lead to deviations of the exchange distributions from the Poisson distribution. According to Wolff (1959) underdispersion occurs when one deals with a limited number, n , of sites where exchanges can take place. In this case one obtains a binomial distribution of the probability to observe v aberrations in

*The term "relative variance" has frequently been used for $\sigma^2/\overline{v}$; it is avoided here because in microdosimetry it denotes the ratio of the variance and the square of the mean of a distribution.

a cell:

$$P_v = \binom{n}{v} p^v (1-p)^{n-v} \quad (5)$$

where $p = \eta/n$ is the probability for an observable exchange per site. The second moment of the binomial distribution is:

$$\overline{v^2} = n^2 p^2 + np - np^2 \quad (6)$$

and with the mean $\eta = np$ one obtains the relative dispersion:

$$\frac{\sigma_v^2}{\overline{v}} = \frac{\overline{v^2} - \overline{v}^2}{\overline{v}} = \frac{\overline{v^2}}{np} - np = 1 - \frac{\eta}{n} \quad (7)$$

This formula is valid for a fixed site number, n . The relative dispersion is larger if one deals with a varying site number, n .

If N and σ_N^2 are the mean and the variance of n , one obtains by averaging over Eq.(6):

$$\begin{aligned} \overline{v^2} &= \overline{n^2} p^2 + \overline{np} - \overline{np^2} \\ &= \sigma_N^2 p^2 + Np - Np^2 + N^2 p^2 \quad \text{with } p = \eta/N \end{aligned} \quad (8)$$

Therefore one has:

$$\frac{\sigma_v^2}{\overline{v}} = 1 - \frac{\eta}{N} \left(1 - \frac{\sigma_N^2}{N} \right) \quad (9)$$

The result corresponds to the formula given by Atwood (1963); it

implies that underdispersion is less expressed if the site number varies stochastically. The formula simplifies if the site number varies according to a Poisson or a binomial distribution. If sites are formed randomly wherever two chromosome arms are in sufficient proximity, their number should be distributed according to Poisson; the relative dispersion, σ_N^2/N , of the number of sites is then equal to 1 and consequently the relative dispersion of chromosome exchanges is 1. If, however, n varies according to a binomial distribution with a maximum number M of sites, the relative dispersion of the site numbers equals $1 - N/M$ (in analogy to Eq.(7)), and accordingly one obtains the relative dispersion of chromosome exchanges:

$$\frac{\sigma_v^2}{n} = 1 - \frac{n}{M} \quad (10)$$

This means that underdispersion can not merely be due to the fact that the mean site number is small. It can only occur if the mean number of sites is small and the distribution of site numbers is narrower than a Poisson distribution. This would require that there is an upper limit to the number of possible sites, and according to Eq.(10) the index of the observed binomial distribution of chromosome exchanges is to be identified with this maximum number of sites, not with the mean number of sites. It also implies that the notion of a finite site number and the concept of distortion (Savage, 1967, 1970)

are essentially equivalent. One should note that the relative dispersion of the distribution of site numbers can exceed 1 in all those cases where one deals with an inhomogeneous population of cells. For example one should observe increased dispersion if one deals with a population which is not perfectly synchronized at the time of irradiation. Variations of the site number are however not the only factor which may increase dispersion. The statistical fluctuations in energy deposition, especially with densely ionizing radiation, can also lead to an increase of dispersion.

Increase of dispersion due to fluctuations of energy deposition

The probability that a site is affected, or more generally speaking, the expected number, η , of observable exchanges in a cell need not be a unique function of absorbed dose; it may also depend on the cell age or on other variables in the irradiated population. Suitable choice of the experimental technique can, at least in principle, reduce such statistical variations which lead to an increase in dispersion. Intrinsic stochastic variations are however due to the fact that the absorbed dose, D , is only a mean value and that the corresponding random variable, the specific energy, z , in the cell nucleus, can deviate significantly from D . Because η depends on the energy actually laid down in the nucleus or in a sensitive subnuclear region it must be considered as a function of

z rather than D . It is therefore itself a random variable, and this leads to an increase in dispersion. In the following this increase will be calculated for different radiation qualities. For this purpose a simplified situation will be considered. It will be assumed that the yield, η , of aberrations depends only on the total energy deposited within the cell nucleus (or a subregion of the nucleus), and that for a given energy deposition, i.e. for a given z , the actual number of aberrations follows a Poisson distribution around η . Although more complicated, it is possible to analyze the case where for a given z the dispersion is less than 1. The simple case may, however, serve to demonstrate the influence of radiation quality more clearly, and bring out the basic point that dispersion should increase with increasing LET. The assumption that only the total energy within the sensitive region of the nucleus matters, is, of course, only an approximation. If one were to make a specific assumption concerning the number and the spatial distribution of sites in the nucleus, one might find that for a given z the yield depends also on the length or the radial extension of the particle tracks. It can, however, not easily be judged what form such a dependence would take, and it is therefore appropriate to consider the simplest case.

Formal relations

Although the focus of experimental studies has been on the

determination of the dispersion of 2-break exchanges and only little experimental information is available on the dispersion of chromosome breaks (Lea (1946)), both cases will be considered. Since the case of the 1-break aberrations is simpler, it will be treated first.

The yield of chromosome breaks depends on the energy deposited in a sensitive region, either the cell nucleus or a part of it. In the following the term sensitive region will be used with this connotation, and it will be left open whether it is the whole nucleus or a part of it, such as a narrow region near the nuclear membrane. One can assume that at a given specific energy, z , in the sensitive region the number of breaks follows a Poisson distribution with the mean:

$$\eta = cz \quad (11)$$

While at a given z one deals with a simple Poisson distribution of the number, v , of breaks, the actual distribution of v at a given absorbed dose D is a superposition of Poisson distributions with different mean values according to the relative frequency of the different values of z . Superposition of Poisson distributions does not again lead to a Poisson distribution but instead to a distribution with a relative dispersion larger than 1.

The relative frequency of different values of z is determined by the microdosimetric distribution $f(z;D)$; accordingly one obtains

the following expression for the probability of v breaks:

$$P_v = \int_0^{\infty} e^{-cz} \frac{(cz)^v}{v!} f(z;D) dz \quad (12)$$

The microdosimetric functions $f(z;D)$ are known for different radiation qualities and different sizes of the reference region.

It will in the present context not be necessary to evaluate Eq.(12); only the mean and the second moment have to be calculated.

This is done by integrating the mean, cz , and the second moment, $c^2 z^2 + c z$, (see Eq.(2)) of the Poisson distribution over $f(z;D)$:

$$\begin{aligned} \bar{v} &= \int_0^{\infty} cz f(z;D) dz \\ \overline{v^2} &= \int_0^{\infty} (c^2 z^2 + c^2 z) f(z;D) dz \end{aligned} \quad (13)$$

The mean of z is equal to the absorbed dose:

$$D = \int_0^{\infty} z f(z;D) dz \quad (14)$$

and the mean of the square, z^2 , at an absorbed dose D is given by the relation:

$$\overline{z^2} = \zeta D + D^2 \quad (15)$$

where ζ is the energy average of the distribution $f_1(z)$ of increments of specific energy, z , brought about by a single charged particle track (see for example Kellerer and Rossi, 1972).

Accordingly one has:

$$\bar{v} = cD \quad (16)$$

$$\overline{v^2} = c^2(\zeta D + D^2) + cD \quad (17)$$

and the relative dispersion of the number of 1-break aberrations is:

$$\frac{\sigma^2}{\bar{v}} = \frac{\overline{v^2} - \bar{v}^2}{\bar{v}} = 1 + c\zeta \quad (18)$$

This means that the number of 1-break aberrations should show a constant overdispersion which is equal to the mean number of such aberrations produced per cell by a dose of size ζ . The quantity ζ is the energy average of the specific energy z produced by a single particle track in the sensitive region.

Eq.(18) implies that, if, for example, gaps are scored, overdispersion should be largest for densely ionizing radiations, for small cell nuclei (or sensitive regions), and for the most sensitive method of scoring.

For 2-break aberrations one can set the expected yield in a cell proportional to z^2 , since the number of exchanges is proportional to the square of the concentration of breaks (see for example Conger, 1965):

$$\eta = kz^2 \quad (19)$$

Accordingly the mean number of 2-break aberrations per cell at the absorbed dose D is obtained by averaging η over the distribution of z :

$$\bar{\nu} = k \int_0^{\infty} z^2 f(z;D) dz = k(\zeta D + D^2) \quad (20)$$

For the second moment one has, in accordance with Eq.(2):

$$\overline{\nu^2} = \int_0^{\infty} (k^2 z^4 + kz^2) f(z;D) dz \quad (21)$$

Therefore, if the averages of z and its higher powers over $f(z;D)$ are indicated by a horizontal bar, the relative dispersion of the number of the exchanges is:

$$\frac{\sigma^2}{\bar{\nu}} = \frac{\overline{\nu^2}}{\bar{\nu}} - \bar{\nu} = 1 + k \frac{\overline{z^4}}{\bar{\nu}} - \bar{\nu} \quad (22)$$

The evaluation of this expression as a function of dose is complicated. Using the relations between the moments of $f(z;D)$ and $f_1(z)$ (see Kellerer, 1969) one obtains:

$$\frac{\sigma^2}{\bar{\nu}} = 1 + k \frac{\zeta_3 + 4D\zeta_2 + 2D^2\zeta_2 + 4D^3\zeta}{\zeta + D} \quad (23)$$

where ζ_2 and ζ_3 are the second and third moment of the distribution

$f_1(z)$ of specific energy in single events while ζ , as defined in Eq.(16) is the mean of this distribution.

For large doses, i.e. if D is large as compared to ζ, ζ_2 , and ζ_3 , this reduces to the simpler expression:

$$\frac{\sigma^2}{\bar{v}} = 1 + 4k\zeta D \quad (24)$$

This means that for 2-break aberrations the increase of relative dispersion due to the statistics of energy deposition is proportional to dose. As in the case of 1-break aberrations the increase in dispersion is greatest for densely ionizing radiation and for small sensitive regions.

Numerical evaluation

Eqs.(18) and (24) express the increase of relative dispersion due to the fluctuations of energy deposition in terms of the single microdosimetric quantity ζ . However, it remains to be seen whether the simplified Eq.(24) is a sufficient approximation for the more accurate Eq.(23). General comparison of the two equations is not possible, since the ratio of the quantities ζ_3, ζ_2 and ζ is different for different radiation qualities. One may however consider the simple special case of a radiation with constant LET. In this case only LET and the statistical fluctuations of the chord lengths in

the sensitive region determine the moments ζ_3 , ζ_2 and ζ . Particularly one has (Kellerer and Rossi, 1969):

$$\zeta = 34.4 \frac{L}{d^2} \quad (25)$$

where ζ is measured in rad, the linear energy transfer, L , is in keV/ μ m, and the diameter d of the sensitive region is in μ m. Fig.1 is a plot of the relative dispersion of 2-break aberrations for different LET values in this case according to Eq.(24) (solid curves). For the purpose of this example the value $7 \cdot 10^{-6}$ (rad⁻²) has been assumed for k ; this value is somewhat arbitrary, but it lies near the middle of the range of values obtained in experimental studies of various plant and animal cells (see Bauchinger, 1972). Furthermore it is assumed that the diameter of the sensitive region is 7 μ m which corresponds approximately to the diameter of the nucleus of a human lymphocyte. A considerably larger increase of dispersion is obtained if it is assumed that the sensitive region is only part of the nucleus; on the other hand, one may obtain smaller values in plant material, for example Tradescantia, where cellular nuclei may be considerably larger. For comparison with the simplified equation the curves which result according to the more accurate Eq.(23) are given as broken lines in Fig. BP-7-1.

BP-7-1

One concludes from Fig./ that the simplified Eq.(24) is a

Fig. BP-7-1 Theoretical overdispersion of two-break aberrations in the absence of factors, such as limited site number of distortion, which lead to a reduction in dispersion. Parameter of the curves is the linear energy transfer. The broken curves correspond to the accurate equation (23), the solid line to the approximate relation (24).

conservative but perhaps often close approximation. For actual numerical estimates in a particular experiment one must use whatever actual value of k is observed; one must also note that in one and the same biological object this value need not be the same for different radiation qualities.

An approximate value of ζ can usually be obtained by entering the dose average linear energy transfer, $\bar{L}_D$, in Eq.(25). This leads, however, to overestimates for low or intermediate energy neutrons, because the range of the charged particle recoils is frequently smaller than the diameter d of the sensitive region. One then has to use actual microdosimetric data. From such data (see Kellerer and Rossi, 1972) one concludes that for neutrons the resulting overdispersion at an exchange frequency near 1 is quite significant, while the values of ζ for x rays are so small that no observable overdispersion is to be expected.

Up to this point only spherical sensitive regions have been considered. Several recent investigations suggest that at least in certain phases of the cell cycle DNA is concentrated near the nuclear membrane; it is therefore of interest to derive the quantity ζ for this configuration. The calculation, in terms of LET, is elementary but leads to somewhat complicated integrals. The evaluation has therefore been performed on the computer. ^{BP-7-2} Fig. / represents the

Fig. BP-7-2 Ratio $\rho(\epsilon)$ of the mean event size ζ in a spherical shell and in the corresponding sphere. The ratio of shell thickness and shell radius is ϵ .

result in a form which permits ready comparison with the case of a spherical volume. The quantity, ζ , for a spherical shell of diameter d , and of a ratio ϵ of its shell thickness to its radius can be given in a form similar to Eq.(25):

$$\zeta = 34.4 \rho(\epsilon) \frac{L}{d^2} \quad (26)$$

The factor $\rho(\epsilon)$ which distinguishes the case of the spherical shell from that of the sphere is given in Fig.2 as a function of ϵ . One finds that in the range of practical interest ($0.1 < \epsilon$) the values of ζ for the shell are remarkably close to those for a sphere of equal radius.

No microdosimetric data are available for other than spherical volumes; it is therefore not possible to give values of ζ for a spherical shell and for neutrons. The approximation in terms of LET is, however, acceptable even for neutrons, because most of the recoils in the energy range of practical interest have ranges comparable to or larger than the thickness of the shell if the latter is of the order of fractions of a micrometer.

Summary

One concludes that in the absence of factors which reduce dispersion 1-break and 2-break chromosome aberrations should be over-dispersed due to the statistical nature of energy deposition. The

amount of overdispersion for a 1-break aberration is $c\zeta$, where c is the mean number of observed aberrations per cell and per unit dose and ζ is the energy average of the increments of specific energy produced by a single charged particle track in the sensitive region of the nucleus. In a first approximation ζ is proportional to the dose average LET of the radiation in question and inversely proportional to the square of the diameter of the sensitive region. For 2-break aberrations the overdispersion amounts to $k\zeta D$, where k is the mean yield of these aberrations per cell and unit dose, ζ is defined as above, and D is the absorbed dose. The increase of relative dispersion is therefore not constant as in the case of 1-break aberrations, but is proportional to dose. The more exact formulation in Eq.(23) shows that there is overdispersion even at very low doses, but it is insignificant except for very densely ionizing radiation.

The results account only for the influence of the fluctuations of energy deposition on the distribution of aberration, i.e. they determine the dispersion for a homogeneous population in the absence of mechanisms which lead to a decrease in dispersion. That such mechanisms play a role for 2-break aberrations has been shown in numerous studies with x-rays or γ -rays. Present experimental data for different radiation qualities do not permit a quantitative asses-

sment of the various factors. However the theoretical results explain the observation (Bhatt et al.(1968)) that in Tradescantia microspores significant underdispersion occurs with x-rays while the distributions are close to Poisson distributions for fast neutrons; the results are also in agreement with the recent finding (Chmelevsky et al. (1971)) that dicentrics in lymphocytes are underdispersed for x-rays but, at least partly, overdispersed for a mixture of γ -rays and neutrons.

Generally one concludes that the factors which have been previously invoked to explain underdispersion (Wolff (1959), Atwood (1963), Pershad (1961), Savage (1967, 1970)) may play a role even in those cases where with densely ionizing radiations no underdispersion is observed. Furthermore it follows that, at least for densely ionizing radiations, there is no reason to fit the observed data to one of the commonly considered distributions. A quantitative analysis would have to be based on Eq.(12) which depends on the microdosimetric functions $f(z;D)$; any agreement of the result with, for example, the negative binomial distribution in the case of overdispersion or the binomial distribution in the case of underdispersion would be fortuitous. It is therefore in general more meaningful to analyze the moments of the observed distribution than to attempt a numerical fit to analytic expressions. This is in agreement with the proposal made by Savage and Papworth (in Savage, 1970) for the statistical analysis of exchange aberrations.

The recently developed chromosome banding techniques may lead to better quantitative analysis because they permit the scoring of multiple exchanges, which according to Savage's distortion concept are not detected in the conventional technique. The resulting values of the relative dispersion should be larger. But even if distortion is eliminated or reduced, the dispersion could still be less than predicted by Eq.(24). A reason for this is the potential delay of heavily damaged cells in reaching the first mitosis after irradiation. There have been ambiguous reports about this factor in the literature (Wolff (1959), Pershad (1961)) and, as pointed out by Wolff, it may be of little importance in plant cells. In the relatively sensitive lymphocytes correlation between chromosome damage and division delay can however not be neglected. Haag (unpublished) has found a significant increase of the relative dispersion of dicentrics and rings when irradiated swine lymphocytes are kept for increasing temporal intervals before they are arrested in mitosis. This confirms that cells with multiple aberrations are delayed in their progress towards mitosis and that this factor can reduce the observed dispersion.

The situation may be less complex in the case of 1-break aberrations, where distortion plays no role and where the correlation to division delay may be less important. Overdispersion should be proportional to the yield per unit dose and should therefore be most

easily detected if all observable 1-break aberrations are scored. On the other hand, one may be dealing with smaller sensitive regions if gaps or minute deletions are scored in only one particular chromosome. This would lead to larger values of ζ and correspondingly larger increments in dispersion. Comparison of the relative dispersion of the number of breaks throughout the chromosome complement of a cell and in individual chromosomes could therefore contribute information on the spatial configuration of chromosomes in the interface nucleus.

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BP-8 Analysis of Japanese Leukemia Data

A.M. Kellerer and H.H. Rossi

In its recently published report (1), the Advisory Committee on the Biological Effects of Ionizing Radiation of the Division of Medical Sciences of the National Academy of Sciences (the BEIR Committee), has derived risk estimates for leukemia with the assumption of a fixed RBE of either 1 or 5 for the radiation at Hiroshima (where neutrons predominated) relative to that at Nagasaki (where only gamma radiation was significant). On the other hand the report of the United Nations Scientific Committee on the Effects of Ionizing Radiations (the UN Committee) specifically rejects the notion of a constant RBE and questions the validity of linear extrapolations (2). Although the BEIR Committee mentions such objections to its treatment of the problem, it proceeds to derive risk estimates and to term these "most likely values." There is now evidence that these values are considered to be sufficiently firmly established to form the basis of large-scale national planning.

We have decided to perform an analysis of the Japanese data because of the evident importance of the subject and in view of the fact that the assumption underlying the BEIR Committee estimate would make human leukemia the only somatic radiation effect known that does not conform to the general rule that neutron RBE increases with decreasing dose.

The Data

One of the difficulties in establishing dose-effect or dose-RBE relations for the Hiroshima and Nagasaki observations is that available dosimetric information concerns principally the dependence of tissue kerma in free air ("air dose") on distance^{*}. The mean absorbed dose in the bone marrow is less than kerma, the reduction being larger for neutrons than for gamma rays and dependent in either case on body orientation. However, since it appears that the radiation spectrum changed little with distance from the epicenter of the explosion, the ratio between kerma and dose can be considered a constant. The shape of the dose-effect curve at Nagasaki (where only gamma radiation was of importance) should therefore be the same as that of the kerma-effect curve. At Hiroshima where the proportions of neutron and gamma radiation varied with distance the shapes should differ, particularly at low values where the dose-effect curve should be somewhat steeper.

The RBE is the ratio of doses for equal effects. We are employing for the purposes of this discussion the symbol R for the corresponding kerma ratio. If the ratio of mean dose to the marrow and kerma is denoted by c , i.e. if

^{*} In the following the tissue kerma in free air will be simply referred to as kerma (K).

$$c_Y = D_Y/K_Y \text{ and } c_n = D_n/K_n$$

it follows from

$$RBE = D_Y/D_n \text{ and } R = K_Y/K_n$$

that

$$RBE = (c_Y/c_n) R.$$

The RBE is larger than R because $c_Y > c_n$, but the ratio RBE/R should be at least approximately constant.

The observed leukemia prevalences at Hiroshima and Nagasaki during the observational period from October 1950 to September 1966 are shown in Fig. BP-8-1 as function of kerma. The data are taken from the paper by Ishimaru et al (3). In this compilation the populations were divided into categories which received kermas within certain ranges that were represented by nominal values. The broken lines correspond to a least squares fit which will be discussed below. The 95% confidence limits are indicated by the solid bars.

An overall fit of the data, both in Hiroshima and Nagasaki, is complicated by the unknown influence of lethality and the corresponding flattening of the curves in the range of high doses. A comparison of the kerma-effect relation in Hiroshima with that observed in Nagasaki does, however, indicate a characteristic difference, and we have therefore applied a more direct statistical analysis which is not based on

Figs. BP-8-1a and 1b: Prevalences of leukemia for the period from Oct. 1950 to Sept. 1966 versus kerma at Hiroshima (a) and Nagasaki (b). The bars represent 95%-confidence ranges; the shaded area is the 95% confidence region for the un-irradiated population. The broken curves are the result of a least squares fit as described in the text.

Fig. BP-8-1a
-249-

Fig. BP-8-1b
-250-

the fitting of the kerma-response curves to certain equations, but instead on the comparison of the effects of each of the nominal kermas in Hiroshima with each of the nominal kermas in Nagasaki. In this way one obtains a confidence region for the dependence of R on kerma for the radiation in Hiroshima with respect to that in Nagasaki. Details of this statistical procedure are described elsewhere (4).

If K_H is a particular kerma in Hiroshima and K_N a particular kerma in Nagasaki, I_H and I_N are the observed incidences, and I_H is significantly larger than I_N , then the R at the kerma K_H must be larger than K_N/K_H . Such a result of the comparison of two doses is indicated in Fig. BP-8-3 by a solid bar which covers the range of values of R which are rejected, i.e., all values below K_N/K_H . Conversely, if I_N is significantly larger than I_H , R must be smaller than K_N/K_H , and the result is then indicated by a solid bar extending from the top of the figure downwards, so that it covers all values of R which must be rejected. If the difference between the incidences at K_N and K_H is not statistically significant, the result of the comparison is merely indicated by a wedge. The actual kerma relation should lie between the two sets of bars in Fig. BP-8-2.

The statistical test used for the comparison of two observed incidences is the χ^2 -test for all those cases where in both groups the number of observed cases of leukemia was larger than 6; in all other cases

Fig. BP-8-2 Relative biological effectiveness of the radiation in Hiroshima compared to that in Nagasaki as a function of kerma in Hiroshima. The bars represent those values which can be excluded with 95% confidence; the broken bar stands for a level of significance of 86%. The broken curve is the result of the least squares fit described in the text.

Fig. BP-8-2
-253-

Fisher's 'exact probability test' has been applied. The two groups of survivors in the ABCC master sample which have been assigned the kermas of 31 and 69 rad of gamma rays in Nagasaki, have been combined into one group in order to make this group sufficiently large for comparison with the group of persons who were assigned the kerma of 10 rad in Hiroshima. Nevertheless, it has been found that the incidence in the Nagasaki group is lower than that in the Hiroshima group only on a level of statistical significance of 87%. To indicate this fact, the result of the comparison has been represented by a broken bar in Fig. BP-8-2.

Although the possibility of a constant R of about 3 can only be rejected with a significance level of 87%, the trend of the data indicates an increase of R with decreasing neutron kerma as has also been postulated by Poston et al (5). Furthermore this dependence appears to be of the same form which has been found in all the various systems we have analyzed in the past. We therefore conclude that it is not justified to use the linear approximation to represent the leukemia data in both Hiroshima and Nagasaki.

It should be noted that the direct analysis of R does not only lead to a somewhat sharper statistical discrimination between the data in Hiroshima and Nagasaki, but that it also has the advantage of avoiding assumptions on the overall shape of the kerma-effect relations. Such assumptions are especially problematic in the present case because

one deals with considerable uncertainties both in the range of low and of high kermas. In the range of low kermas the absolute number of observed leukemia cases is very small and the statistical uncertainties are correspondingly large. In the range of high kermas on the other hand, one deals with considerable uncertainties in the assignment of kerma values. This is due to the fact that those persons who received the largest doses are a highly selected group of survivors from regions near the epicenter of the explosion where the mortality was high. The direct analysis of R as a function of kerma has, however, the disadvantage that it refers to the comparison of a mixture of neutrons and gamma rays in the case of Hiroshima with the gamma radiation which was prevalent in Nagasaki. It does not permit the direct estimate of the R-kerma relation for the neutrons alone compared to gamma rays.

A Simple Approximation to the Kerma vs. Incidence Curves

Although the analysis of the dose-RBE analysis establishes the difference between the dose-effect curves in the two cities, their individual shapes can only be roughly approximated on the basis of linear and quadratic terms. In order to obtain an indication whether a linear dose-effect relation is more likely for neutrons or for gamma rays, separate linear regression analyses have been performed for the data in Nagasaki and Hiroshima. Fig. BP-8-3 gives the results of this

Fig. BP-8-3 95% confidence regions for the linear component, a , and the quadratic component, b , in the kerma-incidence relation of leukemia in Hiroshima and Nagasaki. The solid curves result if kermas up to 132 rad (Hiroshima) and kermas up to 147 rad (Nagasaki) are used in the analysis; the broken curves result if kermas up to 234 rad (Hiroshima) and 241 rad (Nagasaki) are used.

The ellipses are the projections of the confidence ellipsoids for the 3 parameters a , b , and control incidence, I_0 . The estimates of I_0 are $4.6 \cdot 10^{-4}$ (Hiroshima) and $6.2 \cdot 10^{-4}$ (Nagasaki) independent of the choice of the upper limit of kerma considered in the analysis.

Fig. BP-8-3
-257 -

analysis. The ellipses represent the 95%-confidence domains for the parameters a and b in the equation:

$$I(K) = I_0 + aK + bK^2$$

The analysis is based on Fisher's F-distribution (see for example (6)). It has been performed for the 3 parameters I_0 , a , and b . The ellipses in Fig. BP-8-3 are the projections of the confidence ellipsoids for these 3 parameters into the plane of a and b . The solid curves result if all kermas up to 132 rad (Hiroshima) and 147 rad (Nagasaki) are included; the broken curves result when kermas up to 234 rad (Hiroshima) and 241 rad (Nagasaki) are considered. The centers of the ellipses represent the least square estimates of the parameter.

A linear relation is found to be well within the 95% confidence region for Hiroshima where neutrons were the dominant radiation component. On the other hand a linear relation is clearly excluded for Nagasaki where nearly all of the dose was due to gamma rays. In fact the confidence domain for the Nagasaki data lies in the region of negative values of the linear component, a . This would indicate that a sufficient fit requires either a negative linear component, or a higher power than 2 of kerma in the incidence-kerma relation. However, in view of the few points on which the analysis is based and in view of the uncertainties in the kerma values it may not be justified to draw such

detailed conclusions. The essential result is that a linear relation must be excluded for the data from Nagasaki.

In order to obtain better statistics one may pool the data from Nagasaki and Hiroshima. The simplest assumption is then that of a linear relation for the neutron component K_n and of a quadratic relation for the gamma component K_Y :

$$I(K_n, K_Y) = I_0 + a K_n + b K_Y$$

A separate assessment of the neutron component and the gamma component of the kerma in Hiroshima and Nagasaki has been given by Ishimaru et al (3). On the basis of their values one obtains the estimated probability of 2.2×10^{-4} for leukemia during the observation period per rad of neutrons and of 8.7×10^{-8} per rad^2 of gamma radiation. In the least square fit which results in these values, only the kermas below 200 rad in the case of Hiroshima and below 300 in the case of Nagasaki have been considered. Higher dose values have been excluded because of the possible distortion of the kerma values due to selection. Another reason is that there is evidence suggesting an actual decline of incidence at high kerma (2). The 95%-confidence region of the parameter a and b (and in broken line the region of standard deviation) is given in Fig. BP-8-4. As in the cases discussed above this is the projection of the ellipsoid which represents the

Fig. BP-8-4 95%-confidence region for the linear coefficient, a , for the leukemia incidence due to the neutron component of kerma and the quadratic coefficient, b , for the incidence due to the gamma component. The broken curve represents the region of the standard deviation.

The ellipses are the projections of the ellipsoids for the 3 parameters a, b , and the control incidence, I_0 .

The estimate of I_0 is $4.8 \cdot 10^{-4}$.

Fig. BP-8-4
- 261 -

95%-confidence domain for the 3 parameters I_0 , a , and b , and which is derived on the basis of Fisher's F-distribution. It is somewhat doubtful whether it is justified to use the same control incidence, I_0 , for Hiroshima and Nagasaki, but it was felt that it may not be warranted to fit the limited data to more than 3 statistical parameters.

From the values estimated for the linear-quadratic model, one can calculate the theoretical kerma-R dependence for the actual mixture of neutrons and gamma rays which occurred in Hiroshima. The resulting curve as well as the curve for Nagasaki are given as a broken line in Fig. BP-8-1.

It must be stressed that the values estimated above are based on an assumption of linear dependence of leukemia incidence on neutron kerma. Although this assumption, together with the generally observed dependence of the RBE of low energy neutrons on neutron dose, results in better agreement with observations in the Japanese cities, it may well be only a rough approximation.

It is even more important that the relations given here not be extrapolated beyond the range in which meaningful observations were made. According to our basic theoretical approach (7) the RBE should become constant for gamma-ray doses of the order of perhaps a few rads with the result that below such doses the functional dependence of incidence on dose should be the same for both radiations except for a

constant applied to the dose. The question whether this dependence is linear can not be answered at this time with any degree of assurance.

The conclusions made here support the position taken by the UN Committee (2). They are at variance with the approach taken by the BEIR Committee (1). A more detailed report of this analysis has been submitted for publication.

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BP-9 Theory of Dual Radiation Action

A.M. Kellerer and H.H. Rossi

The theory of dual radiation action has been presented in detail in a chapter in "Current Topics in Radiation Research" (1); its application to the experiments performed with high energy heavy ions is the object of a recent paper (2). The following remarks concern some points which are still under consideration.

A. Observed Effect and Primary Damage

It is one of the main conclusions of the theory that the primary cellular damage is proportional to the square of the specific energy, z , in a sensitive region whose diameter is comparable to the diameter of the cell nucleus:

$$\epsilon(z) = k z^2 \quad (1)$$

The possibility that there is an additional linear component in this relation (see next section) will be neglected for the purpose of the present discussion.

From the quadratic dependence on specific energy one deduces a dependence on dose of the form:

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

where ζ is the energy average of the increment of z produced in a single event (passage of a charged particle through the nucleus). In certain cases, such as the production of dicentric chromosomes and centric rings or an induction of pink mutants in Tradescantia, one can assume that the observed yield is proportional to the primary damage. In general this may, however, not be the case, and it is certainly not the case in somatic effects such as the skin reaction or the opacification of the lens. The observed effect, E , may then depend on ϵ in a more complicated form, and it remains to examine the role of the auxiliary concept of primary damage, ϵ , under this condition.

The basic observation of the theory is that the variety of different dose-effect relations obtained with different radiation qualities in a variety of experimental systems reduces to a set of curves which depend essentially only on radiation quality if one considers RBE as a function of dose (3). This finding is equivalent to the statement that dose effect relations contain two separate functional dependences of which the one, $\epsilon(D)$, depends only on radiation quality while the other, $H(\epsilon)$, characterizes the experimental system and is the same for different radiations:

$$E(D) = H(\epsilon(D)) = H(\zeta D + D^2) \quad (3)$$

While no general statements can be made under functional dependence $H(\epsilon)$, the dependence on radiation quality is of simple form and contains only the characteristic parameter ζ (the fact that ζ can also depend on the experimental system, insofar as it reflects different site diameters or interaction distances, is considered in the next section).

It is the simplicity of Eq. (3) which justifies the connection of the term $(\zeta D + D^2)$ with the concept of primary damage. One should, however, note that it is an auxiliary concept which still lacks biological interpretation. It is also important to note that Eq. (3) is based on direct observation. It is not merely a theoretical conclusion derived from Eqs. (1) and (2); in fact, it follows from these equations only under the condition that the observed effect E depends on the mean value of ϵ at a given dose and not otherwise on its distribution. This condition:

$$E(D) = H(\overline{\epsilon(D)}) = H(\zeta D + D^2) \quad (4)$$

is trivial neither in the case of cellular effects nor in the case of effects on the tissue level. It may in fact have important implications for the identification of the cellular alterations which represent the primary damage.

B. Dependence of the Dose Effect Relation on Cell Age

There are two alternative explanations which may equally account for the typical shape of the inactivation curves obtained when mammalian cells in vitro are exposed to x rays or gamma rays in different stages of their generation cycle.

One hypothesis is that in the more sensitive phases of the cycle, i. e. for hamster cells in G_1 and G_2 (see (4)), the primary damage depends not only on the square of the specific energy z but has also a component which depends linearly on z :

$$\epsilon(z) \sim k_1 z + z^2 \quad (5)$$

One then obtains the dose dependence:

$$\epsilon(D) \sim (k_1 + \zeta) D + D^2 \quad (6)$$

If one assumes that the survival probability decreases exponentially with ϵ , one has:

$$S(D) = e^{-k((k_1 + \zeta) D + D^2)} \quad (7)$$

This means that the survival curves have larger initial slope and less of a shoulder in those phases of the cell cycle where the coefficient

k_1 is comparable to ζ or larger than ζ . The hypothesis would therefore be in agreement with the observed shapes of the dose effect curves at small to intermediate doses. In particular one would obtain a smaller limiting value of RBE at small doses, namely the value $(k_1 + \zeta_n) / (k_1 + \zeta_x)$ instead of the ratio ζ_n / ζ_x of the ζ -values for the densely ionizing radiation and for x rays which would be expected if no linear component were present. This is in agreement with those experimental findings (5) which indicate a limiting neutron RBE for the survival of mammalian cells which is considerably smaller than the value predicted on the basis of a purely quadratic dependence on z .

An alternative hypothesis (in the following termed the second hypothesis) can be based on the assumption that DNA is the critical target and on the observation that in the S-phase DNA is distributed near the nuclear membrane, while in G_1 and G_2 as well as in M it is condensed in subnuclear regions. It has been shown in Section BP-7 that the values of ζ for a spherical shell are close to the values of ζ which result for a sphere of the same diameter. In S one deals accordingly with very small values of ζ (for x rays) of the order of a few rads; this would explain the broad shoulder observed for cells irradiated in S phase. On the other hand, one obtains values of ζ for x rays of 100 rad or more in G_1 and G_2 if the effective diameter of the regions of condensation of the DNA material is less than

1 micrometer; the initial slopes are then large and the shoulders correspondingly small as follows directly from the equation:

$$S(D) = e^{-k(\zeta D + D^2)} \quad (8)$$

According to the second hypothesis the limiting value of RBE at very low doses should be equal to the ratio of ζ for the two radiation qualities being compared. However, this may not necessarily lead to the possibility of an experimental discrimination between the two hypotheses. The reason is that due to an increased saturation effect the effective value of ζ for densely ionizing radiation may be smaller than the microdosimetric value whenever one deals with very small critical volumes. To decide this question one would have to obtain cellular inactivation cross-sections in track segment experiments performed with synchronized mammalian cells.

One experimental method to discriminate between the two hypotheses would be based on comparative experiments with x rays and gamma rays in the low dose region. The microdosimetric quantities ζ are different by almost a factor of 2 for low energy x rays and gamma rays; one should accordingly find a larger initial slope of the survival curve with x rays if the second hypothesis is valid.

C. Inactivation Cross-section of Mammalian Cells

The functional dependence of the inactivation cross-sections of mammalian cells on the lineal energy y has been discussed previously (1,2). It has been found that an exponential dependence on the square of y is in close agreement with the experimental data:

$$\bar{\sigma}(y) = \sigma_{\infty} (1 - e^{-(y/y_0)^2}) \quad (9)$$

where the value of y is taken for a sensitive region with a diameter of one to several micrometers, and where y_0 has a value of 120 keV/ μ m. Eq. (9) is only an approximation; a more accurate formulation will depend on such factors as the spatial distribution of DNA in the cell nucleus in the different phases of the generation cycle (see preceding section). At present one can aim only at an approximate treatment since the data on inactivation cross-sections as a function of cell age and of ionization density are incomplete.

The so-called track segment experiments, i.e. cellular inactivation studies with monoenergetic charged particles, (6,7) are a useful basis for the analysis because one can in this case assume a fixed value of y corresponding to the stopping power of the particles. This avoids the integration of Eq. (9) over the full spectrum of y -values which is necessary in the analysis of our experiments performed in Princeton or our neutron experiments. However, even with some of the

track segment data, namely those obtained by Todd (7) one cannot neglect the distribution of y -values. The reason is that Todd's results were obtained with particles fast enough to produce delta rays whose range is not negligible compared to the diameter of the cell nucleus. This may at least partly account for the observation that at very high LET cross-sections are found which exceed the geometrical cross-section of the cell nucleus significantly. It would be of interest to apply microdosimetric analysis to Todd's data, but for the present discussion it is sufficient to refer to Barendsen's data which are obtained with particles of low velocity and with correspondingly small delta-ray ranges.

Eq. (9) corresponds to the solid line A in Fig. BP-9-1.a. This relation applies to aerated conditions. The simplest assumption to account for the oxygen effect is that of a constant reduction factor, ρ . The cross-section under hypoxic condition then takes the form:

$$\sigma_H(y) = \sigma_\infty (1 - e^{-(\rho y/y_0)^2}) \quad (10)$$

This equation corresponds to solid line B in Fig. BP-9-1.a. Barendsen's data for the aerated case are in close agreement with line A (see (1)), while in the hypoxic case the experimental values lie clearly above curve B in the range of y -values between 100 and 200 keV/ μ m.

Fig. BP-9-1 a) Cellular inactivation cross section as a function of y under aerated (A) and hypotonic (B) condition.
b) Dependence of the saturation parameter y_0 on lineal energy y which results in the broken lines in Fig. a.

Fig. BP-9-1
-273 -

Two possible explanations for this disagreement between experimental values and theoretical predictions could be considered. The first possibility is that the reduction factor, ρ , disappears for a densely ionizing particle ($y > 100 \text{ keV}/\mu\text{m}$). The fact that the OER approaches 1 at high values of y would then not be simply explained by saturation; it would instead point to characteristic differences, for example on the radiochemical level (the possibility of oxygen production in high-LET tracks has been considered).

If this explanation applies, one would have to expect the disappearance of the reduction factor, ρ , for all particles which produce sufficiently high values of y . The analysis of experimental data obtained with nitrogen ions (8,2) has, however, led to the conclusion that Eqs. (9) and (10) are valid. The values of OER obtained with fast nitrogen ions are significantly larger than those expected on the basis of a reduction factor, ρ , disappearing at high values of y . This supports a second possible explanation, namely the assumption that the deviations of Barendsen's data from Eq.(10) with constant ρ are due to the fact that in his experiments the radial extension of the particle tracks decreases with increasing LET.

An accurate quantitative analysis is at present not possible, if only for the reason that the experimental data refer to non-

synchronized cells, i.e. to a mixed population. A qualitative argument may, however, serve to bring out the essential point. In the experiments (6) higher values of LET are achieved by the use of slower particles, this is associated with tracks which are more narrow. Although the change of the radial profile of the tracks has little effect on the y -values measured over the relative large critical region of one or more micrometers, it may still lead to an increased saturation effect (corresponding to smaller values y_0). The change of y_0 which is represented in Fig. BP-9-1.b. results in the dotted lines for the cross-section in Fig. BP-9-1.a. One notes that the change is quite significant in the hypoxic case but much less significant in the aerated case; this is in agreement with the experimental observations.

A more accurate assessment of the saturation effect should be possible in experiments with synchronized cell populations and particularly in the planned experiments described in section BP-1.

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RADIOBIOLOGY

- B-1 Analysis of OER and RBE data from irradiation of Vicia seedlings with monoenergetic neutrons.
A.M. Kellerer and E.J. Hall
- B-2 OER and RBE studies with ²⁵²californium and radium at low dose-rate.
E.J. Hall, L.A. Roizin-Towle and R.D. Colvett
- B-3 Irradiation of mammalian cells with fast neutrons.
E.J. Hall and J. Novak
- B-4 Split dose experiments with hypoxic cells.
E.J. Hall, S. Lehnert and L.A. Roizin-Towle
- B-5 Dose response relationship for in vitro transformation of mammalian cells by x rays.
Carmia Borek
- B-6 The quinacrine fluorescence and giemsa banding of normal and neoplastic rat liver cells.
Carmia Borek. In collaboration with D.A. Miller, O.J. Miller and V.G. Dev (Department of Human Genetics)
- B-7 Surface alterations in transformed epithelial cells.
Carmia Borek. In collaboration with Max Burger (Biology Department, Princeton University)
- B-8 The relationship of intracellular cyclic AMP to radiosensitivity.
Shirley Lehnert
- B-9 Inhibition of repair of potentially lethal damage in mammalian cells by medium harvested from cells in plateau phase.
H. Norwint, I. Horowitz and E.J. Hall

B-1 Analysis of OER and RBE Data from Irradiation of Vicia seedlings with Monoenergetic Neutrons.

A.M. Kellerer and E.J. Hall

In last year's annual report a project was described in which the goal was to determine the oxygen enhancement ratio (OER) and the relative biological effectiveness (RBE) as a function of neutron energy for the wide range of energies available at RARAF. The radiobiological test system chosen for this investigation was the inhibition of the root growth of seedlings of Vicia faba, the techniques for which have been well established over a period of years (2 to 5). The experimental protocol was detailed last year, and the raw data presented. Since then the data have been subjected to a rigorous statistical analysis using novel methods.

Fig. B-1-1 shows the dose-response relationships for the nine neutron energies studied; these experiments were spread over a period of eighteen months. X ray experiments were performed at the beginning, at the end, and halfway through this period to insure that there was no systematic change in the radiation response of the system; the x ray results are all pooled in Fig. B-1-2.

The experimental endpoint is the growth of the primary root in ten days, G. The dose-effect curves are given in plots of the logarithm of the growth relative to controls, G/G_0 , versus dose, D. For

Fig. B-1-1 Dose-effect curves for Vicia seedlings exposed to neutrons at RARAF

Fig.B-1-2 Dose effect curves for Vicia seedlings exposed to 210 kv x rays.

the neutron experiments least square fits to exponential functions are inserted:

$$G = G_o e^{-aD} \quad (1)$$

The treatment is in accordance with the normal usage in the analysis of dose-effect curves, but it is a departure from the earlier approach chosen for the analysis of the Vicia faba data (5,6,7). In the earlier approach plots of G vs. $\ln D$ were used and straight lines in this plot were chosen for least square fitting. This corresponds to the equation,

$$G = G_o - s \log(cD) \quad (2)$$

The two approaches are not mutually exclusive because for the values of s which have been experimentally observed, Eq. (2) results in curves which are very near to an exponential function for G/G_o from 0.8 to 0.2.

In the earlier analysis, Eq. (2) has been chosen because multiplication of the dose by a constant factor then merely corresponds to a parallel shift of the straight line in the G vs. $\ln D$ plot. This reduces the statistical analysis of OER to a parallel line assay. In the present study, the statistical method for the derivation of OER has been retained and the resulting OER values will be

given further below. With the exception of this special application the representation of the $\ln G$ vs. D has been used as the basis of the present analysis; this is not only in line with the more common treatment of dose-effect curves but also eliminates the difficulty which arises from the fact that Eq. (2) diverges at low doses.

According to the theory of dual radiation action (8,9) the elementary lesions underlying a wide range of cellular radiation effects increase with the square of the specific energy in sites comparable to, but somewhat smaller than, the nuclear volume. This leads to a linear-quadratic dependence on dose; the linear component represents the interaction of lesions produced in one and the same particle track while the quadratic component stands for that part of the effect which is due to the interaction of lesions produced by independent particle tracks. In the case of neutrons the linear component dominates in the range of small and intermediate doses, as evidenced by the frequently observed exponential dose-effect relations. For x rays, on the other hand, the quadratic component dominates except at very small doses. In the general case, one must account for the possibility that both for sparsely and densely ionizing radiations one may deal with a linear as well as a quadratic component in dose. Accordingly, a least-square fit of the experimental data both for x rays and the various neutron energies has been performed for the

logistic equation.

$$G = G_0 e^{-aD - bD^2} \quad (3)$$

Linear regression techniques can be applied for this purpose because the logarithm of G depends linearly on the three parameters, $\ln G_0$, a , and b :

$$\ln G = \ln G_0 - aD - bD^2 \quad (4)$$

In addition to the least-square estimates of the parameters, the joint 95% confidence regions for the parameters have been determined on the basis of Fisher's F distribution. The analysis has been based on the complete data of all individual roots and not merely on the mean values obtained for different dose values in a given experiment.

For the pooled x-ray data, representing a total of approximately 500 roots, the estimates $a = 2.5 \times 10^{-3} \text{ (rad}^{-1}\text{)}$ and $b = 1.2 \times 10^{-5} \text{ (rad}^{-2}\text{)}$ have been obtained for the experiments in air, and the values $a = 0.63 \times 10^{-3} \text{ (rad}^{-1}\text{)}$ and $b = 0.25 \times 10^{-5} \text{ (rad}^{-2}\text{)}$ have been obtained for the hypoxia experiments. The 95% confidence regions for the parameters a and b , as indicated in Fig. B-1-3, are wide, but it is important to note that both parameters are strongly correlated. It

Fig. B-1-3 Last square estimates and 95% confidence regions for the parameter a and b .

is therefore decisive to give the joint confidence intervals and not merely, as is commonly done, confidence intervals for the individual parameters. In the following the estimated values of the parameters will be used for comparison with the results of the neutron experiments.

In the analysis of the neutron data, it has been found that the resulting confidence ranges for b are very broad. This is to be expected because for neutrons, the linear component in Eq. (4) is in all cases dominant over the quadratic component, and the least-square fit is therefore not significantly affected by the quadratic term. Accordingly, there is no indication that the quadratic component is different in the case of neutrons from that observed for x rays. Since, however, the contribution of the quadratic term is negligible the simple fit to an exponential function with only two free parameters G_0 and a has been applied for all neutron data:

$$G = G_0 e^{-aD} \quad (5)$$

and the resulting curves have been inserted in Fig. B-1-1.

The estimate and 95% confidence regions of the parameter a are given in Fig. B-1-4 for various neutron energies. The broken line merely serves to show the trend of the data, it has no mathematical significance.

Fig. B-1-4 Least square estimates of the parameter "a" and corresponding 95% confidence ranges for various neutron energies

The RBE at low doses is equal to a_z/a_n , where a_z and a_n are the linear coefficients for x rays and neutrons. The values of RBE as a function of neutron energy are given on the right ordinate scale of Fig. B-1-4. The confidence intervals are based solely on the regression analysis of the neutron data and on the estimated value of the parameter a_z for x rays; the uncertainty in the value of a_z is disregarded. According to the confidence regions in Fig. B-1-3 the curves of RBE could therefore be shifted with regard to the RBE scale. The shape of the curves in this logarithmic representation is, however, unaffected by such a shift. Moreover one obtains the same curve shape if one considers the RBE at a certain effect level, instead of the extrapolated value of RBE at very low doses; this merely introduces a constant factor in the RBE values as can be seen in the following way. From Eq. (3) for x rays and Eq. (5) for neutrons one has the following relation for the RBE of neutrons:

$$\text{RBE} = a_n/a_x + b_x D_x. \quad (6)$$

Thus RBE is always proportional to a_n , while the factor $(a_z + b_x D_x)^{-1}$ which connects the two quantities RBE and a_n decreases with increasing effect level. For any chosen effect level the value D_x is read off Fig. B-1-2 or is calculated from Eq. (3) and the estimated parameters a_x and b_x . In the aerated case one obtains

Fig. B-1-5

Least square estimates of the OER and the corresponding 95% confidence ranges for various neutron energies

$$RBE = a_n/a_x = 400a_n \quad (7)$$

for very small doses, while for 50% growth reduction one has $D_x = 160$ and accordingly,

$$RBE = a_n/a_x + D_x \ell_x = 226a_n \quad (8)$$

From the broken lines in Fig. B-1-4 an estimate of the dependence of the oxygen enhancement ratio (OER) on neutron energy can be obtained. This estimate is given as a broken line in Fig. B-1-5; it has, however, as little precise mathematical meaning as the broken lines in Fig. B-1-4, and it remains, therefore, to obtain the actual confidence intervals for the values of OER. Such confidence limits cannot readily be obtained from the confidence ranges of the RBE values. This is due to the fact that the experiment in air and nitrogen at a given neutron energy share the same control group. The estimates of the two parameters a_{Air} and $a_{Nitrogen}$ are therefore not independent and the confidence range for $OER = a_{Air}/a_{Nitrogen}$ cannot be obtained directly from the two confidence ranges for a_{Air} and $a_{Nitrogen}$. For this reason the confidence ranges for OER are derived by a parallel line assay according to Eq. (2). The results are superimposed to the broken line given in Fig. B-1-5. Application of this particular statistical test has the additional advantage that one obtains a cross check between the results based on two different analytical

approximations of the data. One finds that the results are in sufficient agreement. Although the 95% confidence ranges for the values of OER are broad, there is a clear indication of an increase of OER with increasing neutron energy. Whether the OER also increases when the energy of the neutrons falls below 100 kV cannot be decided on the basis of the present data. It should be pointed out that the neutrons have been essentially monochromatic for higher energies but that for the two lowest energies one is dealing with a broad spectrum ranging from zero energy up to 110 kV in the one case, and from zero energy up to 60 kV in the other case. The energies of 50 kV and 80 kV have been taken as rough estimates of the effective energy in each case.

It should be noted that one cannot a priori assume that for each radiation quality the OER is a constant, independent of dose. In the present experiments with neutrons a dependence of OER on dose can, however, not be detected because both under oxygenated and hypoxic conditions the dose-response curves do not significantly differ from exponential functions; if all response curves are exponential the OER is independent of dose. As an additional check, however, a more direct statistical test has been employed which is not based on the choice of a particular equation for the response curves. The test consists of a

direct non-parametric comparison of each dose given under oxygenated conditions with each dose given under hypoxic conditions. This analysis is applicable both to the evaluation of OER and RBE as a function of dose. Results for the RBE analysis will be given below; as far as the OER values are concerned, it is sufficient to state that the results are in good agreement with the results represented in Fig. B-1-5, and that no dependence of OER on dose can be detected in the present experiments. This does, of course, not exclude a possible dependence of OER on dose which might be revealed by a more extensive series of experiments, or in an experimental system where the response can be studied over a wider range than in the growth reduction studies of Vicia faba.

For the RBE of neutrons a strong dependence on dose has been shown in various systems. Such a dependence is also found in the present study. Figs. B-1-6 and B-1-7 give the confidence regions and the statistical estimates of RBE as a function of neutron dose. These results are also obtained by the nonparametric test which has been applied (10) to an analysis of the RBE of neutrons in the opacification of the murine lens. One finds that at intermediate and higher effect levels the results are all consistent with the assumption that the curves in this double-logarithmic representation have a slope of -0.5,

Fig.B-1'-6 Estimates of the dose RBE dependence and 95% confidence regions for various neutron energies under aerated conditions.

Fig. B-1-7 Estimates of the dose-RBE dependence and 95% confidence regions for various neutron energies under hypoxic conditions.

as has been postulated in the theory of dual radiation action (8). At small doses the results indicate a leveling out of RBE, as is also expected on the basis of the theory. The confidence regions are, however, wide and consequently the shape of the RBE curves cannot be determined with great accuracy. One must also note that all curves relate to the same x-ray data and that the results are therefore not statistically independent. Nevertheless the results are useful because they complement the results obtained from overall curve fitting; in Fig. B-1-4 confidence limits are given which are valid only for the quantity a_n and not for RBE. The results represented in Fig. B-1-7 are also of interest insofar as they show that the direct statistical analysis of the dose dependence of RBE yields confidence intervals for RBE at intermediate and high doses which are even more narrow than the confidence intervals for the parameter a_n obtained by curve fitting and which are given in Fig. B-1-4.

As can be seen from Fig. B-1-5, the oxygen enhancement ratio decreases as the neutron energy is decreased between 15 MeV and 220 keV. The data for the two low-energy spectra are less accurate than for the higher energies. This is a result of technical difficulties involved with these two low energies. As plotted in Fig. B-1-5 the OER does not appear to decrease further below 220 keV, and indeed there is a possibility that it begins to rise again.

There are a number of other reports in the literature concerning the dependence of OER on neutron energy. The situation is very complex but some comparison with the results presented here is clearly called for. The present data indicate a greater change of OER with neutron energy than was reported by Broerse, Barendsen and Van Kersen (11), using mammalian cells in culture over a range of neutron energies from 1 to 15 MeV.

Using P388 leukemia cells in vivo, Berry (12) found an increasing OER with neutron energy from 1 to 25 MeV. The increase from 6 MeV upwards is quite similar to that reported here, but in striking contrast to the present data, and indeed contrary to other reports in the literature, Berry found a very low OER at 1 MeV. Too much importance cannot be ascribed to this difference since, not only were the biological systems totally different, but the 1 MeV neutrons described in the present report are essentially monoenergetic whereas Berry used a spectrum of fission neutrons.

A further possible factor could be a dose dependence of OER, especially for 15 and 25 MeV neutrons. At these high neutron energies and at small doses, the biological effect is determined mainly by heavy recoils and the densely ionizing parts of the proton tracks; this should result in a low OER. At higher doses, the quadratic component due to the sparsely ionizing tracks of the fast protons becomes more

significant and consequently a higher OER might be expected. Kellerer and Rossi (9) have calculated the dependence of OER on neutron energy on the basis of microdosimetric data and of the cellular inactivation cross-sections obtained by Barendsen (13) for low doses of heavy charged particles. These calculations lead to a nearly constant OER from a neutron energy of 300 keV to 14 MeV and are in essential agreement with the experimental results of Broerse et al (11).

Berry, on the other hand, has obtained large values of OER for neutrons of 25 MeV in experimental studies which involve high doses of the order of 2000 rad. The dose range in the present study is intermediate and the moderate increase of OER at higher neutron energies may therefore reflect a partial contribution of the fast protons to the effect, although the statistical accuracy of the survival curves for 14-MeV neutrons is not sufficient to establish the quadratic component.

Dennis and Boot (14), scoring pink mutations in Tradescantia, found a rapid reduction in OER between 6 MeV and 100 keV which is in reasonable agreement with the results presented here; however, two other features of their work were not observed here. They found that the OER decreased again as the neutron energy rose from 6 MeV to 15 MeV, and they also found a large OER in excess of 2, for 440 keV neutrons which they attributed to oxygen resonance; neither of these findings was confirmed in the present series of experiments.

Variation of RBE as a function of neutron energy shows a rapid increase of RBE as the neutron energy is reduced from 15 MeV down to 440 keV. The curve then goes through a maximum at around 350 keV and for lower energies the RBE falls again. The data are in substantial agreement with the dependence of RBE on neutron energy which has been predicted on the basis of microdosimetric data (9).

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B-2 OER AND RBE STUDIES WITH $^{252}\text{CALIFORNIUM}$ AND RADIUM AT LOW DOSE RATE

E.J. Hall, L.A. Roizin-Towle and R.D. Colvett

The increasing availability of the man-made nuclide californium-252 has made possible a number of studies of its radiobiological properties. Plant seedlings, mammalian cells cultured in vivo and in vitro, as well as normal tissues in experimental animals have all been used as test system to investigate the oxygen effect for ^{252}Cf and to compare the effectiveness to the mixed neutron and gamma ray emission from this nuclide with more conventional radium gamma rays. Many of the previous studies, including our own, have been unsatisfactory for a variety of reasons. The present study was designed to satisfy many of the criticisms leveled at previous work, although radiobiological measurements at low dose rate, particularly if hypoxia is involved, inevitably involve considerable technical difficulties.

The irradiation set-up used was designed to mimic as far as possible the conditions pertaining to an implant in a clinical situation. The radioactive sources and the cell cultures were located within a block of tissue equivalent material to reproduce conditions of scatter and attenuation. The radiation dose rates used were those commonly employed with radium and projected for an equivalent biological effect for $^{252}\text{californium}$.

V79 Chinese hamster cells were used, and cultured using the conventional in vitro techniques. For each experiment cells from a number of actively growing stock cultures were prepared into a suspension containing 3.3×10^6 cells per ml. For irradiation, cells were contained in plastic vials fabricated from Falcon 1 ml disposable pipettes. The pipettes are made of polystyrene and have an internal diameter of 2 mm. The pipettes were cut into 7 cm lengths, one end sealed by heating, filled with 0.3 ml of the cell suspension described above (i.e. 10^6 cells) and then the top of each vial heat sealed. After an appropriate dilution, a second series of vials were seeded with 10^3 cells. The vials were stored upright, so that cells settled out into a thin layer on the bottom of each, a process fastened and made more complete by gentle centrifugation.

The vials containing the high concentration of cells rapidly become hypoxic because the cells metabolize and use up the oxygen present in the small volume of culture medium. At the lower cell concentration this does not occur at least over a period of 24 hours. The validity of this technique has been tested many times in experiments using x-rays at high dose rate.

After the completion of all irradiations in an experiment each vial was treated in the following way. First it was agitated for one minute on a mechanical vibrator, before being opened and its contents pipetted vigorously. Aliquots

of the resulting suspension were seeded into plastic flasks containing fresh growth medium, and incubated at 37°C to test for the ability to form visible colonies. The setup of the low dose rate irradiations is illustrated in Figure B-2-1. In the case of radium, six 5 milligram tubes were used, while for californium six sources with a nominal content of 6 micrograms were available. In either case the radioactive sources were arranged around the periphery of a circle 4 cm in diameter, which was slowly rotated to eliminate variations in the activity of the individual sources. Vials containing the cells were located in sixteen holes in a block of tissue equivalent plastic above the radioactive sources. The cells, which had sedimented to the bottom of the vials, were positioned around the circumference of a circle of 3 cm in diameter, parallel to and spaced 1 cm above the plane of the radioactive sources. This entire fixture, fabricated from tissue equivalent plastic, was maintained at 20°C inside a temperature control incubator.

The dose rate in the position occupied by the cells was measured by means of small (1/4" diameter) spherical ionization chambers. Two such chambers were used, identical in size, but one had walls of aluminum and other of tissue equivalent plastic. For measuring the dose rate from radium the tissue equivalent chamber alone was used; the dose rate was 50 rads/hr. For measuring the dose rate from ²⁵²Cf,

Fig. B-2-1
Cut-away drawing of the
set-up for irradiating
cells with radium or
 $^{252}\text{Californium}$ at low
dose-rate

both ionization chambers were used and from their differential sensitivities it was possible to calculate the neutron and gamma ray components of the radiation field; the neutron dose rate was found to be 12 rads/hr while the corresponding gamma ray dose rate was 6 rads/hr.

The data from low dose rate experiments with radium and ^{252}Cf are listed in Tables B-2-1 and B-2-2. In Figure B-2-2 the mean surviving fraction relating to each dose is plotted for both types of radiation; the lines were fitted to the data by method of least squares. The resulting oxygen enhancement ratios, calculated as the ratio of the slopes of the lines under hypoxic and aerated conditions, are 1.42 for ^{252}Cf and 2.0 for radium.

The relative biological effectiveness (RBE) of the mixed neutron and gamma emission from ^{252}Cf , compared with radium, is 3.5; this is calculated from the ratio of the slopes of the lines fitted to the aerated data for radium and ^{252}Cf , i.e., 771 divided by 221. If the biological effect of the ^{252}Cf gamma rays is ignored, then the RBE of the ^{252}Cf neutrons alone compared with the radium gamma rays becomes approximately 5.9.

There are described in the literature a number of determinations of the oxygen enhancement ratio for the mixed neutron and gamma ray emission from ^{252}Cf . They all involve simple laboratory systems such as plant seedlings or mammalian cells

Fig. B-2-2 Survival curves for cells irradiated with either radium gamma-rays or the mixed neutron and gamma-ray emission from ^{252}Cf .

cultured in vitro or in vivo; Table B-2-3 lists the available data.

In summarizing the OER values obtained to date it should be emphasized that when ^{252}Cf is used for an interstitial implant caution will dictate treatment plans comparable to those of the radium it replaces. This will involve a total dose rate (neutron + gamma rays) of certainly less than 20 rads per hour. At these low dose rates the biological effectiveness of the gamma ray component is vanishing small, and the value of the OER is determined almost exclusively by the neutron component. A value of about 1.5 would be expected, comparable to that obtained with a beam of fission neutrons at high dose rate. This is indeed found to be the case in the first five experiments listed in Table B-2-3 where low dose rates were used comparable to those that might be used clinically for an implant. At much higher dose rates the biological effectiveness of the gamma ray component cannot be ignored; the overall OER of the mixture of neutron and gamma rays is then elevated by the characteristically higher OER of the gamma ray component. This is seen to occur in the last three experiments listed in Table B-2-3, where the higher OERs which were found may be attributed to the higher dose rates used.

The RBE of ^{252}Cf compared with radium varies with dose rate, and depends upon the biological system chosen. The available data are listed in Table B-2-4. The variation of RBE with dose rate results largely from the changing effectiveness

of the radium gamma rays as the treatment time is protracted. In choosing an RBE from experimental studies with which to predict interim clinical doses, it is important to consider only data gathered at a relevant dose rate. RBE values obtained with such systems as the skin of swine, or the gut of the mouse probably provide the safest extrapolation to the clinical situation, since both systems are known to effectively repair sublethal damage, which is thought to be the case for the limiting tissues in the human. Of the in vitro cell lines used, hamster cells which exhibit a survival curve with a large shoulder are to be preferred to (for example) HeLa cells which are usually characterized by a smaller shoulder. In Table B-2-4 in vivo experiments with pig skin and mouse gut, together in vitro experiments utilizing hamster cells result in RBE values which are similar. It is upon data such as these that interim guidelines for dosage levels in the human must be based, until such time that they are supplemented by a body of accumulated clinical experience.

Table B-2-1 Summary of Radium Data

Dose (rads)	Experiment Number	Surviving Fraction (aerated)	Mean and SE	Surviving Fraction (hypoxic)	Mean and SE
224	200	.855	$.78 \pm 0.021$	.925	$.87 \pm 0.080$
	232	.745		.882	
	234	.805		.890	
	236	.755		.570	
	201	.750		1.060	
898	199	.333	$.337 \pm .051$	.605	$.600 \pm 0.047$
	204	.182		.665	
	209	.300		.410	
	232	.463		.745	
	234	.500		.561	
	236	.235		.582	
1178	199	.268	$.201 \pm .034$	.527	$.450 \pm .048$
	204	.163		.461	
	209	.171		.362	
1403	200	.174	$0.171 \pm .018$	.455	$.400 \pm .044$
	201	.154		.324	
	203	.166		.223	
	232	.127		.314	
	234	.251		.500	
	236	.153		.452	

Table B-2-2 Summary of Californium Experiments

Dose (rads)	Experiment Number	Surviving Fraction (aerated)	Mean and SE	Surviving Fraction (hypoxic)	Mean and SE
109	194	.635	$.566 \pm 0.054$	.715	$.757 \pm .076$
	195	.478		.726	
	198	.680		.975	
	225	.471		.620	
437	210	.090	$.137 \pm .02$	.256	$.280 \pm 0.013$
	238	.140		.300	
	242	.181		.284	
518	194	.077	$.094 \pm .007$	.174	$.193 \pm .006$
	195	.104		.184	
	198	.095		.186	
	210	.053		.182	
	238	.092		.201	
	242	.132		.224	
	244	.104		.202	
655	194	.059	$.058 \pm .006$	.105	$.120 \pm .008$
	195	.056		.133	
	198	.070		.125	

Table B-2-3 OER Values

Author	Ref.	Biological system	Californium dose-rates rads/hr.	Californium OER	Radium dose-rate rads/hr.	Radium OER
Present paper		Hamster cells	18	1.42	50	2.0
Fairchild et al (1970)	(5)	HeLa cells	16	1.3	37.3	2.5
Drew et al (1972)	(14)	HeLa cells	19.4	1.55-1.60	37.3	2.35-2.50
Hall & Fairchild (1970)	(4)	Vicia seedlings	16	1.66	46.9	2.02
Hall (1972)	(12)	Hamster cells	16.5	1.4	-	-
Djorjevic et al (1973)	(17)	HeLa cells	20	1.55	60 rads/hr.	2.7
Bushong et al (1970)	(6)	Hamster cells chromosome aberrations	60	1.8	-	-
Berry (1972)	(9,10)	P388 cells	high dose rate 248	1.9	300 to 500	2.3
Bushong et al (1973)	(16)	Hamster cells chromosome aberrations	167 522-406	1.76 1.81	79 327	2.32 2.39

Table B-2-4 RBE Values

Author	Ref.	Biological system	Cf dose-rate rads/hr.	Ra dose-rate rads/hr.	RBE of ²⁵² Cf relative to Ra	
					Total dose	Neutrons only
Present paper		Hamster cells	18	50	3.5	5.9
Atkins et al (1970)	(2)	Pig skin	12-13	35	4.9	7
Withers et al (1971)	(7)	Mouse jejunal crypt cells	26.5	160	3.3	4.9-5.4 estimate at clinical d/r.7
Hall (1972)	(11)	Hamster cells	1.14 to 21.6	35.7	4.3	6.7
Fairchild et al (1969)	(1)	HeLa cells	16	31	2.86	4.8
Djorjevic et al (1973)	(17)	HeLa cells	20	64	2.1	2.7
Atkins et al (1972)	(15)	Pig skin	~10	50	4.5	6.6
Berry (1972)	(8,10)	P388 lymphocytic leukemia cells	248-279	336-520	-	2.7-3.8
Bushong et al (1973)	(16)	Hamster cells chromosome aberrations	167 522-406	79 327	1.77 3.44	- -

B-3 IRRADIATION OF MAMMALIAN CELLS WITH FAST NEUTRONS *

E.J. Hall and J. Novak

This project was designed to provide basic data concerning the dependence of RBE on neutron energy. Such data are a necessary input to test and extend the theory of dual radiation action.

V79 Chinese hamster cells were used, irradiated in suspension, with the cells contained in small plastic vials fabricated from short lengths of Falcon disposable plastic pipets. These polystyrene vials are 5 cm in length and have an internal diameter of 2 mm.

For each experiment cells from an actively growing stock culture were prepared into a suspension in growth medium containing 3.3×10^4 cells/ml. One third of an ml of this suspension was transferred into each of a large number of the plastic vials described above. Cells sedimented to the bottom of each vial forming a thin layer of cells. Cells may be stored at room temperature in this way for periods of at least 24 hours, and subsequently recultured with plating efficiencies characteristically in excess of 50%.

Using this biological system survival data have been obtained for nine neutron energies at RARAF; 15, 6, 2, and 1 MeV as well as 660, 440, 350, 220 and 100 keV. The details of the irradiation set-up and the dosimetry are described in detail

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in the physics and dosimetry section. Because the cells occupy a small volume at the bottom of each vial, a sufficient number of vials could be exposed to a uniform dose-rate even at the lower neutron energies where short treatment distances are necessitated by the limited output. For each neutron energy, 16 vials could be irradiated simultaneously at a uniform dose-rate; some were removed after various times and others inserted so that a total of seven doses, with four replicate vials per dose, were available. In the later experiments a parallel experiment with 250 kv x rays was performed, using cells from the same suspension and common controls. In this way, inter-experiment error was eliminated from the calculation of RBE values.

In addition to the experiments at RARAF a visit was made to the TAMVEC accelerator in Texas to measure the RBE of the neutron beam produced by 50 MeV deuterons on beryllium. The same cell system was used, and dosimetry measurements were made by Mr. Steven Marino who was also responsible for the dosimetry for the RARAF experiments; data obtained with the two accelerators may, therefore, be compared with confidence.

Most of the data have been collected but as yet a rigorous statistical analysis has not been performed.

Figure B-3-1 is a preliminary plot of the RBE (calculated at a cell surviving fraction of 10^{-2}) as a function of neutron energy. The shape of the curve in this double logarithmic

Fig. B-3-1 RBE as a function of neutron energy - as determined with Chinese hamster cells in vitro.

plot is not dissimilar to that obtained for Vicia seedlings and described in section B-1. The absolute values of the RBE are of course much lower because of the considerably larger doses used for mammalian cells compared with the more sensitive plant seedlings.

B-4 SPLIT DOSE EXPERIMENTS WITH HYPOXIC CELLS; IMPLICATIONS FOR
RADIOTHERAPY*

E.J. Hall, S. Lehnert and L.A. Roizin-Towle

There is no question that in the majority of situations a multifraction dose schedule results in a higher therapeutic ratio than a single dose, i.e. greater tumor destruction with less normal tissue damage. The advantages conferred by fractionation are probably a result of several quite distinct factors. Repair of sublethal damage and reoxygenation are usually considered to be dominant, while repopulation (or proliferation) and changes in the distribution of cells in the cell cycle with attendant variations in radiosensitivity, are generally assumed to play a secondary role.

Repair of sublethal damage has been demonstrated unequivocally in a wide range of biological systems both in vivo and in vitro. Reoxygenation has been shown to occur in the several animal model tumor systems which lend themselves to this kind of complex study. To what extent this occurs in the human is not, of course, known.

The success of a multifraction regime in improving the therapeutic ratio is readily understood in terms of these effects. For the tumor population, a fractionated regime results in more damage than the same total dose given in a single fraction, because the process of reoxygenation can occur between dose fractions. During the protraction of the

*This investigation was supported in part by Public Health Service Research Grant No. CA-12536 from the National Cancer Institute.

of the treatment, resistant hypoxic cells become sensitive aerated cells, at least for the later doses of the multifraction protocol. In the case of normal tissues a fractionated regime saves compared with a single dose, because repair of sublethal damage and repopulation may occur during the time intervals between dose fractions.

A reduced repair of sublethal damage under hypoxic conditions would constitute an alternative factor favoring the use of treatment regimes involving multiple fractions. For the most part hypoxic cells are tumor cells, while normal tissues are well supplied with oxygen. Consequently, the use of many fractions allows increased killing of hypoxic cells relative to well oxygenated normal tissues; in some instances this process can completely obviate the decreased sensitivity of the hypoxic cells.

The repair of sublethal radiation damage has been studied extensively by means of split dose experiments. The role played by oxygen in this process has been the subject of considerable controversy during the 13 years since Elkind's and Sutton's first demonstration of sublethal damage repair. The experiments reported here were designed to elucidate the effect on repair of hypoxia induced by the crowding of mammalian cells of high cell concentrations.

V79 Chinese hamster cells we use throughout, cultured according to the well established techniques. On the day of an experiment, a

large number of cells were harvested by trypsinization, spun down to remove excess trypsin and washed with fresh medium. Two cell suspensions were prepared, one containing 2×10^6 cells per ml and the other 10^4 cells per ml. A series of glass ampoules were filled from each cell suspension. The ampoules containing cells at the lower concentration were heat-sealed immediately and left to stand undisturbed at room temperature; it was found by subsequent experiments that cells at this concentration did not become radiobiologically hypoxic. The ampoules containing cells of the higher concentration were then handled in the following way, illustrated diagrammatically in Figure B-4-1. High purity nitrogen plus 5% CO_2 was bubbled into the glass ampoule through a long cannula to displace the air above the solution. With the nitrogen still flowing, the cannula was partly withdrawn and the ampoule heat-sealed. After the gassing and sealing processes were completed these ampoules were tumbled and reciprocated violently from side to side to keep cells in suspension, and at the same time the temperature was raised to 37°C ; this treatment was maintained for 30 minutes to allow the cells to scavenge the oxygen remaining in the medium by normal metabolism. Following this procedure the ampoules were left to stand undisturbed for at least 1 hour at room temperature so that the cells could settle out into a thin layer on the bottom of each ampoule before any irradiations were started. Single or split doses of gamma-rays were given by means

Fig. B-4-1 Illustration of the method used to produce hypoxia.

of a cobalt-60 teletherapy unit, which was directed vertically upward so that the cells were irradiated through the bottom of the ampoules (see Fig. B-4-1). At a treatment distance of 40 cm the dose-rate was computed to be 430 rads per minute.

Immediately after the conclusion of all irradiation procedures, each ampoule was agitated in a vortex mixture for one minute to resuspend the cells, after which it was opened and the contents pipetted vigorously to break up clumps of cells; various aliquots of this cell suspension were plated into petri dishes and incubated for 8 days to test for their ability to form visible colonies.

Three types of experiments were performed.

a) Graded single doses of gamma-rays to aerated and hypoxic cells.

Four replicate experiments were performed in which graded doses of gamma-rays were delivered to aerated and hypoxic ampoules. The accumulated data are shown in Fig. B-4-2; the survival curves were fitted by eye. The results of these experiments have not yet been subjected to a rigorous statistical analysis, but it is evident from a cursory examination that the oxygen enhancement ratio is close to 3.2 and that this value is maintained for all dose levels. There is no indication from these experiments of a difference in the survival curve's shape between aerated and hypoxic conditions.

Fig. B-4-2 Survival data for hamster cells irradiated under aerated and hypoxic conditions.

b) Split dose experiments with aerated and hypoxic cells.

Ampoules containing 10^4 cells (aerated) were exposed to a single dose of 1200 rads or two doses of 600 rads, separated by a time interval of 1, 2, 3 or 4 hours. The results of this experiment are shown in Fig. B-4-3. Under aerated conditions repair of sublethal damage takes place during the interval between split doses. There is a recovery factor of about 3-1/2 with a time interval with 3 or 4 hours between split doses. Under hypoxic conditions, maintained during the radiation exposures and throughout the interval between, the repair of sublethal damage is almost completely inhibited.

c) The effect of oxygen admitted between split doses delivered under hypoxic conditions.

In this experiment ampoules containing cells at the high concentration (i.e. hypoxic) were given a single dose of 4000 rads or the first of two doses of 2000 rads. They were then opened, the cells removed, and allowed access to air for a period of 3 hours. Following this time interval they were then returned to ampoules, made hypoxic again by the procedure described above, and a second dose of 2000 rads was delivered to the appropriate ampoules. A parallel series of vials were irradiated in single and split doses containing the lower cell concentration. The results of this experiment are shown in Fig. B-4-4. The extent of the repair of sublethal damage during the 4 hour interval

Fig. B-4-3 Split-dose experiments with aerated and hypoxic cells.

Split-dose experiments with cells aerated throughout, or hypoxic during irradiations and aerated between dose fractions.

Fig. B-4-4

between split doses is similar for both series of cells. The fact that the doses were delivered under hypoxic conditions appears to be irrelevant so long as the cells had access to oxygen between the split doses; in this case repair appears to take place as efficiently as in the case of cells that were aerated throughout both irradiations and the storage period between the split doses.

This project has not yet been completed and further experiments are in progress. However, the results so far appear to demonstrate unequivocally that access to oxygen is essential for the process of repair of sublethal damage to take place. The presence or absence of oxygen during the radiation exposures appears to be irrelevant; the important factor is the availability of oxygen during the storage interval between split doses. The technique described allows a low level of hypoxia to be achieved, and repeated from one experiment to another. Cells irradiated and stored between split doses at this low level of hypoxia are completely unable to repair sublethal damage.

B-5 DOSE RESPONSE RELATIONSHIP FOR IN VITRO TRANSFORMATION OF
MAMMALIAN CELLS BY X RAYS *

Carmia Borek and Eric J. Hall, assisted by Allan L. Whitlow

Earlier studies have described the induction of malignant cell transformation in short term cultures of hamster embryo cells by a dose of 300 rads of x rays (1-4). The conditions for the fixation of this event as a hereditary property of the cells, and the tumorigenicity of the transformed cells were also described, as well as their characteristics in vitro.

A knowledge at a cellular level of the relationship between the dose absorbed and the probability of neoplastic conversion is essential for the elucidation of the mechanism of radiation carcinogenesis. The present work deals with the dose response relationship over a dose range of 1 to 600 rads.

Midterm Golden Hamster embryos served as the source of normal cells. Primary cultures were established by progressive dissociation of the minced fresh tissue, and by seeding 10^7 cells, resuspended in medium, into 100 mm petri dishes. 3 day old primary cultures were dissociated and cloned onto rat feeder layers at seeding levels ranging from 2000 to 10,000 cells, depending upon the dose to be administered, thus allowing for cell killing. 24 hours later the single cell cultures were irradiated with a single dose of 600, 300,

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150, 75, 50, 25, 10 or one rad. The source of x rays was a 210 kV Westinghouse constant potential generator. Non-irradiated cells served as controls. Following irradiation the cells were incubated for 9-10 days after which the clones were fixed and stained (1). Transformed clones were identified by their appearance. The transformed clones differed from the controls in their piled up morphology and the random orientation of the cells which indicates a lack of contact inhibition.

Photographs of representative clones are shown in Figs. B-5-1 to B-5-3. In several experiments transformed clones were isolated and grown into mass cultures. The ability of the transformed cells to grow in semi soft agar and their agglutinability by plant lectins (Concanavalin A and wheat germ agglutinin) were used as additional criteria for the transformed nature of the cells as compared with control cultures. While all the isolated transformed cells were agglutinable by 50 μ g of the plant lectins, only cells from 2 isolated colonies with an epithelioid morphology were able to grow in semi soft agar.

The data of cell survival are shown in Fig. B-5-4. The shape of the survival curve is characteristic of that commonly observed for mammalian cells exposed to x-irradiation, with a broad initial shoulder at low doses becoming straight on this semi-logarithmic plot at higher doses indicating that cell survival in this region

Fig. B-5-1 A 9 day old clone of normal embryo cells.
Giemsa stain X15.

Fig.B-5-2 A 9 day old clone of hamster embryo cells transformed in vitro by 300 rads of x irradiation.

Fig. B-5-3 A 9 day old clone of hamster embryo cells transformed in vitro by 1 rad of x irradiation. Note the random orientation on the circumference.
Giemsa X15.

B-5-4 Survival of reproductive integrity of hamster embryo cells as a function of x ray dose. Circles describe points calculated from the transformation experiments (Table BP-5-1). Triangles describe points established from a separate cell survival experiment (see text). n denotes the extrapolation number, defined to be the point in the surviving fraction axis to which the straight portion of the surviving curve extrapolates. D_0 is defined as the dose required to reduce the population to a fraction of 37%, and is used as a measure of the slope in the linear portion of the survival curve.

Fig. B-5-4

is an exponential function of dose.

The dose response data for transformation are shown in Table B-5-1 and Fig. B-5-5. A complex relationship is indicated. Transformation has been observed for x ray doses as low as one rad. As the dose increases the percentage of transformed cells increases up to a plateau of about 1% between 150 and 300 rads. A further increase in dose results in a decrease in the percentage of cells transformed.

A preliminary study of the karyotype of the transformed cells indicates that they tend to be aneuploid, but there appear to be no gross chromosomal aberrations.

Experiments kindly carried out by I.B. Weinstein, using reported methods (5) indicated that there were no C type virus particles in x ray transformed cells which have proved to be tumorigenic.

Our results demonstrate that neoplastic transformation can be induced in vitro in single cells with doses of x rays as low as 1 rad. The proportion of killed cells is low at any of the doses used. This, along with the fact that no transformation was observed in the controls, indicate that transformation is directly induced by the x-irradiation and not a result of spontaneously occurring transformed cells.

B-5-5 - Incidence of hamster embryo cell transformation following exposure in vitro to x irradiation. For doses at which more than one experiment was performed, the data were pooled; the mean value together with the standard deviation is plotted in the figure (see Table BP-5-1 for results of individual experiments). The broken line is drawn by eye to the mean data points; the full line has a slope of +1 and passes through the error bars of each datum point.

Fig. 8-5-5

The shape of the dose response curve obtained in the present in vitro experiments is not unlike that characteristic of in vivo data (6-8). The major asset of the in vitro system is that at low doses one can overcome the difficulties present in the in vivo system where inordinately large numbers of animals are required to detect tumors, by irradiating a large number of plates and scoring thousands of clones for transformation. At high doses, the in vitro system inherently accounts for cell killing so that transformation is scored from among the survivors.

In the ascending part of the curve a linear dependence of induction cannot be ruled out. A plateau between 150 and 300 rads resembles that often reported for in vivo experiments (6). The descending limb of the curve, also observed in vivo (6,7), and attributed to overall cell killing at these high doses, can be interpreted here to be due to a preferential killing of the potentially tumor cells and supports some of the ideas proposed by Gray (8). Cells that have been altered by the absorption of radiation and are destined to become transformed are more susceptible to being killed by the accumulation of additional damage at higher doses. The striking similarity between the in vitro and the in vivo dose response relationships, in addition to the fact that more than one type of transformed cell can be detected, makes this system suitable for further

studies on the carcinogenic action of x rays, and other ionizing radiation present and increasing in our environment.

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Table B-5-1 Neoplastic Cell Transformation of Hamster Embryo Cells Following X Irradiation in Vitro

Exp. No.	<u>Control</u>			Dose rads	<u>Irradiated</u>			
	Number of clones counted	Plating* efficiency	Number transformed clones		Number of clones counted	Number of transformed clones	% transformed** clones	Surviving*** fraction
1	1600	1.6	0	600	920	2	0.22	0.12
2	1002	2.2	0	600	3020	8	0.27	0.13
3	1012	1.3	0	300	1130	8	0.71	0.75
4	1220	1.6	0	300	1960	18	0.92	0.75
5	2500	4.5	0	150	6200	44	0.71	0.89
6	4020	2.1	0	150	3268	25	0.77	0.86
7	1620	2.0	0	75	2132	11	0.52	.92
8	4100	1.2	0	75	2200	10	0.46	.95
9	1025	1.05	0	50	5402	10	0.185	0.95
9	1025	1.05		25	5503	8	0.145	1.00
6				10	10,200	4	0.039	1.00
8				1	6800	1	0.015	1.00
10	9600	2.5	0	1	9200	1	0.011	1.00
10	7200	1.9	0	1	7900	1	0.013	1.00

* Plating efficiency (P.E.) = $\frac{\text{number of clones counted}}{\text{number of cells plated}} \times 100$

** % cell transformation = $\frac{\text{number transformed clones}}{\text{number clones counted}} \times 100$

*** Surviving fraction = $\frac{\text{number of cells plated}}{\text{number of clones counted} \times \text{P.E.}}$

B-6 THE QUINACRINE FLUORESCENCE AND GIEMSA BANDING OF NORMAL AND
NEOPLASTIC RAT LIVER CELLS *

Carmia Borek in collaboration with D.A. Miller, O.J. Miller
and V.G. Dev (Department of Human Genetics)

Cytogenetic studies of the rat have been limited by the lack of an accurate method for individual identification of many of the similar chromosomes. The recent development of the fluorescent and Giemsa banding techniques for chromosome identification (1) were applied here for determining the normal karyotype of the rat rattus novегicus in cells derived from fresh embryo cultures of Fischer rat and in normal adult liver cells cultured and cloned from buffalo rat liver (2). Chromosomal alterations following neoplastic transformation in vitro were studied on these liver cells following their neoplastic conversion in vitro under conditions of nutritional stress (2). In order to compare the karyotype changes found in cells transformed in vitro with those occurring in liver cells transformed in vivo, we studied the karyotype of the H₄ IIEC line derived and cloned from rat hepatoma (3). We found the banding patterns to be the same in the normal diploid and the malignant aneuploid cells. The diversion from normal pattern in the tumor and the transformed cells was in the number of copies of specific identifiable chromosomes. The transformed cell karyotype pattern was aneuploid and suggested that transformation was

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advocated with a process of tetraploidization of the cells along with a random loss of some chromosomes and the appearance of an unidentifiable chromosome not present in the untransformed parental culture. The hepatoma cell line was also aneuploid with about 9 abnormal marker chromosomes per cell (4).

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B-7 SURFACE ALTERATIONS IN TRANSFORMED EPITHELIAL CELLS IN CULTURE *

Carmia Borek in collatoration with Max M. Burger (Biology Department, Princeton University)

Differences in the chemical composition and structure of the cell surface membranes between normal and neoplastic cells can be detected using plant agglutinins (Lectins) as surface probes. Microstructural alterations have been shown in fibroblasts following transformation (1,2). No studies have been carried out with these lectins on transformed epithelial cells as compared to their normal parental cells, nor on cells of epithelial tumors even though the preponderance of epithelial tumors (carcinomas) over fibroblastic tumors (sarcomas) is unequivocal. Amongst malignant tumors in liver one finds a variety of types ranging from the "minimal deviation" hepatomas which are differentiated and have retained most of the biochemical and some of the cytological characteristics of normal liver, to the "multiple deviation" hepatoma (3) which are completely dedifferentiated.

In earlier studies we have shown that the surface of hepatoma cells in culture differ from those of the normal cells in their ganglioside content (4) in the permeability to small ions and in their escape from growth control (5). We have also found these

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changes to hold true for liver cells transformed in vitro. In the present study (6) we have investigated the minimal deviation hepatoma cells (H₄11EC and H₅123) and the liver cells transformed in vitro for additional alterations in the surface membranes detectable by 5 different plant agglutinins; wheat germ agglutinin, concanavalin A, phytohemagglutinin, Ricinus Communis, great northern bean, and lense culinaris.

We compared them on one hand to cultured cells derived from the novikoff "multiple deviation" hepatomas (3) and on the other hand to normal differentiated hepatocytes growing in culture.

In general the degree of agglutinability of the neoplastic epithelial cells was comparable to that reported for transformed fibroblasts, though one of the agglutinins (wheat germ agglutinin) reacted more with the undifferentiated hepatomas than with the differentiated ones. The normal cells were not agglutinable but became so after mild treatment with proteolytic enzymes, thus suggesting that in the transformed state specific carbohydrate sites are unmasked and react with the plant lectins. Inhibition of protein synthesis in confluent non growing cultures of the normal cells lead to their agglutinability to the same degree as the novikoff hepatoma cells. Since under these conditions the proteolytic activity in the cells was increased it is suggested that these enzymes play a role in the surface alterations occurring in malignantly transformed cells.

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B-8 The Relationship of Intracellular Cyclic AMP to Radiosensitivity.*

Shirley Lehnert

Cyclic 3'5' adenosine monophosphate (cyclic AMP) regulates many physiological functions (1,2). Addition of dibutyryl cyclic AMP to the media of cultured mammalian cells results in changes in cellular morphology (3-6), in the restoration of controlled growth to transformed cells, (3), in changes in the surface architecture of the cell (3,7) and in induced axon formation in neuroblastoma cells (8).

The cessation of growth at confluency which is observed in density dependent or contact inhibited cells is accompanied by a concomitant rise in intracellular cyclic AMP. When confluent monolayers are exposed to serum or protease which stimulates further division a transient reduction in cyclic AMP occurs (9). In transformed cells where growth rate is unchanged at confluency no change in intracellular cyclic AMP is seen, addition of dibutyryl cyclic AMP to the culture medium however restores controlled growth to some transformed cells.

A further indication that cyclic AMP may have a regulatory effect on cell growth is the fact that intracellular cyclic AMP levels change in a characteristic way during the cell cycle, the lowest level being observed during mitosis (10,11). The radiosensitivity of mammalian cells is, to some extent, dependent on the stage of the cell cycle so it is of interest to see whether the level of intracellular cyclic AMP has any effect on radiosensitivity.

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The level of intracellular cyclic AMP in the cell may be modified in two ways. Firstly by activating the enzyme adenylyl cyclase which converts ATP to cyclic AMP, and secondly by inhibiting the enzyme cyclic AMP phosphodiesterase which converts cyclic AMP to 5' AMP and thus produces an intracellular accumulation of cyclic AMP. (Table B-8-1)

Four different drugs have been used to produce elevated intracellular cyclic AMP, one of them, prostaglandin E, activates adenylyl cyclase, while the other 3 listed in Table B-8-1 are inhibitors of cyclic AMP phosphodiesterase. Also shown in Table B-8-1 are the levels of intracellular cyclic AMP found after exposure of V-79 cells to appropriate concentration of these drugs for 1 hour or 3 hours at 37°C. Cyclic AMP was measured using a radio-immune assay supplied by Schwarz/Mann.

X-ray survival curves for asynchronous cells treated for one hour with each of these drugs are shown in Figure B-8-1. Treatment of cells prior to irradiation with any of the drugs produced somewhat similar changes in terms of radiosensitivity, in all cases there was an increase in the size of the shoulder of the survival curve, with the result that survival following low doses of radiation was greater for drug treated cells than for controls. Beyond the shoulder region, however, the slope of the exponential portion of the curve was greater than that of the control curve, consequently at higher doses drug treated cells

Fig. B-8-1 Survival curves for V-79 cells with 210 Kv x-rays 24 hours after attachment to Falcon flasks. Cells exposed to drugs for one hour prior to irradiation. Medium exchanged for control medium immediately before start of radiation.

were more sensitive to irradiation. Drug treated cells are thus characterized by a higher extrapolation number and higher D_0 than are control cells; x ray survival curves for control and drug treated cells typically "cross-over" at about 1000-1200R.

Since the drug is removed from the medium prior to irradiation, changes in radiosensitivity of the cells are not due to any direct action of radiation on the drug. Moreover the four compounds used have little in common, either chemically or biologically, except their capacity to raise levels of intracellular cyclic AMP in mammalian cells, hence the conclusion that the changes in radiation response of drug-treated cells is directly related to their elevated cyclic AMP content is inescapable.

It should be pointed out that, although cyclic AMP probably exerts a regulatory effect on the passage of the cell through the cell cycle, in experiments of the type described here, i.e. a relatively brief exposure of asynchronous cells to cyclic AMP - inducing drugs, no synchronization of the cells at the time of irradiation was not observed and changes in radiation sensitivity cannot be explained on this basis.

Some experiments were performed to determine whether the increased size of the shoulder on the survival curve was related to an increased ability to repair sub-lethal damage or, simply to an

increased ability to accumulate such damage. Figure B-8-2 shows the survival of drug-treated or control cells following a dose of 1200R, delivered either as a single dose or as two doses of 600R. The results are plotted in terms of the recovery factor, that is the ratio of the survival following a split dose to that following a single dose, against time between split doses. The extent of recovery between split doses was almost twice as great for cells treated either with prostaglandin E, or DL 150 as was that of controls. Cells containing high cyclic AMP then, have an enhanced capacity for repair of sub-lethal damage.

It is known that increased levels of cyclic AMP produce, amongst various effects, an increased capacity to assemble 100° A° microtubules, one of the elements of the cytoskeletal system. This is evidenced when cyclic AMP is elevated in the cell by the morphological detransformation of transformed cells, by the extension of elongated process by fibroblasts, and by the production of axons by neuroblastoma cells. As a start to a study designed to determine why repair of sub-lethal damage is enhanced in high cyclic AMP cells, an experiment was done using vinblastine sulfate, an alkaloid which inhibits the assembly of microtubules. If 0.0005 µgm/ml of vinblastine sulfate was added between split doses to either control or DL 150 treated cells, (Figure B-8-3) no repair of sub-lethal damage was observed in either

Fig. B-8-2 Recovery between split doses of V-79 cells. Drugs were added 1 hour prior to first dose of x-rays and removed immediately before the second.

Fig. B-8-3 Recovery between split doses of control V-79 cells and cells treated with DL150. Vinblastine sulfate 0.005 MEM/ml was added between doses to cells treated with DL-150.

group. This would indicate that organization of the cytoskeletal system was important to the repair of sub-lethal damage. Unfortunately, vinblastine sulfate has effects other than that on the assembly of microtubules, so that the observed suppression of repair could be a non-specific effect. Further studies of this and other ways in which cyclic AMP could influence the repair of sub-lethal damage are being undertaken. Attempts are also being made to determine whether variations of radiosensitivity and cyclic AMP content during the cell cycle are related.

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TABLE B-8-1 Cyclic AMP levels in V-79 Chinese Hamster Cells.

Compound	Mode of Action	cAMP, pMoles/ 10^6 cells in treated V-79 cells.	
		1 hour	3 hours
None	-	5.7 ± 0.6	
Prostaglandin E, (Upjohn Co.) 10 $\mu\text{gm/ml}$	Activation of adenyl cyclase	16 ± 3.9	
1-methyl-3-isobutyl xanthine, 0.5m Molar (G.D. Searle Co.)	Inhibition of cAMP phosphodiesterase		
Diethylaminoethyl-1- reserpine, DL-150 10^{-5}M (Clin-Byla, Paris)	"	15 ± 2.6	14.6 ± 6.0
4-(3-butyoxy-4- methoxy benzyl)-2- imidazolidinone. Ro-20-1724, 200 $\mu\text{gm/ml}$ (Hoffman LaRoche)	"	21.5 ± 4.2	12.5 ± 4.0

B-9 ENHANCEMENT OF REPAIR OF POTENTIALLY LETHAL DAMAGE BY CONDITIONED
MEDIUM HARVESTED FROM CELLS IN PLATEAU PHASE

I. Horowitz, H. Norwint and E.J. Hall

The component of radiation damage which can be influenced by post-irradiation conditions has been termed potentially lethal damage. It is potentially lethal in that it may be expressed and lead to cell death provided the cells are maintained under optimal growth conditions. It is not inevitably lethal in that it may be repaired under certain circumstances.

It is known that cells incubated in balanced salt solution, or at reduced temperature, for several hours after irradiation will repair potentially lethal damage (1-4). In general, More cells will survive a given dose of radiation if sub-optimal culture conditions exist for a few hours after irradiation, than is the case if ideal conditions of temperature, pH, and nutrition, etc. prevail during this period. The probable explanation for this is that normal cell metabolism, progression through the cycle, and/or preparation for mitosis must be able to occur for the radiation damage to be fully expressed. Under sub-optimal culture conditions, at least part of this damage may be repaired before the cell attempts any complex process, the failure of which may lead to cell death.

Little (5) recently described experiments linking the repair of potentially lethal damage and plateau phase cultures. When mammalian

cells are allowed to reach plateau phase their subsequent growth is markedly reduced. Even when the culture is regularly "fed" with fresh growth medium, the total number of cells in a given culture vessel remains essentially constant; under these conditions population size is restricted by overcrowding and is said to be density-limited. It has been suggested that conditioned medium taken from such plateau phase cultures may contain a substance, released from the crowded cells, which can inhibit protein synthesis (3) and can depress both cell multiplication and DNA synthesis in exponentially growing cells (4). Little (5) found that the repair of potentially lethal damage was inhibited by conditioned medium from plateau cultures in two cell lines investigated, namely Chang liver cells and Chinese hamster cells. The experiments to be described here were designed to follow up this observation, extend it to other cell lines, and possibly to shed some light on the mechanisms involved.

This topic is of more than academic interest since more recent experiments by Little, Hahn, Frindel and Tubiana (6) convincingly demonstrated that potentially lethal damage is a factor in the radiation response of solid tumors in animals; there is no reason to suppose that it is not also involved in the response of human tumors treated with radiation.

X ray dose response curves have been obtained for two cell lines

carried routinely in the laboratory; Chinese hamster V79 cells and Morris cells derived originally from a rat hepatoma. The results are shown in Figures B-9-1 and B-9-2. For each cell line, survival data were obtained under 3 conditions:

- a) Immediately after x-irradiation, the growth medium was removed from the cultures and replaced for 6 hours with "conditioned" medium harvested from stationary phase cultures of the same cell line. After this treatment, fresh growth medium was returned to the cultures which were incubated for 8 days to test for colony formation.
- b) Same as (a) except that the conditioned medium was heated at 60°C for one hour before being added to the irradiated cultures.
- c) The third replicate survival curve was obtained with dishes handled in the same way as above, and subject to the same medium changes, except that fresh medium was used throughout.

In both cell lines used, the 6-hour post-irradiation incubation with "conditioned" medium significantly enhanced the survival. The fraction of cells surviving large doses of x rays (about 1000 rads, for example) is increased by a factor of two when conditioned medium was added after exposure to x rays. This reflects the enhanced ability of the cells to repair potentially lethal damage under these conditions. The data have not been subjected to a detailed statistical analysis, and probably do not warrant any such treatment, but at first sight it appears that the

Fig. B-9-1 Survival curves for V-79 Chinese hamster cells with various media replaced for 6 hours after radiation.

Fig. B-9-2 Survival curves for "Morris" cells with various media replaced for 6 hours after radiation.

major effect of the conditioned medium is to increase the D_0 of the survival curve. These results agree essentially with those of Little (5), and therefore extend his observation to other cells lines.

It has been suggested that the efficacy of "conditioned" medium in enhancing the repair of potentially lethal damage is due to the presence of some diffusible substance released into the culture medium from the density-limited plateau phase cells. It is important to distinguish between this explanation and the alternative possibility that "conditioned" medium is simply depleted or exhausted of vital nutrients as a result of its use to support dense plateau phase cultures; in the latter case the conditioned medium would be acting essentially like buffered saline which is known to enhance the repair of potentially lethal damage (1).

To detect, much less identify, a foreign substance in tissue culture medium is a formidable task, since it already contains a galaxy of known ingredients, not to mention the complex and variable composition of the added fetal calf serum. To distinguish between medium depletion and the presence of a diffusible substance in the conditioned medium as the explanation for its effectiveness, a third survival curve was obtained for each cell line and these are also shown in Figures B-9-1 and B-9-2; in this case the conditioned medium was heated for one hour at 60°C before being used for postirradiation treatment. The rationale was as follows. If the heat treatment reversed the affect of the

conditioned medium, it would argue strongly in favor of a diffusable substance which is heat degradable. On the other hand, if the heat treatment were ineffective the experiment would not be diffinitive. It is evident from the Figures B-9-1 and B-9-2 that the result is somewhat equivocal. In general the points relating to the heat treated conditioned medium fall between the survival curves for fresh medium and unheated conditioned medium. The heat treatment does indeed appear to have some effect and strongly suggests that the presence of a substance in the conditioned medium, derived from the plateau phase cultures cannot be ruled out.

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TEACHING

During the Fall of 1972 a course was given in radiobiology for residents in radiology, followed during the winter months by a course in radiological physics. Both lecture series were attended by 40 to 50 residents from Columbia Presbyterian Medical Center and associated hospitals, as well as other institutions in the surrounding area. Introductory lectures have also been given to medical and dental students in radiation effects.

The practice has been continued whereby radiotherapy residents spend a period of 6 months full-time in the laboratory during the final year of training. During this period they are taught basic tissue culture techniques, given a small research project, and encouraged to read the literature extensively.

A series of seminars has been organized intermittently during the year to take advantage of the presence of the continuous stream of visitors from home and abroad, many of them leaders in their fields, who pass through New York.

The principal teaching activity of the laboratory has been an advanced course in Radiation Biophysics. This was offered for the first time in June 1973, and evoked considerable of interest. The teaching faculty consists predominantly of members of this laboratory, supplemented by a few eminent visitors from other institutions.

Invited Seminars and Papers at Scientific Meetings

Dr. C. Borek

American Society for Cell Biology, St. Louis, Missouri, Nov. 1972

Radiation Research Society, St. Louis, Missouri, May 1973.

Host Environment Interactions in the Etiology of Cancer in Man,
Primosten, Yugoslavia, Aug. 1972.

Chester Beatty Inst. for Cancer Res., London, Aug. 1972

Hunter College, Oct. 1972

Am. Tissue Culture Assoc., Boston, June 1972.

Dr. J.F. Dicello

Radiation Research Society, St. Louis, Missouri, May 1973

Dr. W. Gross

Radiation Research Society, St. Louis, Missouri, May 1973

Dr. E.J. Hall

Cospar Space Biology meeting, Madrid, Spain, May 1972

International Congress of Medical Physics, Göteborg, Sweden, Aug. 1972

International Union of Biophysics, Moscow, USSR, Aug. 1972

Seminar, Hammersmith Postgraduate Medical School, London, England,
Aug. 1972

Seminar, Univ. of Wisconsin, Madison, Wis., Oct. 1972

Radiological Soc. of N.A., Chicago, Ill., Nov. 1972

Seminar, Harvard Medical School, March 1973

Radiation Research Society, St. Louis, Mo., May 1973

Dr. A.M. Kellerer

German-Austrian Roentgen Congress, Vienna, Austria, April 1973

Radiation Research Society, St. Louis, Missouri, May 1973

Dr. S. Lehnert

Radiation Research Society, St. Louis, Mo., May 1973

Dr. H.H. Rossi

Conference on Particle Accelerators in Radiation Therapy,
Los Alamos, New Mexico, Oct. 1972

IAEA Panel on "Fast Neutron Dosimetry: Characterization,
Standardization and Intercomparison." Nov. 1972,
Vienna, Austria

Bureau of Radiological Health, Rockville, Md., May 1973

NSF Neutron Workshop, Boston, Mass., May 1973

Radiation Research Society, St. Louis, Mo., May 1973

Dr. A. Underbrink

Radiation Research Society, St. Louis, Mo., May 1973

Graduate course in radiation biology, Adelphi University

OTHER ACTIVITIES

Several visiting scholars were at the Laboratory for varying periods of time: Mr. Jean Brenot, Mr. M. Chemtob from the French Atomic Energy Commission; Mr. Eckhardt Krüger from the University of Munich, Germany; and Dr. S. Pszona from the Institute of Nuclear Research, Swierk, Poland.

A bibliography of publications is attached.

In addition, members of the Laboratory were active on the following Committees and Commissions:

International Commission on Radiation Units and Measurements:

Main Commission

Committee to Review Nominations for the ICRU Gray Medal (Committee)

Technical Area: Radiobiology (Co-Sponsor)

Radiation Physics - Neutrons and Heavy
Particles (Sponsor)

Report Committee on High Energy and Space
(Radiation Dosimetry (Chairman)

Report Committee on Average Energy Required
to Produce an Ion Pair

Theoretical Aspects (Sponsor)

Report Committee on Fundamental Quantities and Units

Report Committee on Concepts and Principles of
Radiation Protection Measurement
(Cooperative Effort with the ICRP) (Chairman)

Intercomparison of Neutron Instrument (Sponsor)

National Council on Radiation Protection and Measurements

Board of Directors

Main Council

Scientific Committee 1 Basic Radiation Protection Criteria

Scientific Committee 4 Heavy Particles (Neutrons, Protons and Heavier) (Chairman)

Scientific Committee 13 Safe Handling of Bodies Containing Radioactive Isotopes (Chairman)

Scientific Committee 15 Radiation Protection in Teaching Institutions

Scientific Committee 29 Brachytherapy

Scientific Committee 49 Radiation Protection Guidance for Paramedical Personnel (Chairman)

Advisory Committee on Ionizing Radiation for the Mayor of the City of New York (Chairman)

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

Advisory Committee for Radiation Biology Aspects of the Supersonic Transport, FAA (Chairman)

Review Committee for Biological and Medical Research, Argonne Universities Association

AEC Consultants Committee on Medical Use of Nuclear Powered Pacemakers

Dept. of Health, State of New York, (Consultant)

Radiobiological Advisory Panel of the National Academy of Sciences

AEC Ad Hoc Committee on the Hazards of Californium

AEC Ad Hoc Working Group to Assess the Factors Determining the Doses to be Employed in the Clinical Application of Californium-252

Low Level Radiation Group

During the year Drs. Rossi, Kellerer and Hall and Mr. L. Goodman 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.

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