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RADIATION PHYSICS, BIOPHYSICS AND RADIATION BIOLOGY

Progress Report, October 1, 1978—September 30, 1979

July 1979

Work Performed Under Contract No. EP-78-S-02-4733

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

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RADIATION PHYSICS, BIOPHYSICS
AND RADIATION BIOLOGY

Progress Report
for Period October 1, 1978 - September 30, 1979

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TABLE OF CONTENTS

INTRODUCTION	6
ACKNOWLEDGEMENTS	8
STAFF	10
 RADIOLOGICAL PHYSICS	 12
A) <u>Dosimetry</u>	
PD-1 Investigation into the thermal defect of tissue-equivalent plastic L.J. Goodman	13
PD-2 W_n and neutron kerma for methane based tissue-equivalent gas L.J. Goodman	19
PD-3 The W-value for electrons and photons in tissue-equivalent gases D. Srdoc	37
PD-4 Radial energy deposition profile of proton tracks R.E. Mills	51
PD-5 Measurement of the Fano Factor in tissue- equivalent and other gases D. Srdoc	58
PD-6 Neutron dosimetry for rat irradiations R.E. Mills	64
 B) <u>Microdosimetry</u>	
PM-1 The response of the proportional counter to unit charge D. Srdoc	65
PM-2 Influence of amplifier time constant on proportional counter calibration P.J. Kliauga	72

PM-3	Microdosimetry at the Bevalac P.J. Kliauga, Y.M.P. Lam, H.H. Rossi, and L.J. Goodman	76
PM-4	Microdosimetry in the Nanometer Region M.H. Biavati, H.H. Rossi, P.J. Kliauga and P.E. Goldhagen	88
PM-5	Microdosimetry measurements at RARAF D. Srdoc, R.E. Mills, and S.A. Marino	95
BIOPHYSICS		102
BP-1	A rigorous formulation of the site model Y.M.P. Lam	102
RADIOBIOLOGY		106
RB-1	Oncogenic transformations with split doses of x-rays R.C. Miller and E.J. Hall	108
RB-2	Neoplastic transformation following split doses of x-rays C. Borek	115
RB-3	The intercomparison of relative biological effectiveness and oxygen enhancement ratio at clinically used neutron facilities E.J. Hall (in collaboration with Dr. A. Kellerer, Institut Für Med. Strahlenkunde, Würzburg, West Germany)	120
RB-4	Sister chromatid exchanges: Interaction of a retinoid (Ro-10-9359) and a protease inhibitor (antipain) with x-rays M.R. Freeman and C.R. Geard	128
RB-5	The effect of neutron induced recoil protons of restricted energy and range on chromosome aberration formation C.R. Geard	132

RB-6	Effects of 1.91 MeV monoenergetic neutrons on <i>Vicia faba</i> chromosomes: Microdosimetric considerations C.R. Geard	145
RB-7	Sequential exposures with high and low-Let radiations R.P. Bird	154
RB-8	The associated ion experiment R.P. Bird	158
RB-9	Irradiations with the track segment facility and the analysis of data R.P. Bird and P.J. Kliauga	162
RB-10	The modulating effects of the protease inhibitor Antipain on x-ray induced transformation C. Borek and R.C. Miller	170
RB-11	A comparison of two nitroimidazoles as hypoxic sensitizers, radiosensitizers and cytotoxic agents E.J. Hall and M. Astor (in collaboration with Drs. P. Shapiro and L. August, Naval Research Laboratory, Washington, D.C.)	179
RB-12	MTDQ as a hypoxic cell radiosensitizer alone and in combination with misonidazole M. Astor, E.J. Hall and R.S. Osmak	193
RB-13	Combination studies with Bleomycin, Misonidazole and x-rays L. Roizin-Towle and E.J. Hall	207
RB-14	The effect of hyperthermia alone, or combined with x-rays, on the incidence of oncogenic transformations <i>in vitro</i> R.C. Miller, L. Harisiadis and E.J. Hall	216

RB-15	An investigation <i>in vitro</i> of the clastogenic effects of misonidazole L.E. Schwartz and C.R. Geard	222
RB-16	Regulation of retinol-binding protein metabolism in cultured rat liver cell lines C. Borek	242
TEACHING		249
INVITED SEMINARS AND PAPERS AT SCIENTIFIC MEETINGS		250
LOW LEVEL RADIATION GROUP		253
OTHER ACTIVITIES		253
BOOKS		256
BIBLIOGRAPHY		257

INTRODUCTION

The fundamental aim of the Radiological Research Laboratory is to pursue research in radiation physics and cellular biology, with the aim of understanding the basic mechanisms of radiation effects on biological materials, with particular reference to carcinogenesis. In the hope of making progress towards this end, a broad based experimental program is underway, designed to measure the pattern of deposition of radiation energy at the micrometer and nanometer levels, and to investigate the effects of radiations of a wide spectrum of ionization densities on mammalian cells, scoring cell killing, chromosome aberrations and oncogenic transformations as biological endpoints. The experimental program is carried out against a strong theoretical background, that adds cohesion to the program and suggests special thrusts in particular areas.

Certain areas of research have immediate pragmatic value of special note, as well as contributing to the overall understanding of radiobiological effects. The study of oncogenic transformations *in vitro* falls into this category. This project is central to the interests of the laboratory, and by its nature, consumes a sizeable portion of the budget. This *in vitro* model of carcinogenesis allows the effects of radiation to be studied in the low dose range of 1 to 10 rad, as well as the investigation of agents that promote or inhibit the effectiveness of radiation. Another example of a project with immediate practical usefulness is the study of chemotherapeutic agents and hypoxic cell radiosensitizers, used in radiotherapy. The discovery in this laboratory during the past year that the cytotoxic, and possibly the carcinogenic,

properties of misonidazole can be inhibited by antioxidants, has been taken up in a number of other laboratories and is likely to find a clinical application in the near future.

The past year has been successful and productive. The number, and more important the quality of scientific papers presented at National and International meetings, as well as appearing in refereed journals, has continued to be satisfactory. There have been a number of staff changes, and a few additions as well. The overall size of the laboratory, as well as the blend of physics and biology, is now close to optimal. Further expansion would not be desirable and is not planned. During the year Dr. Yuk-Ming Lam has left to take up an appointment in the computer industry; his place has been taken by Dr. Marco Zaider, who will be principally responsible for the statistical analysis of data, and developments of a theoretical nature. A new project has been initiated during the year to study the biological effects of the soft radiation emitted by ^{125}I iodine. For this work we were fortunate to obtain the services of Dr. Michael Freeman, trained in Radiation Biology at Colorado State, and Dr. Paul Goldhagen who has a strong background in high energy physics. Dr. Goldhagen will also be concerned with the development of nanodosimetry. The study of the radiobiological effects of neutrons and charged particles of defined LET will be greatly strengthened by the addition to our staff of Dr. Paul Furncinitti, who will be located at Brookhaven.

Of particular note was the help received from our two consultants. During the Fall, Professor J.W. Boag spent a week in the laboratory, consulted with most of the scientific staff, and made available his special expertise in statistical methods. During the Spring, Professor Albrecht Kellerer spent 3 weeks in the laboratory, sufficient time to materially advance several joint projects between this laboratory and the Institut Fur Med. Strahlenkunde in

Wurzburg. The counsel and advice of these consultants proved to be extremely valuable, and was of significant benefit to the program.

ACKNOWLEDGMENTS

Much of the research effort of this laboratory centers around the Radiological Research Accelerator Facility, operated by the Safety and Environmental Division of the Brookhaven National Laboratory. We would not miss this opportunity to thank Dr. V.P. Bond, Mr. C. Meinhold, Dr. N. Rohrig, Mr. S. Marino, and the Brookhaven staff who are responsible for the operation of the machine.

Because of our interest in high LET radiations, we have used a number of accelerators in various parts of the U.S. as well as overseas. We are grateful to the following who made this possible: Drs. Bondelid, Theus, August, and Shapiro at the Naval Research Laboratory, Washington, D.C.; Drs. Griem and Kuchnir at the University of Chicago; Drs. Harrison and Holgren at the University of Maryland; Drs. Bewley, Parnell, and Field of the Hammersmith Hospital, London.

Research projects in both microdosimetry and radiobiology have been carried out at the Bevalac facility of the University of California in Berkeley and we are indebted to Drs. J. Howard and H. Grunder for their assistance.

In the area of electron affinic drugs, Roche Products generously supplied substantial amounts of several products, while Dr. Ged Adams and Dr. Martin Brown made available small amounts of newly synthesized compounds. Bristol Laboratories supplied us at no cost with Bleomycin and a number of other chemotherapy agents were supplied by the Division of Cancer Treatment, of the National Cancer Institute.

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

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

CA 12536: PD-6

PM-3, PM-4, PM-5

RB-1, RB-2, RB-4, RB-5, RB-6, RB-7, RB-10, RB-11, RB-12

RB-13, RB-14, RB-15, RB-16, RB-17

CA 15307: RB-8, RB-9

CA 09053: RB-1

CA 18506: RB-3

CA 23952: RB-1, RB-10, RB-15

Additional permanent support is furnished by Columbia University. Some of the personnel listed are partly or wholly supported by such other sources.

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RADIOLOGICAL PHYSICS

A) Dosimetry

- PD-1 Investigation into the thermal defect of tissue-equivalent plastic
L.J. Goodman
- PD-2 W_n and neutron kerma for methane based tissue-equivalent gas
L.J. Goodman
- PD-3 The W-value for electrons and photons in tissue-equivalent gases
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- PM-4 Microdosimetry in the Nanometer Region
M.H. Biavati, H.H. Rossi, P.J. Kliauga and P.E. Goldhagen
- PM-5 Microdosimetry measurements at RARAF
D. Srdoc, R.E. Mills, and S.A. Marino

A) DOSIMETRY

PD-1 Investigation into the thermal defect of tissue-equivalent plastic

L.J. Goodman

A. Introduction

Calorimetric dosimetry has the potential of serving as a standard for measurements of neutron absorbed dose in tissue. The calorimeters in use or planned for such measurements employ a radiation absorber made of A-150 tissue equivalent plastic.¹ This material has a significant thermal defect, defined as the fraction of absorbed radiation energy which is not converted to heat in the absorber but is dissipated in endothermic radiophysical and radiochemical effects in the plastic. Studies of the thermal defect of A-150 plastic have been undertaken in collaboration with the Biophysics Laboratory of the Memorial Sloan Kettering Cancer Center, New York, NY, utilizing the charged particle beams available from the 4 MV Van de Graaff accelerator at the Radiological Research Accelerator Facility (RARAF) of Brookhaven National Laboratory.

Annual Report COO-3243-5 discussed plans for making these measurements using the same calorimeter as used by Fleming and Glass (1969). Annual Report COO-3243-6 reported the results of density and elemental composition studies made on the variety of molded A-150 absorber discs available for measurements of thermal defects (Goodman, 1978). It was concluded that differences in the molding techniques used by the four suppliers of the discs, including extremes of temperature and pressure, had no significant effect on the uniformity of the density and composition of the discs. That report also described the calorimetry instrumentation, on-line minicomputer program, and preliminary measurements. Last year's Annual Report COO-4733-1 discussed instrumentation problems and modifications and described the difficulties in obtaining acceptable calorimeter stability and reproducibility.

¹Supplied by Physical Sciences Laboratory, Lisle, IL.

Measurements

The thermal defect of an A-150 absorber disc is measured by determining the time rate of temperature rise produced by the total absorption of a beam of charged particles of constant energy and current, and comparing this rate to that produced by absorption of the beam in an aluminum disc mounted on the opposite face of the absorber assembly. The thermal defect of a test disc is thus determined relative to that of aluminum. Additional measurements for aluminum relative to gold or platinum are used to verify that that thermal defect of aluminum is essentially zero.

Figure PD-1-1 shows a typical computer-produced plot of absorber temperature (TB) versus time (T). Before any measurements are made the computer checks to verify that the calorimeter has achieved temperature stability. Then, starting at time $T = 0$, the computer makes 20 measurements spaced 6 seconds apart to document that, in the absence of the particle beam, the absorber temperature is constant. At 120 seconds the beam stop is removed and the absorber is irradiated resulting in a temperature rise which is linear following a small initial lag (about 12 seconds). Measurements continue to be made every 6 seconds until 22 beam-on data points have been acquired. The beam stop is then inserted and 7 additional measurements are made. Before plotting the data, the computer calculates linear least-squares fits to the data points through which straight lines have been drawn in Fig. PD-1-1. The slope of the line obtained during the beam-on time gives the time rate of absorber temperature rise. While this data is being accumulated, the computer also acquires data on the total beam charge delivered to the absorber during each 6 second time interval and computes a least-squares line whose slope is the time rate of beam energy deposited in the absorber.

Figure PD-1-1: Computer-produced plot of absorber temperature (TB) versus time (T).

The quotient of time rate of absorber temperature rise by the time rate of beam energy deposited in the absorber is calculated to yield the parameter R, the absorber temperature rise per unit energy input (i.e., K/J).

Although such a run takes only about 5 minutes, about one hour is then required to cool the absorber to the starting temperature and to allow for acceptable stability to be attained.

Determinations of R, including replicates, are made with the charged particle beam impinging on the test disc and on the aluminum reference disc. The thermal defect, δ , is then given by

$$\delta = \frac{R_{Al} - R_T}{R_{Al}} \quad (1)$$

As will be discussed later, in some cases the discs on both sides of the absorber assembly were covered with thin (0.7 μ m) nickel foils. If these foils are assumed to have no thermal defect, it can be shown that in this case equation 1 becomes

$$\delta = \left[\frac{R_{Al} - R_T}{R_{Al}} \right] \frac{1}{1-\psi} \quad (2)$$

where ψ is the fraction of the particle energy which is deposited in the foil.

Results

Although much time and effort have been expended to obtain good results with this calorimeter system, to date the overall results have been disappointing due to inconsistencies and lack of reproducibility. Because of this only rough, tentative conclusions can be given at this time.

Measurements made with 1.7 and 4 MeV protons indicate that the extrapolated zero dose thermal defect of A-150 plastic is ~4 percent, in agreement with the value found by Fleming and Glass (1969) using 1.7 MeV protons. No significant thermal defect was observed when aluminum was compared to gold

or when gold was compared to platinum. For these latter measurements it was found necessary to cover both absorber faces with a thin (0.7 μm) nickel foil so that both surfaces would have the same emissivity. This had not been done for the A-150 discs and an approximate correction was determined by comparing pure graphite to aluminum with and without nickel foils.

The thermal defect of A-150 does not appear to depend on the supplier, but it was found that low molding temperature and pressure yield a thermal defect about twice as high as discs molded under optimal conditions or at high temperature and pressure.

The thermal defect of A-150 was found to decrease slightly as the absorbed dose increases, but recovers within 1 to 2 days if the plastic remains in vacuum. Exposure of A-150 to air within about two days after irradiation results in a permanent decrease in the thermal defect. This has little practical effect on the use of A-150 in a calorimeter designed for neutron dosimetry since about 10 Mrad of 1.7 MeV protons resulted in a decrease of the thermal defect of only about 0.2 percent and the absorbed doses normally utilized for practical calorimetric neutron dosimetry are usually only a few hundred rads.

Some measurements were also made employing beams of 4 MeV ^3He particles. The results indicated that the thermal defect of A-150 for these heavier, higher LET particles is somewhat less, ~3 percent, than for protons.

Attempts to determine the thermal defect for 0.5 MeV protons were unsuccessful due to excessive scattering of the protons in the nickel entrance foil of the calorimeter. The scattered protons strike the walls of the adiabatic jacket producing a secondary electron emission which interferes with proper control and measurement of the proton beam current. An attempt will be made to overcome this problem by operating the calorimeter without the

entrance foil.

It is also planned to repeat some of the measurements made with 1.7 and 4 MeV proton beams but with the absorber faces covered with thin nickel foils. This may reduce the uncertainty of the emissivity correction required for the earlier measurements.

References

1. Fleming, D.M. and Glass, W.A. (1969). Endothermic processes in tissue-equivalent plastic. Radiat. Res. 37, 316-322.
2. Goodman, L.J. (1978). Density and composition uniformity of A-150 tissue-equivalent plastic. Phys. Med. Biol. 23, 753-758.

L.J. Goodman

Introduction

The principal dosimeter used to determine the absorbed dose of neutrons employed in radiobiology and radiotherapy is the homogeneous tissue-equivalent (TE) ionization chamber. A methane-based TE gas mixture is most commonly used in the chamber cavity. This instrument is used to measure the charge liberated in the gas by the secondary charged particles set in motion by the neutrons. Conversion of this charge to absorbed dose requires knowledge of W_n , the mean energy expended to form an ion pair in the gas by the initial spectra of secondary particles produced by the neutrons. Last year's Annual Report (COO-4733-1) presented data on W_n computed from recent measurements of W for charged particles. The present report, prepared in collaboration with Joseph J. Coyne of the Center for Radiation Research, National Bureau of Standards, is similar to last year's Report but differs in two important respects:

1. The computation of W_n requires that secondary charged particle spectra calculated from neutron interaction cross sections and energetics be employed for each neutron energy used, as is done in this report. For the earlier computation such spectra were used for only seven neutron energies; the remainder of the W_n data were obtained by an approximation method employing useful but inaccurate simplifying assumptions.

2. The computation of W_n also requires that experimental data on W for each type of secondary charged particle be available. One of the principal sources of this data (3) has been significantly modified and augmented since the previous report was prepared. These data had little influence on the

evaluations of W made for the earlier report due to the large discrepancy they displayed relative to the other available data. However, their recent revision (4) puts them in sufficient agreement with the other data that they are here given equal weight in the evaluations of W.

Further, this report discusses the relative kermas contributed by the various types of charged particles. By combining computed charged particle spectra with the evaluations of W, the number of ion pairs produced in the gas by each type of charged particle is derived, from which W_n is computed as the quotient of the total kerma by the total number of ion pairs.

CHARGED PARTICLE SPECTRA

Material composition

The values of W and W_n presented will be those for the TE gas mixture containing 64.4% methane, 32.4% carbon dioxide, and 3.2% nitrogen by partial pressures (5). This gas is used in the cavity of an ionization chamber with walls of A-150 TE plastic.¹ The elemental compositions of these materials are shown in Table PD-2-1 along with that for ICRU muscle tissue (1). It can be seen that the composition of the gas differs somewhat from that of the chamber wall, particularly the amounts of carbon and oxygen, and that in both of these materials there is a substantial substitution of carbon for oxygen in comparison with the composition of ICRU muscle tissue.

Table PD-2-1

Elemental Compositions (1)

<u>Material</u>	<u>Percent mass</u>						
	<u>H</u>	<u>C</u>	<u>N</u>	<u>O</u>	<u>Fl</u>	<u>Ca</u>	<u>Other</u>
TE gas	10.2	45.6	3.5	40.7	---	---	---
A-150 plastic	10.1	77.6	3.5	5.2	1.7	1.8	---
ICRU muscle	10.2	12.3	3.5	72.9	---	0.007	1.1

¹Supplied by Physical Sciences Laboratory, Lisle, IL.

Figure PD-2-1: Kerma relative to total kerma for ions produced in methane-based TE gas by neutron bombardment.

Although the initial spectra of secondary charged particles produced in the gas will in general consist of particles created in the wall and in the gas, it will be assumed in most of the following that the spectrum is that which results from charged particle equilibrium in the gas. The effect of this assumption on the computation of W_n will be tested and discussed in a later section.

Kerma factors

Coyne (19) has computed the initial spectra of charged particles using cross sections averaged over energy bins of finite width. The bins are the same as those used to prepare the table of kerma factors in ICRU Report 26 (1). The initial spectra of charged particles were used to compute the kerma factors $K_j(E_n)$ for the j th particle type given by

$$K_j(E_n) = \int_0^{E_{jm}} n_j(E, E_n) E dE \quad (1)$$

where $n_j(E, E_n)$ represents the initial differential spectrum of the number of charged particles of type j produced by a fluence of one neutron per cm^2 (particles $\text{MeV}^{-1} \text{g}^{-1}$ per neutron cm^{-2}) of Energy E_n . The integration is up to the maximum energy of the particles of type j , E_{jm} . Summing over all of the type j particles yields the total kerma.

$$K(E_n) = \sum_j K_j(E_n) \quad (2)$$

It is of interest to examine the kerma contribution of each particle type to the total kerma, as shown in Fig. PD-2-1. It can be seen that protons dominate. Ions of carbon, nitrogen and oxygen are second in importance up to 13 MeV above which their contribution is exceeded by that of alpha particles. However, alpha particles are of little importance until neutron energies of several MeV are reached. All other ions, including the e^+e^- pair, contribute

little to the total kerma.

W for charged particles

All of the available experimental data on W in TE gas for protons and for ions of helium, carbon, nitrogen, and oxygen are presented in Figs. PD-2-2 through -4. The uncertainty bars shown are the overall uncertainties given by the various experimentors. Where no bar is shown the uncertainty was comparable to or smaller than the plotting symbol, except for the data of Kemmochi (11) where no uncertainty was given.

Intercomparing the data and the uncertainties of the various experimentors it is apparent that in some cases the uncertainties are underestimated.

To arrive at an overall evaluation of W for each type of particle, least squares trial fits were made of the data to various functions. For protons and helium ions the functions which simulated the data best were accepted. For carbon, nitrogen and oxygen ions the functions were empirically adjusted until they were judged to give the fairest representation of the several sets of data available for each particle type. This was done in an attempt to remove the bias inherent in calculating a common curve fit for data sets of different numbers of measurements having different systematic errors. The results of these judgments are shown by the curves drawn in Figs. PD-2-2 through -4 and represented by the following empirical functions:

$$\text{Protons} \quad W_P = 29.28(\ln E)^{-2} + 29.99 \quad (3)$$

$$\text{Helium-4 ions} \quad W_\alpha = 49.96(\ln E)^{-0.2418} \quad (4)$$

$$\text{Carbon-12 ions} \quad W_C = 15.4 + 50.0(\ln E)^{-\frac{1}{2}} - 10.5(\ln E)^{-1} \quad (5)$$

$$\text{Nitrogen-14 ions} \quad W_N = 15.4 + 50.6(\ln E)^{-\frac{1}{2}} - 9.3(\ln E)^{-1} \quad (6)$$

$$\text{Oxygen-16 ions} \quad W_O = 15.4 + 53.5(\ln E)^{-\frac{1}{2}} - 8.8(\ln E)^{-1} \quad (7)$$

in which W is in eV and E is specific energy in keV per nucleon. Eqs. 5 to 7 apply for $E \geq 1.3$ keV per nucleon; for lower energies a linear extrapola-

tion in $\ln E$ was used.

It must be emphasized that these functions have been empirically derived to provide a reasonable compromise of the data sets; no physical significance is to be attached to the analytic forms of Eqs. (3) to (7). Each of the curves in Figs. PD-2-2 through -4 has been extended beyond the highest energy for which experimental W data are available. This liberal extrapolation is felt to be justified for protons and helium ions because the values of W at lower energies clearly indicate the trend of the data. For ions of carbon, nitrogen and oxygen the extrapolations to high specific energy shown in Fig. PD-2-4 are more speculative. However, it will be shown later that all ions except protons make only a minor contribution to W_n .

The proton data of Chemtob et al. (4) in Fig. PD-2-2 do not show an increase in W_p at low energies. The data of Leonard and Boring (7) and Sidenius (17), by contrast, shown an increase in W_p consistent with the expectation of rising W for protons with an energy less than about 25 keV corresponding to the electron velocity in the first Bohr orbit of hydrogen. The fitted curve was, therefore, made to follow at low energies the trend indicated by the latter two sets of data.

Calculation of W_n

For a given neutron energy, E_n , the computed data on the initial differential spectrum of charged particles of type j , $n_j(E, E_n)$, is combined with the W data for the type j particles, $W_j(E)$, to yield the number $N_j(E_n)$ of ion pairs produced by a fluence of one neutron per cm^2 in one gram of TE gas.

$$N_j(E_n) = \int_0^{E_{jm}} n_j(E, E_n) E \, dE / W_j(E) \quad (8)$$

Figure PD-2-2: W_p for methane-based TE gas.

Figure PD-2-3: w_{α} for methane-based TE gas.

Figure PD-2-4: W_C , W_N , and W_O for methane-based TE gas.

The value of W_n is calculated as the quotient of the sum of the kermas from all particle types by the sum of the numbers of ions's pairs produced by all particle types.

$$W_n(E_n) = \frac{\sum_j K_j(E_n)}{\sum_j N_j(E_n)} \quad (9)$$

The spectra $n_j(E, E_n)$ included protons, deuterons, and ions of He, Li, Be, B, C, N, and O. Since W_j was available only for the more important ions (^1H , ^4He , ^{12}C , ^{14}N , and ^{16}O), the following approximations were applied for the remaining ions: for deuterons the W for protons was used at the corresponding specific energy; for Be and B ions the W for C ions was used at the corresponding specific energy; the small number of Li ions produced by nuclear reactions with nitrogen are included with Be ions; although several isotopes of B ions are produced they are all included as ^{11}B ions. These approximations provided a means for including types of ions for which data on W are not available. These ions, however, contribute very little to the total kerma, as demonstrated in Fig. PD-2-1, and hence their effect on W_n is very small.

The results of the computations for W_n are shown in Fig. PD-2-5. For reference, and for use in ionization chamber calibrations, the right ordinate shows W_n normalized to W_e , the mean energy required by energetic electrons to create an ion pair in TE gas. $W_e = 29.3$ eV has been used. This is the average of measurements by Leonard and Boring (7) (29.2 eV) and by Waker and Booz (14) (29.4 eV).

Resonances and minimums in the energy transferred to carbon and oxygen produce the corresponding sharp changes in W_n evident in Fig. PD-2-5. The triangular data points shown on the graph are values of W_n evaluated at point energies of prominent resonances or minimums. These values are also listed in the third column of Table PD-2-2. They serve to indicate extreme limits

for W_n when the incident neutrons have an energy at a resonance or minimum rather than a spectrum spread out over at least one energy bin. The differences are 1.5% or less, except at 1.651 MeV where a difference of 2.6% was obtained.

Table PD-2-2

Comparisons of W_n obtained using initial charged particle spectra produced in the TE gas to W_n obtained using spectra produced in the A-150 plastic wall.

E_n /MeV	Condition ^a	W_n /eV		% Difference
		Gas Spectra	Wall Spectra	
0.445	R in O	33.03	32.25	-2.4
1.000	R in O	32.31	31.79	-1.6
1.315	R in O	31.80	31.57	-0.7
1.651	R in O	32.29	31.58	-2.2
2.350	M in O	31.02	31.26	0.8
2.944	R in C	31.44	31.74	0.9
3.764	R in O	31.95	31.74	-0.7
4.594	R in O	31.55	31.31	-0.8
5.120	R in O	31.56	31.17	-1.2
5.908	R in O	31.36	31.11	-0.8
6.295	R in C	31.36	31.61	0.8
7.750	R in C	31.46	31.65	0.6
10.25	M in C	31.04	31.00	-0.1
13.80	X in O	31.12	31.11	0

^aR, M and X denote respectively resonance, minimum and maximum.

Discussion of results

Fig. PD-2-5 shows an overall decrease in W_n of 5.5 percent as E_n increases from 0.1 to 5 MeV. Thereafter W_n is almost constant at 31.1 eV with only

Figure PD-2-5: W_n for methane-based TE gas. Solid line: histogram of energy-bin average W_n .
Triangles: values of W_n at point energies of resonances or minimums.

minor fluctuations of about 0.5%, and this is probably true for neutron energies somewhat higher than 20 MeV. The increase in W_n at low neutron energies is due to the pronounced rise in W_p (Fig. PD-2-2) at low proton energies. As E_n increases a greater fraction of the kerma is contributed by alpha particles and by ions of C, N, and O (Fig. PD-2-1) which generally have higher W than W_p . However, as E_n increases above 5 MeV the W of alpha particles and of ions of C, N, and O changes slowly and approaches W_p . This accounts for the flattening of W_n for $E_n > 5$ MeV. It is instructive to compare W_n to the values $W_n(P)$ which are obtained when only the secondary protons are considered, and to the values $W_n(P+\alpha)$ obtained when only secondary protons and alpha particles are considered. This is done in Fig. PD-2-6 where it can be seen that the computations for protons alone yields values which differ from W_n by only 2 or 3%, except at energy transfer resonances where the differences are still limited to 5%. Including alpha particles as well as protons in the computations has little effect on these differences. Thus, Fig. PD-2-6 demonstrates the importance of W_p in determining W_n .

The values of W_n in Fig. PD-2-5 apply strictly only when the initial spectra of secondary charged particles originate in the gas, that is, when there is no contribution from the ionization chamber walls. To set an upper limit to the effect on W_n that wall secondaries could have, W_n was computed at prominent resonance and minimum energies using initial charged particle spectra generated in A-150 plastic. The results are compared in Table PD-2-2. The differences obtained are small and would be even smaller at neutron energies away from resonances or minimums, or when a spectrum of energies is considered. The data of Fig. PD-2-5, therefore, can be used with little error even when wall secondaries contribute significantly to cavity ionization, that is, for high neutron energies or small gas cavities.

Figure PD-2-6: $W_n(P)$ and $W_n(P+\alpha)$ relative to W_n for methane-based TE gas.

The overall variation of W_n with E_n and the resonance fluctuations require that W_n be evaluated for each neutron spectrum for which accurate dosimetry is required. This may be done by an appropriate weighting procedure using the W_n data of Fig. PD-2-5. ICRU Report 26 (1) recommends $W_n/W_e = 1.05 \pm 0.05$ overall uncertainty. Based on the present computations, a single value of $W_n = 31.9 \pm 0.9$ eV for applications not requiring the best accuracy or when neutron spectra are poorly known is recommended. This results in $W_n/W_e = 1.09 \pm 0.03$.

Table PD-2-3 shows at selected neutron energies comparisons of W_n computed in this report with values derived in earlier studies. The data of Goodman (2) are all lower but differ by less than 1%, except at the lowest energy. The computations of Bewley (15) were based on much less input data than were available for this report. His values for W_n also were all lower but by no more than 1.7%. The greatest differences evident are with the work of Dennis (16) whose computations for energies below 3 MeV were higher by up to 6.9%, and above 3 MeV were lower by as much as 2.7%. This is a consequence of the inaccurate function he used for W_p which was based on the meager information available at that time.

It is difficult to assess rigorously the uncertainty of the calculated values of W_n since this involves uncertainties in the neutron cross sections and energetics used to compute the initial spectra of charged particles and uncertainties in the evaluations of W for these particles. However, it has been demonstrated that protons dominate the calculations for W_n , and the overall uncertainties for hydrogen cross sections and energetics should be less than 2% and usually less than 1%. For the proton data of Fig. PD-2-2 overall uncertainties in W_p of 2% for $E_n > 20$ keV and 5% for $E_n < 20$ keV are estimated. Because both the kerma and the number of ion pairs produced

Table PD-2-3

Comparisons of W_n values

E_n/MeV	W_n/eV <u>This report</u>	<u>Percent difference from W_n in this report</u>		
		<u>Goodman(2)</u>	<u>Bewley(15)</u>	<u>Dennis(16)</u>
0.10	32.78	-1.2	-	-
0.30	32.15	-0.8	-1.1	6.4
0.45	32.52	-0.1	-0.1	-
0.49	31.91	-0.3	-	6.9
0.60	31.74	-0.4	-1.7	-
0.63	31.72	-0.4	-	6.6
1.00	32.09	-0.3	-0.6	6.0
1.20	31.60	-0.6	-1.6	-
1.34	31.58	-0.6	-	5.8
2.00	31.30	-0.3	-1.6	-
2.21	31.13	-0.1	-	3.1
3.64	31.70	-0.3	-	-0.6
4.00	31.38	-0.3	-0.9	-
6.00	31.10	-0.6	-	-1.3
9.90	31.12	-0.7	-	-2.3
14.00	31.14	-0.1	-0.1	-2.7
19.00	31.02	-0.1	-	-

are weighted by energy, the larger uncertainty for low proton energies has little effect on W_n at least down to the lowest neutron energy (100 keV) considered here. In sum, an overall uncertainty for the computations of W_n of 3% is estimated.

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PD-3 The W-value for electrons and photons in tissue-equivalent gases

Dusan Srdoc

Tissue-equivalent gas mixtures have been extensively in use in dosimetry for more than 20 years, yet the most important physical property of these gas mixtures for radiation measurement - the energy required to form an ion pair - has never been measured precisely. Several scattered data in literature (Booz 1967, 1975, 1978; Leonard and Boring 1973; Glenn 1974) on the W-value for electrons prove the above statement: The published W-values for the same gas mixture are not consistent (Booz), or the experimental method has not been described precisely enough to allow critical assessment of the published data (Leonard and Boring, Glenn). A more satisfactory situation exists in measurement of the W-value for protons where the energy per ion pair has been centered around 30.5 eV for 1 MeV protons (Rohrig and Colvett 1978).

The reason for the existing situation could be only guessed. It is very likely that the researchers of Jesse's era were more attracted by monoatomic gases with the prospective of theoretical calculations of W-values. Modern physicists are reluctant to repeat "old fashioned" experiments using DC ionization chambers and unpredictable gas multiplication counters.

Whereas counting of protons or alpha particles entering a gas filled chamber is relatively easy thanks to ample ionization and modern low noise preamplifiers, precise counting of total number of electrons and measuring of total charge may present an experimental problem. For instance, in case of slow electrons ($E < 10$ keV) one is dealing with charges per particle comparable to the noise RMS deviation of modern commercially available FET preamplifiers. Gas multiplication of primary charges is unavoidable to bring the signal above the preamplifier noise. A different problem arises when dealing with fast electrons. Here a large volume,

high pressure chamber is required and charge collection is hampered by traces of electronegative impurities and slow signal rise time. In spite of these difficulties, numerous and valuable data on W for fast electrons have been collected in 1950 as presented in the review articles by Valentine (1958) and White (1963). However, the W-values for slow electrons as well as the W-values for special gas mixtures such as tissue-equivalent gas remained unknown for a long time.

Another experimental approach has been attempted by the author in the late 1960's (Srdoc 1969) with modest success. The basic idea was to measure precisely the so-called single electron spectrum using a proportional counter. Calculation of the first moment of the pulse height distribution (i.e., the mean pulse height $\bar{P}$) enables one to calibrate the measured spectra in terms of number of electrons per pulse. The energy of the incident radiation such as slow electrons or mono-energetic soft X-rays is known from an independent source. Knowing the energy of incident particles or photons and measuring the mean number of electrons they produce in a gas enables one to calculate the W straightforwardly.

Early measurements lacked precision in calculation of the mean pulse height of a spectrum, especially in case of single electrons. Various sources of errors will be discussed in the following paragraphs in details. It is sufficient to say that a complete knowledge of processes governing ionization and charge multiplication in gases is required in order to process the measured spectra adequately.

First attempts to exploit the new experimental method (Srdoc 1969) did not result in the W-values for tissue-equivalent gases; instead, they showed that the W for low energy photons in many gases including the tissue-equivalent mixtures is relatively insensitive to the photon energy in the range of 0.3 through 6 KeV. The conclusion was that the W for photons for methane and propane based tissue-equivalent mixtures; the frequently used "stock item" gas mixture Ar + 10% CH₄ and for propane was constant down to 0.3 KeV within the limits of accuracy of the

measurement which was estimated at $\pm 3\%$.

At the same time Booz (1967) published the W-value ($W_{\infty} = 31.3 \pm 0.7$ eV) for methane based tissue-equivalent mixture using essentially the same technique. As judged on the basis of recently compiled data and the data from this report, both statements (the absolute value and the dependence on the photon energy) were correct within 1 or 2 σ .

In 1973 the author published more complete data on energy dependence of W for photons in propane. The experimental technique and the data processing have been described in some detail. The energy range has been extended down to 85 eV revealing an increase of the W with decreasing photon energy. The following years brought several new experimental data. Leonard and Boring (1973) published a paper on W for heavy ions in tissue-equivalent gas putting the W for 250 KVp x-rays at 30.2 ± 0.7 eV and that for ^{60}Co gamma rays at 29.2 ± 0.6 eV. The emphasis in their paper has been on the W for heavy ions and the description of experimental procedure to measure the W for x and gamma rays is missing. Glenn (1974) reported measurement of W for ^{60}Co gamma rays (29.5 eV) and neutrons (31.0 eV) and was quoted by Bichsel (1975). No numerical values or experimental details are available in the published abstract. Waker and Booz (1975) reported measurement of W for electrons using a different technique. They arrived at a peculiar dependency of W on electron energy which was later revoked (Smith and Booz 1978). Table 1 shows the W values for electrons and x rays in methane and propane based tissue-equivalent gas including data from this paper.

Experimental

The experimental technique follows closely that described in a previous paper (Srdoc 1973). However, several components of the experimental system have been modified and perfected resulting in a high gain, low noise pulse height analyzing system with long term overall stability. The preamplifier noise has

Table 1

Authors	Radiation	Energy (KeV)	W(eV)		W(eV)		Comment
			CH ₄	TE gas	C ₃ H ₈	TE gas	
Booz (1968)	photons	10	31.3 ± 0.7				Proportional counter technique
		1.5	31.5 ± 0.5				
		0.3	34.4 ± 1.6				
Leonard and Boring (1973)	⁶⁰ Co gamma rays x-rays	MeV					Ionization chamber technique
		1.1	32.2 ± 0.7				
		1.3					
Waker and Booz (1975)	electrons	250 KV peak	29.2 ± 0.6				Data taken from graph ref. Waker, Booz. 1975. Ionizat. chamber technique
		6	30				
		1.2	32.5				
Glenn (1974) See also Bichsel 1975)	⁶⁰ Co gamma rays	0.26	36				Ionization chamber technique
		MeV					
		1.1	29.5 ± ?				
Smith and Booz (1978)	electrons	1.3					Ionization chamber technique
		2.0	28.3 ± 1.5				
		1.5	28.2 ± 1.5				
		0.30	34.0 ± 2.6				
		0.25	35.8 ± 2.3				
		0.10	41.8 ± 2.4				
Srdoc this paper	photons	0.03	61 ± 9				Proportional counter technique
		5.9	30.7 ± 0.3		27.3 ± 0.3		
		1.49	31.3 ± 0.3		27.8 ± 0.3		
	electrons	0.277	32.3 ± 0.3		28.8 ± 0.3		
		5.6	30.7 ± 0.3		27.3 ± 0.3		
		1.2	31.3 ± 0.4		27.9 ± 0.4		
		0.25	34.8 ± 0.5		30.4 ± 0.5		

been reduced close to the lowest practically obtained limits ($\sim 60 e^-$ RMS deviation) without cooling the FET input circuit. This achievement was crucial for the measurement of single electron spectra, making it possible to determine exactly the entire shape of the spectrum. Gas flow system and pressure reading have been substantially improved by replacing the old Manostat pressure regulating device with Granville-Philips gas flow regulator and the MKS Baratron head. The processing of data has been completely computerized.

A typical set of experimental data has been taken in the following way: After prolonged pumping the whole system consisting of gas lines, gas flow regulating device and the counter was flushed overnight with the investigated gas at the chosen pressure. This procedure was essential to reach a steady state, which could be checked by frequent measurement of the ^{55}Fe test spectrum. Generally, the drift of the ^{55}Fe peak due to changes of gas and electronic gains did not exceed 1% per day. After checking for a constant overall gain, four spectra were usually taken: The single electron spectrum, Carbon and Aluminum x-ray spectra and the ^{55}Fe spectrum. Details on the single electron source, the soft x-ray sources and radioisotope source preparation are described in a paper by the author and Clark (1970).

The data were stored on magnetic tapes for further processing. A computer program was available to make possible the following operations: fitting of the Gamma function to the experimental data, calculation of the mean value of the pulse height distribution and its standard deviation and several other practical features like normalization of spectra, plotting etc. Fitting of a Gamma function of the general shape $f(x) = Ax^B e^{-Cx}$ to the experimental data was required to extrapolate the measured spectrum toward low and high energy "tails". Namely, the low energy tail is always overlapped by the electronic noise and/or small pulses originated from truncated tracks, neighboring peak etc., whereas the

high energy tail is cut off because of the finite dynamic range of the ADC unit of the pulse height analyzer. The choice of the Gamma function for fitting the data was based on the comprehensive study of the proportional counter response to single electrons and monoenergetic soft x-ray radiation (Marino and Srdoc 1977 and Srdoc, this Annual Report). The error in calculation of the mean value of a spectrum can amount from ± 5 up to $\pm 10\%$ depending on the photon energy, if the spectrum is not properly extrapolated. It was this fact which so far prevented the accurate calculation of W-values, since the error in calculation of the mean values propagates directly into the calculation of the W.

Results

Two tissue-equivalent gas mixtures were investigated: Methane based mixture containing 64.4% CH_4 , 32.4% CO_2 and 3.2% N_2 , and propane based mixture containing 55% C_3H_8 , 39.6% CO_2 and 5.4% N_2 . The single electron, soft x-rays and ^{55}Fe spectra were taken repeatedly in order to estimate the reproducibility of the measurement.

An almost ideal agreement between the measured points and the fitted Gamma function is obtained in case of the single electron spectrum in propane based tissue-equivalent gas. Not so ideal are fittings to the Carbon and Aluminum spectra. As expected, a Gamma function will not fit perfectly to a Carbon (or Al) spectrum because such spectra are composed of several superimposed Gamma functions as explained in detail in the report of the author and Marino (1977). The error thus introduced has been estimated to contribute less than 0.5% to the mean value of a spectrum because of a general similarity between the simple and composed functions.

The W-values have been calculated using the following expression:

$$W(E) = \frac{E}{\bar{X}} \frac{G_{SE}}{G_X} \overline{SE} \quad (1)$$

where $W(E)$ is the W-value at the energy E of the source of radiation; $\bar{X}$ is the

mean channel of the pulse height spectrum after extrapolation of tails, G_{SE}/G_X is the ratio of electronic gains at which the single electron spectrum and the radiation spectrum have been taken and $\overline{SE}$ is the mean of the single electron spectrum, also after extrapolation.

A detailed knowledge of ionization processes in gases would allow an exact calculation of the W for electrons from W values for photons. Even though these processes are not known exactly in a tissue-equivalent mixture of gases, some general conclusion could be drawn from the well-studied processes in constituents of these mixtures (CH_4 , CO_2 , N_2). Hamrin et al (1968) studied ionization processes in methane exposed to Mg and Al K photons. More data were collected under leadership of K. Siegbahn (1969) and published in his book on Electron Spectroscopy for Chemical Analysis (ESCA). The aim of these measurements was primarily to determine the orbital energy levels in atoms and molecules; however, in most cases the published experimental data furnish enough information to get a quantitative insight into processes which take place under irradiation. Thus, it is evident from works of Hamrin et al., (1968) that over 95% of interactions of Al-K photons with methane molecules result in expelling the core electrons and the rest are valence electrons. The excited methane molecule returns to its ground state through radiationless Auger process. This process and its efficiency have also been measured in methane (Carlson 1975). From this quantitative picture an attempt was made to calculate the W for electrons. The basis for calculation was the well-defined photoelectron energy and the high efficiency of photoionization, the Auger spectrum and the measured spectra of Al and C K-line photons. The Eq 1 has been modified in the following way:

$$W_e(E) = \frac{E_e \cdot G_{SE}}{(\overline{x} - \overline{y})G_X} \overline{SE} \quad (2)$$

where E_e is the photoelectron energy equal to $E - K$ and $\overline{y}$ is the calculated mean

pulse height due to the Auger electrons. The $W(E)$ for Auger electrons is derived from the Carbon K-line measurement. The above calculation is based on several assumptions such as the similarity of behavior of pure gases and mixture of gases. Yet the results are consistent in terms of a gradual increase of the W with decreasing particle energy. Moreover, an analysis of the dependence of $W(E)$ on electron energy for a large number of gases and wider span of energies which were investigated together with the tissue-equivalent gas mixtures showed a systematic similarity in that dependence. All investigated polyatomic gases (as opposed to noble gas mixtures) obeyed the relationship of the form.

$$W(E) = \frac{W_{\infty}}{1 - \frac{K}{E}} \quad (3)$$

which has been experimentally tested with various degrees of success (Booz 1968, Srdoc 1973). Interestingly enough, the constant K in Eq. 3 has been numerically close to the W_{∞} for each gas. It should be emphasized, though, that the error bars on scattered data points are usually large enough to accommodate a variety of theoretical, semiempirical and empirical relationships between the $W(E)$ and E . On the other hand, solutions of Eq. 3 are very often sought for practical purposes (e.g., neutron dosimetry). Therefore, a relation which satisfies a large number of experimentally tested gases is worth investigating further.

An exact solution of the relation $W(E) = f(E)$ is beyond the reach considering the available experimental data. An approximate and oversimplified scheme leads eventually to the observed dependency of $W(E)$ on energy.

Consider a particle having kinetic energy E and producing a total of $N(E)$ ion pairs along the path in a gas. On the basis of the generally accepted $1/E$ law for fast electron losses and the Fowler equation it is possible to show that the number of ion pairs is proportional to the incident energy of the electron in the first approximation (Inokuti 1975). The Fowler equation can be solved

for relatively high energy particles ($E \gg I$), neglecting the events at the end of the particle travel. Here a point is reached where the particle loses its ionization capability. Taking into account the stochastic processes it seems that the mean energy at which the particle stops producing ion pairs is close to the ionization potential I , although not necessarily exactly I , as supposed by Herring and Merzbacher (1957). It is more likely that a particle near its end-point still shares its energy losses between excitations and ionizations in a similar way as it does along the whole path. If this assumption holds, the mean of non-ionizing energy losses $\overline{\Delta E}$ is closer to W_∞ rather than to I . The collected experimental data on CH_4 and other aliphatic hydrocarbons, C_2H_4 , CO_2 and tissue-equivalent gas mixtures support the above reasoning.

A weak non-linear dependence of the exact relationship $N(E) = f(E)$ is difficult to check experimentally at energies exceeding several KeV. However, below ~ 1 KeV the mean energy loss $\overline{\Delta E}$ of the final step of ionization and excitation sequences becomes perceptible. It should become a significant part of the total energy loss as the incident particle energy approaches the W_∞ value. This fact can be written as follows:

$$N(E) = K(E - \overline{\Delta E}) \quad (4)$$

Next, by definition $W(E) = E/N(E)$, and Eq. 4 becomes

$$W(E) = \frac{1}{K} \frac{E}{(E - \overline{\Delta E})} \quad (5)$$

The mean energy $\overline{\Delta E}$ lost for ionization is of the order of a few tens eV or less.

Therefore, for energetic particles having $E \gg \overline{\Delta E}$ one can write

$$W_\infty = \frac{1}{K} \quad (6)$$

and Eq. 5 becomes

$$W(E) = \frac{W_\infty \cdot E}{E - \overline{\Delta E}} \quad \text{or}$$

$$W(E) = \frac{W_{\infty}}{1 - \overline{\Delta E}/E} \quad (7)$$

which is identical with Eq. 3.

The mean "subionization" energy loss $\overline{\Delta E}$ has been calculated using Eq. 7 and the experimentally obtained data on $W(E)$ in six hydrocarbons ($C_nH_{2n} + 2$; $n = 1 \dots 6$) ethylene, and two tissue-equivalent mixtures. Even though the inherent error of this type of measurement is relatively large, the $\overline{\Delta E}$ values are strikingly close to the W_{∞} values as shown in Table 2. If we replace $\overline{\Delta E}$ by W_{∞} in Eq. 7 for convenience, a simple and practical relation is obtained:

$$W(E) = \frac{E - W_{\infty}}{E - W_{\infty}}$$

which holds for all gases listed in Table 2. It should be pointed out that $\overline{\Delta E}$ has the dimension of energy and W_{∞} is only numerically identical with $\overline{\Delta E}$. For any other application the more rigorous Eq. 7 should be used.

The W -value for photons has been calculated in a straight-forward manner using Eq. 1. The dependence of the W on the photon energy is far less pronounced than for electrons. This can be explained - at least qualitatively - by the contribution of Auger electrons to the total ionization. A photon causing photoionization leaves the atom excited; since we are dealing with low Z elements, the Auger process is dominant. The Auger electrons compensate for $\overline{\Delta E}$ subionization losses-hence a lower W and its less pronounced dependence on E . In addition, the photoelectron itself (or an incident electron) coming to rest represents a substantial fraction of total charge per event, if the total charge consists of ten ion pairs (Carbon x-ray) or even 3 to 4 ion pairs (Beryllium x-ray) as explained in details in a previous work (Srdoc and Obelic, 1975). The measured W_p values for two types of tissue-equivalent gases are listed in Table 1.

Following the same reasoning as for electrons (Eq. 4) one arrives at the

following expression for the dependence of $N_p(E)$ on photon energy:

$$N_p(E) = K(E_p - \overline{\Delta E} + A) \quad (8)$$

where A represents the contribution due to Auger process and/or the thermalized photoelectron. The subscript p denotes relevancy to photons. As opposed to the $N(E)$ function for electrons (Eq. 4), the term in brackets cannot be easily calculated because of the unknown contribution due to Auger electrons. Moreover, this contribution is energy dependent as opposed to the $\overline{\Delta E}$. For instance, more energetic Al-K $_{\alpha}$ photons will create vacancies in Carbon shells (CH₄, CO₂ in tissue-equivalent gases) which will, in turn produce Auger electrons, whereas C-K $_{\alpha}$ photons ($E = 277$ eV) are below the Carbon K-line threshold ($E = 283$ eV) and consequently will not produce Auger electrons. This fact is reflected in the attempt to calculate the constant K (Eq. 3) for photons: It is evident that the "constant" K assumes various values, depending on the photon energy (Table 2). Therefore, Eq. 3 cannot be used for evaluating the $W_p(E)$.

For practical purposes an empirical relation has been found describing the dependency of W_p on E_p fairly accurately. The constants K listed in Table 2 are remarkably close to the $E_p^{1/2}$. If the constant K is replaced by $aE_p^{1/2}$ one obtains:

$$W_p(E_p) = \frac{W_{\infty}}{1 - aE_p^{-1/2}} \quad (9)$$

The new constant a is close to unity and serves - besides small corrections for different gases - to keep the Eq. 9 dimensionally correct. There is no theoretical explanation for such behavior of W_p and Eq. 9 is purely empirical, valid down to the lowest experimental point which was the Beryllium K-line ($E = 108$ eV).

Conclusion

The W-value for electrons and photons has been measured in methane and propane based tissue-equivalent gas mixtures using the proportional counter technique. The results agree within statistical limits with some of the published values.

Table 2

Tissue-equivalent gas mixture	Electron energy (KeV)	K(eV) Eq. 3	Photon energy (KeV)	K(eV) Eq. 3
64.4%CH ₄ ; 32.4%CO ₂ ; 3.2%N ₂	5.6	29.1	5.9	—
	1.2	30.9	1.49	30.7
	W = 30.5 ± 0.3	0.26	32.3	0.277 15.4
55%C ₃ H ₈ ; 39.6%CO ₂ ; 5.4%N ₂	5.6	—	5.9	—
	1.2	30.3	1.49	34.9
	W = 27.2 ± 0.3	0.26	28.2	0.277 15.4
Other gases				
CH ₄	5.6	—	5.9	—
	1.2	28.1	1.49	40.4
	W = 27.3 ± 0.3	0.26	30.6	0.277 15.8
C ₃ H ₈	5.6	—	5.9	—
	1.2	28.7	1.49	42.3
	W = 24.6 ± 0.3	0.26	24.6	0.277 15.7

It is believed that the standard deviation has been narrowed to ± 0.3 eV for W_{∞} which is:

$$W_{\infty} = 30.5 \pm 0.3 \text{ eV/ion pair}$$

for methane based tissue-equivalent mixture and

$$W_{\infty} = 27.2 \pm 0.3 \text{ eV/ion pair}$$

for propane based tissue-equivalent mixture.

The measurements of energy dependence of W resulted in practical formulae describing the $W(E)$ as a function of incident electron or photon energy.

Acknowledgement

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PD-4 Radial energy deposition profile of proton tracks

R.E. Mills

Measurements of the energy deposition distribution around a proton beam, described in previous Annual Reports (C00-3243-5 and -6, C00-4733-1), were continued using the ABC chamber. The three dimensional translator was used to position the chamber center on the proton beam axis at distances from about 4 to 60 cm from the downstream collimating aperture, allowing virtually scatter-free measurements with gas pressures in excess of 100 torr. An experiment was performed to determine the variation of central fraction (the ratio of ionization collected from the inner cylindrical volume to that collected in the entire cylindrical chamber volume) as a function of axial position along the beam axis, holding the incident proton energy and the tank gas pressure constant. This was done for 3.5 MeV protons utilizing gas pressures from 0.2 to 100 torr and for 1.0 MeV protons with pressures from 0.1 to 9.1 torr, thus nearly spanning the range of capability of the RARAF Van de Graaff accelerator.

The expected outcome of this work was to find a range of aperture distances over which the central fraction was constant; at large distances scattering of protons by the gas causes a reduction, and at very small distances the lack of delta-ray equilibrium should result in an increase in central fraction. Thus the derivative of central fraction with respect to distance should never be positive and should be essentially zero over some "plateau" region. The effect of gas scattering was found to be as expected, but a small positive slope was found for small distances. That the effect of nonequilibrium is difficult to detect is a result of the fact that very

few of the delta rays have an energy near the maximum (determined from collision dynamics to be about $1/460$ of the primary proton energy). The slight increase of central fraction with aperture distance is probably the result of slowly decreasing proton energy due to interactions with the gas molecules. It was decided to make subsequent measurements with the chamber positioned near the minimum distance from the aperture to minimize the change of proton energy as a function of tank gas pressure.

All previous central fraction measurements were repeated using the new translator to assure data unaffected by scattered radiation and to have the best conditions of equilibrium and energy determination. Several new data series were also taken, extending the range of incident proton energy from 0.5 to 4.0 MeV, in steps of 0.5 MeV.

In order to compare the results of these measurements to theoretical models of energy deposition, it is necessary to distinguish between the measured central fraction discussed above and the integral distribution function of energy deposited. These two functions are indistinguishable except at low pressures, when the simulated tissue radius of the total chamber volume is smaller than the maximum radial delta-ray range. Since the integral distribution function is the ratio of energy deposited within the inner chamber radius to the total energy deposited, and some energy may be deposited at distances greater than the outer chamber radius and, therefore, not detected, this function may be smaller than the measured central fraction. At high pressures the simulated chamber radius is larger than the penumbral radius, and the two ratios are equal.

It is possible to derive the integral distribution function from central fraction data as follows: Let the ratio of the outer chamber radius to the inner radius be k , a constant. Then

$$C(R) = \frac{I(R)}{I(kR)}$$

by definition, where $C(R)$ is the central fraction for a simulated inner radius R , and $I(R)$ and $I(kR)$ are the ionizations produced within the inner and outer chamber radii, respectively. But since

$$F(R) = \frac{I(R)}{I(\infty)}$$

where $F(R)$ is the integral distribution defined above, we have

$$C(R) = \frac{I(R)/I(\infty)}{I(kR)/I(\infty)} = \frac{F(R)}{F(kR)}$$

or

$$F(R) = C(R)F(kR)$$

By the same reasoning, it can be shown that

$$F(kR) = C(kR)F(k^2R),$$

etc., so

$$F(R) = C(R) C(kR) C(k^2R) \dots$$

Thus if a functional relationship between $C(R)$ and R can be defined (by curve fitting or interpolation of the measured data), $F(R)$ can be calculated. Since $F(R) = C(R) = 1$ for very large R (greater than the penumbral radius), the above

product must only be carried out to sufficiently many terms that all further factors are unity.

Data for 2.5 MeV protons are shown in Fig. PD-4-1, in which triangles represent measured central fractions and diamonds represent the same data corrected as above to give points along the integral distribution. For this purpose, a value of 4.5 (equal to the ratio of mechanical sizes) was assumed for k . The solid line is a theoretical determination of the distribution function (1), which should exhibit a linear relationship to $\ln(R)$ over some range of R . Figure PD-4-2 shows a composite of all the data obtained to date with each curve comprised of line segments whose ends are central fraction data corrected to represent integral distribution functions for each proton energy.

A difficulty remains, however, in the assignment of a value for k , the ratio of chamber radii. The lack of perfect linearity in the chamber's collecting potential field resulting from the location of guard electrodes can be expected to reduce the collecting volume of the chamber at its outer radius, thus lowering the effective value of k . Examination of the values of ionization current produced in the various chamber regions supports this prediction.

In order to determine the effective ratio of radii for the chamber, an experiment was performed using a 50 mg ^{226}Ra isotopic source located inside the tank along the (extended) chamber axis. The chamber was placed at varying distances from the source in order to separate the effects of scattered gamma rays from direct, and corrections were also made for deviations from inverse square response for an extended detector. In general, the effective values of k calculated from the results of this analysis were smaller than the mechanical ratio of chamber sizes, but it was found that this calculated k varied inversely with tank pressure. It is believed that this variation with pressure results from the changing range of photoelectrons produced in the aluminum support rings

Figure PD-4-1: Variation of integrated energy deposited with effective tissue radius. Triangles represent measured central fraction data, and diamonds are the same points corrected to represent the calculated integral distribution function of energy with respect to radius. The solid line represents a theoretical determination of the distribution function (see text).

Figure PD-4-2: Composite of integral distribution functions calculated from data of all proton energies studied. Each curve is comprised of line segments from one calculated point to the next for a particular proton energy. Energies vary from 0.5 to 4.0 MeV (left to right) in increments of 0.5 MeV.

around the chamber periphery. Further analysis will be required to determine the effective value of k , which is required for distribution function calculation.

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Dusan. Srdoc

Measurements of pulse height spectra of very low energy radiation in a proportional counter filled with a polyatomic gas or mixture of gases proved to be an ample source of valuable information on gas properties as well as on some basic physical processes. For example, it is possible to calculate the energy required to form an ion pair in a gas (1), to study the gas multiplication phenomena, to calculate the probability distribution function of primary ionization charges, etc. This report also deals with a basic physical property of gases when irradiated with low energy radiation: The Fano factor which determines the width of the primary ionization charge distribution.

Consider a small cavity exposed to radiation. If the track length of the ionizing particle is much longer than the cavity diameter, the distribution of track segments, or more adequately the distribution of the number of ion pairs per crossing is relatively broad due to several interplaying factors as explained by Kellerer (2) and often measured experimentally in a typical $P(Y)$ vs Y spectrum in microdosimetry. A small fraction of particles which originate or stop in the cavity does not change the shape of a $P(Y)$ vs Y spectrum appreciably. However, if all particles start in the cavity and deposit their entire energy inside the cavity, a completely different picture is obtained. Many features characteristic for straggling theory are absent and the distribution of ion pairs is much narrower. The theory which explains the observed fact has been worked out by Fano (3). He came to the conclusion that the fluctuations of charges produced by monoenergetic particles which are completely absorbed in the medium can be described as follows:

$$V = F\bar{N} \quad (1)$$

V is the variance of the distribution of charges, $\bar{N}$ is the mean number of charges

and F , generally referred to as the Fano factor is a constant typical of each medium. Fano calculated theoretically that F in gases should lie between 0.3 and 0.5, which was confirmed experimentally (4), even though values as low as 0.055 have been reported for special gas mixtures (5).

The method of measurement

The principles of measurement of the Fano factor using a proportional counter can be described as follows. Any pulse height spectrum obtained by measuring a source of monoenergetic particles by means of a proportional counter is broadened by a specific amount due to multiplication of charges in the gas. Since the processes of primary ionization and the subsequent multiplication are independent, one can characterize the resultant distribution as the sum of the variances of each separate process:

$$V_{\text{total}} = V_{\text{ionization}} + V_{\text{multiplication}} \quad (2)$$

To calculate V_i due to ionization it is necessary to know exactly the contribution of the gas multiplication to the spread of the measured spectrum. A detailed calculation of the variance of pulse height spectra in a proportional counter is presented elsewhere by Marino and author (6).

The relative variance due to gas multiplication according to the quoted reference is:

$$V_{\text{rel}} \cong (\bar{N}k)^{-1} \quad (3)$$

where $\bar{N}$ is the mean number of ion pairs per event and k^{-1} is the relative variance of the single electron spectrum. The single electron spectra have been meticulously measured in order to determine the variance. It was found that in tissue-equivalent gas mixtures as well as in all investigated polyatomic gases the relative variance k^{-1} is equal to $\approx 0.64 \pm 0.03$; the relative standard deviation $k^{-1/2}$ which is convenient for calculations is equal to $0.80^{+0.05}_{-0.02}$. Table 1 displays several statistical properties of the single and multiple electron spectra.

Table 1

Single electron spectrum; Best fit to the experimental points			Multiple (N) electron spectrum
$p(x) = \frac{1}{\Gamma k} x^{k-1} e^{-x}$			$p(x,N) = \frac{1}{\Gamma_{Nk}} x^{Nk-1} e^{-x}$
		Experimental values	
Relative standard deviation	$k^{-1/2}$	$0.8 \begin{matrix} + 0.05 \\ - 0.01 \end{matrix}$	$(Nk)^{-1/2}$
Relative variance	k^{-1}	$0.64 \begin{matrix} + 0.07 \\ - 0.015 \end{matrix}$	$(Nk)^{-1}$

Having the numerical data on the variance for experimentally measured spectra and the single electron spectra at hand, it has been possible to calculate the Fano factor by using the Eqs. 1, 2 and 3 in the following way:

$$V_{\text{rel., exp}} = \frac{F}{\bar{N}} + \frac{1}{\bar{N}K} \quad (4a)$$

$$F = \bar{N} V_{\text{rel., exp}} - \frac{1}{\bar{N}} \quad (4b)$$

or, by inserting the frequently used relative standard deviation $\sigma_r = V_r^{1/2}$ one obtains:

$$F = \frac{\sigma_{r, \text{exp}}^2}{\bar{N}} - \sigma_{r, \text{SE}}^2 \quad (4c)$$

Here $\sigma_{r, \text{exp}}^2$ denotes the squared relative standard deviation of the experimental spectrum, $\bar{N}$ is the mean number of ion pairs per event and $\sigma_{r, \text{SE}}^2$ is the squared relative standard deviation of the single electron spectrum. Eq. 4c can be made more practical by inserting $\bar{N} = E/W(E)$:

$$F = \frac{\sigma_{r, \text{exp}}^2}{E} W(E) - \sigma_{r, \text{SE}}^2 \quad (5)$$

Here E denotes the energy of the incident particle totally absorbed in the investigated medium and $W(E)$ is the energy per ion pair at that initial particle energy.

Results

The results of measurements of Fano factors in methane, methane based tissue-equivalent mixture, propane and propane based tissue-equivalent mixture are presented in Table 2. A comparison of the Fano factors of two gases and two gas mixtures listed in Table 2 shows that the experimentally determined Fano factors are very close to the theoretically calculated values. The relatively low spread of Fano factors for various gases and gas mixtures indicate similarity in ionization patterns in these media.

Table 2

Gas medium	Single el. spectrum σ rel	Electron energy: 260 eV		Electron energy: 1200 eV	
		W(E)/eV	Fano factor	W(E)/eV	Fano factor
Methane CH ₄	0.844 $\pm$ 0.005	30.9 $\pm$ 0.3	0.30 $\pm$ 0.03	28.4 $\pm$ 0.3	0.29 $\pm$ 0.03
CH ₄ - based TE gas	0.847 $\pm$ 0.005	34.8 $\pm$ 0.3	0.36 $\pm$ 0.05	31.8 $\pm$ 0.3	0.37 $\pm$ 0.05
Propane C ₃ H ₈	0.80 $\pm$ 0.005	27.7 $\pm$ 0.3	0.28 $\pm$ 0.03	25.7 $\pm$ 0.3	0.29 $\pm$ 0.03
C ₃ H ₈ - based TE gas	0.83 $\pm$ 0.005	30.5 $\pm$ 0.3	0.30 $\pm$ 0.03	27.9 $\pm$ 0.3	0.32 $\pm$ 0.03

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PD-6 Neutron dosimetry for rat irradiations

R.E. Mills

Monoenergetic neutron irradiations of rats described in last year's Annual Report (C00-4733-1) were continued in collaboration with the Department of Radiology of Thomas Jefferson University. Three groups of 24 rats received absorbed doses of 0.43 MeV neutrons corresponding to 300,400, or 560 mGy (30, 40, or 56 rad), respectively, of total tissue kerma in free air at the center of the animal positions. In all cases the absorbed dose contributed by gamma rays was about 3 percent of the total absorbed dose.

In order to alleviate some of the traumatic effects of the experimental procedure upon the pregnant rats, extra air holes were drilled in the aluminum end caps of the rat holders, thus providing increased ventilation for respiration and cooling. It was also found to be desirable to postpone transporting them for one day after irradiation.

B) MICRODOSIMETRY

PM-1 The response of the proportional counter to unit charge

Dusan Srdoc

The stochastic nature of charge multiplication in gaseous detectors as well as in the photomultipliers has attracted a continuing attention of researchers (1...5). The exact knowledge of processes of charge multiplication could lead to the probability density functions resulting from multiplication of charges. A theoretical study (6) showed that the response of a detector to unit charge - the so called single electron spectrum plays the decisive role in any attempt to calculate probability density functions for multiple charges, such as the low LET spectra in microdosimetry or low energy spectra in nuclear and x-ray spectroscopy. Therefore, attention has been concentrated on the experimental measurement of the shape of the single electron spectrum obtained by means of the proportional counter.

Experimental

The experimental technique follows in principle that already described in previous papers (7,8). However, recent development in instrumentation technique, particularly in electronics, gas pressure and flow regulating devices and computer processing of data enabled us to measure more accurately pulse height distributions and to expand the dynamic range of the analyzing system toward low energy events, which was crucial in determination of the shape of single electron spectra.

More than one hundred single electron spectra have been measured and analyzed using a dozen gases and gas mixtures for counter filling. Each measurement included a careful energy calibration of the counter using the Al K-line and an ^{55}Fe source as energy standards. Simultaneously, the counter resolution indicated the gas quality and overall reliability of the measuring system. Depending on the type of gas filling, the counter resolution was better than 30%

and 16% for Al K-line and ^{55}Fe , respectively.

A computer program was available to process the data. Besides standard operations like normalizing, mean and standard deviation calculation, plotting, etc., a number of specific operations were required for processing the spectra. A selected function was fitted to the experimental data to obtain coefficients for the best fit, which were subsequently used for extrapolation at both ends of a measured single electron spectrum. Namely, the low energy part of a single electron spectrum is always overlapped by electronic noise and the high energy part is sometimes cut off due to the finite dynamic range of the pulse height analyzer.

Results

The principal conclusion of the analysis of measured spectra is that the shape of the single electron spectra can be represented by the function of the form $F(x) = x^a e^{-x}$. For practical reasons concerning processing of data the following form has been found more convenient

$$f(x) = Ax^B e^{-Cx} \quad (1)$$

where A, B and C are constants determined by fitting the function (1) to the experimental data. The constant A depends on the number of collected data and has no important physical meaning. However, the constants B and C determine the shape of the spectrum. Table 1 shows the experimental data including the statistical properties of the measured distributions. The numerous investigated gases, listed in Table 1, can be grouped according to their chemical and physical properties as follows:

Saturated aliphatic hydrocarbons having a general chemical formula $\text{C}_n\text{H}_{2n+2}$ ($n = 1, 2, 3, \dots$) behave in a very similar manner when used as the counter filling gas. Their "quenching" property increases with increasing number of carbon atoms resulting in a more stable counter operation. The W-value decreases with increasing number of carbon

TABLE PM-1-1

Gas or Gas Mixture	Single electron spectrum pulse height distribution properties		
	Relative st. dev. σ	B, Eq.1	C*, Eq.1
Methane	0.844 ± 0.02	0.400	0.0235
Ethane	0.810 ± 0.015	0.550	0.0290
Propane	0.810 ± 0.015	0.525	0.027
Butane	0.805 ± 0.015	0.531	0.0295
Heptane	0.814 ± 0.01	0.520	0.0279
Hexane	0.810 ± 0.01	0.526	0.029
Ethylene	0.865 ± 0.02	0.330	0.0233
Carbon dioxide	0.87 ± 0.05	0.337	0.0165
Methane based tissue-equivalent gas	0.850 ± 0.015	0.393	0.0204
Propane based tissue-equivalent gas	0.830 ± 0.015	0.455	0.239

*Coefficient C depends on the choice of the abscissa. Here the energy per channel is kept constant. The variation of the coefficient C reflects the different W-values of each gas.

atoms. There are small fluctuations in otherwise smooth trends, depending on the even or odd number of C atoms. Concerning the shape of the single electron spectra the difference between various gases is vanishing above $n = 3$ (propane). The relative standard deviation of single electron spectra in all higher saturated hydrocarbons is 0.815 ± 0.015 ; methane is an exception having a higher standard deviation of 0.844 ± 0.02 .

Ethylene has been used for counter filling as a typical unsaturated hydrocarbon gas. It has poorer quenching properties and the single electron spectrum standard deviation is 0.865 ± 0.02 .

Carbon dioxide differs markedly from aliphatic hydrocarbons. It seems that very pure CO_2 changes its properties rapidly due to contamination shortly after filling the counter (Jesse effect?). Such behavior introduces large uncertainty in the measurement of the single electron spectrum due to gas gain changes. Fortunately, when a quenching gas such as methane or propane is added to CO_2 the resulting gas mixture becomes stable with respect to the gas gain or the shape of the single electron spectrum. This has been proved in the case of tissue-equivalent gas mixtures, methane and propane based. The shape of the single electron spectra in these mixtures follows closely Eq. 1 which is important for deconvolution calculations.

More complex organic vapors such as methanol and ethanol have been investigated with less success. Due to strong adsorption on insulating surfaces the counter response becomes erratic. However, the shape of the single electron spectrum in these vapors is similar to that of the hydrocarbons and obeys the $x^a e^{-x}$ law.

Figure PM-1-1 shows a typical single electron spectrum in a polyatomic gas.

Contrary to polyatomic organic gases and gas mixtures containing a substantial percentage of these gases, mixtures of monoatomic noble gases with less than 10 to 15% of quenching polyatomic admixture exhibit multiplication process quite different from that described previously. The shape of the single electron spectrum is closer to the simple exponential curve of a general form $f(x) = e^{-x}$, even though the data points do not follow exactly that simple function. No analytic formula has been found which would fit to the data points as good as the Eq. 1 fits into single electron spectra in polyatomic gases. It was found, however, that the higher percentage of quenching admixture to the noble gases or a high total pressure of these mixtures in the proportional counter result in the single electron spectrum, matching very closely the form $f(x) = x^a e^{-x}$. It could be concluded that a complete quenching of gas discharge in polyatomic gases results in the form of single electron spectra described by Eq. 1, as opposed to partially quenched discharges in noble gases with small percentage of polyatomic admixture. Whether the photoionization resulting from neutralization of noble gas ions or photons from the avalanche bring about the change of the multiplication process is not clear. In any case, the first gas discharge is followed by a "train" of subsequent discharges of smaller size, as found by Campion (9), which results in the change of the shape of the single electron spectrum.

Conclusion

There is ample experimental evidence to support the pulse height distribution of the form $f(x) = Ax^B e^{-Cx}$ for single electron spectra in polyatomic gases and in methane and propane based tissue-equivalent gas mixtures. The knowledge of the general analytic form of the single electron spectrum is essential in calculation of the mean value and variance of the experimentally measured single

Figure PM-1-1: The single electron spectrum in propane. Gas pressure: 50 Torr; High voltage: 960 volts; Gas gain $\approx 10^5$. Full line: best fit to experimental points, Eq. 1.

electron spectra. Eq. 1 helps to extrapolate properly the experimental spectra, which in turn allows an accurate calculation of the W-values, the Fano factor and the deconvolution processing.

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Paul J. Kliauga

This note describes the observation of the effects of a particular electronic parameter on the calibration of proportional counters. The effect in question is well-known and in a sense obvious (1), but it is easily overlooked in some applications of proportional counters to microdosimetry. Therefore, it is thought worthwhile to recount an example where this problem did occur, with a view toward helping others avoid such difficulties in the future. The effect will be of greatest importance for large gas pressures and/or small electrode potentials (low gas gain), and for radiation modalities which exhibit a large dynamic range of energy deposition.

This effect was observed while checking calibrations for analysis of the Berkeley data presented in last year's report (2,3). At that time some discrepancies in the analysis led naturally to a check of the counter's calibration data. When no obvious errors could be found, it was decided to test the counter's relative calibration with two often-used standards: 1) an Al x-ray source, yielding 1.49 keV x-rays, and producing a well-defined spectrum peak at all diameters $\gtrsim 0.5 \mu\text{m}$, and 2) a collimated ^{241}Am source, directed along a diameter of the spherical counter, which produces an energy deposition of $\sim 85 \text{ keV}/\mu\text{m}$ from its 5.5 MeV α particles.

Because this study is concerned with relatively large gas pressures ($\approx 70 \text{ Torr}$) the Al x-ray source will produce an almost point-like cluster of ionization from a given photon interaction (entirely photoelectric effect), because of the extremely short range of the secondary electrons ($\lesssim 0.1 \mu\text{m}$). The ^{241}Am source, however, produces a dense cluster of ions along its track core, which is along the counter diameter in this case, and a more diffuse spread due to high energy δ -rays.

The ionization produced by the ^{241}Am α particles, then, is distributed from

one counter boundary to the opposite boundary. Charge collection begins at the point on the wire closest to the ion's track and spreads outwards to the boundaries. The time required for collection of all charge is, of course, a function of p/E , where E is the electric field and p the gas pressure. This time should be shorter than the integration time of the amplifier to ensure that all of the collected charge appears as the analog output voltage signal fed to the pulse height analyzers. If the time constant is too short, the output pulse will obviously be truncated and the calibration will be erroneous.

The time constant most commonly used is 1 or 2 μs , with 1 μs being the conventional figure in most cases. The one-inch wall-less spherical proportional counter mounted inside a four-inch spherical shell as described in ref. 1 was irradiated simultaneously with Al x-rays and ^{241}Am alpha particles. The energy of the ^{241}Am peak was calculated using the formula:

$$E_{\text{Am}} = E_{\text{Al}} \frac{X_{\text{Al}}}{X_{\text{Am}}} \cdot \frac{G_{\text{Al}}}{G_{\text{Am}}}$$

where $E_{\text{Al}} = 1.49$ keV is the energy of the Al K_{α} x-ray, X_{Al} and X_{Am} are, respectively, the channel numbers of the Al and Am peaks, and G_{Al} and G_{Am} are the total electronic gain factors (amplifier and analyzer conversion gain) of the Al and Am spectra.

Figure 1 shows a plot of measured Am peak energies as calculated above, for a pressure of 70 Torr propane TE gas using an amplifier time constant = 1 μs as a function of counter high voltage applied to the voltage divider. Actual electrode potentials are $V_S = 0.80 \times V$ for sphere potential, $V_H = 0.73 \times V_S$ for helix potential.

The pressure of 70 Torr simulates approximately 1 μm diameter unit density material in a one-inch spherical counter. Thus, the expected energy deposition is ~ 340 keV. Note that this is not reached until $V \approx 800$ Volts. At higher voltages, the curve is flat and the proper energy is measured, but at lower voltages,

Figure PM-2-1: Energy of ^{241}Am α -particle peak, calculated as described in the text, as a function of counter voltage (total voltage to voltage divider).

correction must be made for inadequate charge collection. This is obviously a serious problem with very high LET radiations as at the Bevalac where low gas gains must often be used to avoid preamplifier saturation, and where the pulsed machine operation makes small time constants desirable.

Using a 5 μ s time constant, no variation of measured Am energy with counter voltage was detected down to the lowest voltage at which the Al x-ray could still be resolved from the noise ($\sim$ 720 Volts). No systematic study of required time constant vs. E/p has been done yet, but this would obviously be desirable.

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Paul J. Kiauga, Yuk-Ming P. Lam, Harald H. Rossi and Leon J. Goodman

Introduction

Since the successful production of high intensity energetic heavy ion beams at the Bevalac a few years ago, this facility, now the only one in this country, has become a highly productive center of research in the biological and therapeutic uses of heavy ions. The potential usefulness of accurate microdosimetric data on this unique radiation modality is apparent, but has not been realized because of the scarcity of such data.

This scarcity is due in part to the exceptionally severe constraints placed upon a microdosimetric experiment by these particles and in part do logistical constraints brought about by geography and the unique nature of this facility. Some initial measurements on carbon and argon ions were reported in the previous annual report (1), and at the Sixth Symposium on Microdosimetry (2).

These data were taken with wall-less counters of the spherical-grid type, enclosed within tissue-equivalent plastic shells. Since two counters differing in diameter by a factor of four were used (enclosed in the same size spherical shells), comparison of data between the two counters for the same simulated tissue diameter gave an indication of differences due to the "wall effect" (3). As anticipated, this effect is substantial, and confirmed the desirability of using a counter as wall-less as possible for obtaining accurate data on high-energy ions.

Even a grid-structure type of counter, while not suffering a substantial discontinuity in density between the collecting volume boundary and the immediate gas atmosphere surrounding it, can exhibit a "wall effect" from the container walls enclosing the counter and filling gas if the range of delta rays is larger than the radius of the enclosure.

For a 450 MeV/amu particle, the maximum δ -ray energy is given by

$E_{\max} \approx 2 mc^2 \beta^2 / (1 - \beta^2) \approx 1.2 \text{ MeV}$, which corresponds to a range in tissue of $\sim 0.5 \text{ cm}$, or $5000 \text{ } \mu\text{m}$. In a TE gas of density such that $1 \text{ } \mu\text{m}$ tissue corresponds to 1 inch of gas, the would require a container whose dimensions are of the order of 100 meters! Of course, the actual δ -ray distribution favors small energy transfers, and a computer calculation based on a simple model (4) showed that the error in energy deposited would be between 5% and 30% for a counter situated at the center of a tube of radius 30 cm.

A cylindrical container was constructed at the Bevalac out of surplus ventilator shaft parts, whose nominal dimensions are 8 ft long x 19 in diameter. A beam entry "window" was constructed at one end of mylar and dacron sail cloth. The opposite end was a steel plate containing electrical, gas, and mechanical connections. Figure 1 is a schematic of the experimental set up.

Since impact parameter determinations were planned, it was decided to attempt event-mode recording of the data, utilizing the Camac modules available at the Bevalac for pulse height analysis, and using the computer for data storage and analysis, which could be performed not on-line, but at least immediately after a run. Such operation required use of the "Q" operating system, which had only become operational shortly before the scheduled start of the experiment. This was an unfortunate circumstance, because the presence of "bugs" in the Q system initiated computer failures, which in turn caused extensive loss of data. Thus, it was decided on the second day to convert to a simple multichannel analyzer data acquisition system.

Some data were obtained in this fashion before machine problems, equipment problems and human errors (including a partially damaged proportional counter) caused further disruption. Thus, the amount of data collected was not large, and no impact-parameter data were obtained. Furthermore, since no data analysis was

Figure PM- 3-1: Schematic diagram of experimental layout at Bevalac. Tank is approximately 8 feet long by 2 feet diameter. A: Dacron-mylar beam-entrance window. B: Tissue-equivalent plastic disc, 1 foot diameter x $\frac{1}{4}$ inch thick. This is supported on delrin runners C, and is positioned along tank length by rod D. E: Cylindrical wall-less proportional counter, $\frac{1}{4}$ inch long x $\frac{1}{4}$ inch diameter. F: Plastic scintillator strip, $\frac{1}{4}$ inch x 2 inch x $\frac{1}{16}$ inch thick, mounted on conical light pipe, and designed to move perpendicular to the tank and beam axis on a remotely controlled traverse mechanism (not shown).

available, spectrum analysis to check gain settings and calibrations could not be done. This absence was felt particularly keenly because a combination of linear-log amplifiers and an attenuation box was used, and the compound effects of changing various gain factors (including gas gain) was not immediately obvious.

Although the data collected were sparse and not always complete, a comparison of the good data available with those obtained with the spherical wall-less counters as outlined in last year's report (1) reveals some highly interesting features.

Container Wall Effect

One of the primary objectives which was achieved during this experiment was a study of the effect of container size on the microdosimetric spectrum. The spectrum region most sensitive to boundary surfaces near the sensitive volume is the small-event size region, in particular, that part of the spectrum which we often call the "δ- peak", admittedly somewhat erroneously, because particularly for highly energetic heavy ions it is obvious that spallation products contribute a significant part.

In the first set of experiments, gas gain and electronic gain were set to focus on the low-energy part of the y spectrum. A typical spectrum is shown in Figure 2. Part A of the figure is the quantity $y \cdot f(y)$, Part B is $y \cdot d(y)$. Note that these are not normalized.

A moveable TE plastic disc, (B in Fig. 1) 12 inch diameter x $\frac{1}{4}$ inch thick was located at various positions relative to the proportional counter: 4 in, 8 in, 25 in, and 50 in. The parameters examined as a measure of the magnitude of wall effect is the total number of events in the "δ-peak" per incident beam particle, as measured by the scintillation paddle monitor (A in Fig. 1). The integration interval is arbitrarily chosen to be from 0.8 keV/ μ m to 8.0 keV/ μ m (See Fig. 2) The quantity

$$R \equiv \int_{0.8}^8 f(y) dy / \text{monitor counts. Table 1 gives R for the four disc-to-}$$

counter distances (in inches):

Figure PM - 3 -2A:

Figure PM - 3 -2B:

Small - event portion of spectrum of 450 MeV/amu
 ^{40}Ar ions at plateau. Part A is $y \cdot f(y)$.
 Part B is $y \cdot d(y)$.

	4	8	25	50
R	$0.0323 \pm .0003$	$0.0365 \pm .0003$	$0.438 \pm .0003$	$0.0290 \pm .0002$

This shows that as the distance from counter to this artificial "wall" increases, the relative number of small events increases, as is predicted by the δ -ray effect of Kellerer. However, as the wall is moved to still larger distances, the incidence of small events decreases.

This behavior can be readily explained in terms of the spatial distribution of delta radiation. As the separation between the counter and the disk is increased it becomes less probable that long range delta rays traverse the counter together with the primary heavy ions. However, these delta rays are scattered out of the beam by the gas in the tank and there is no compensation for this loss if the beam diameter is too small. An increase from the current diameter of about 8" should therefore, result in less or no decrease of small pulses with increasing separation between counter and disk.

Y Spectra

Upon completion of the wall effect experiments just described, the TE disc was left at a 50" distance from the counter in order to avoid opening up the tank and pumping down. Bragg curve measurements were performed with the disc in place, so water absorber thicknesses were decreased by the water-thickness of the TE plastic.

Only two Bragg curve positions, plateau and ascending, are shown. These correspond to the same position in (1). A comparison of Figure 3 with Figure 4 from Ref (1) shows some similarities but also some striking differences. As before, the $f(y)$ spectra are dominated by a small-event-size peak, but the data taken in the large tank (Fig. 3A) shows this peak shifted toward the left but also much broader, merging with the primary beam peak. This shift cannot be caused by an error in calibration, for inspection of Figure 3B and 4B shows that the primary

Figure PM - 3-3A:

Figure PM - 3-3B:

Microdosimetric spectra of 450 MeV/amu ^{40}Ar at plateau.
 Part A is $y \cdot f(y)$, Part B is $y \cdot d(y)$.

Figure PM-3-4A:

Figure PM-3-4B:

Microdosimetric spectra of 450 MeV/amu ^{40}Ar at plateau, taken with small spherical wall-less counters as reported in Ref (1), and to be compared with corresponding Fig. 3.

beam peak is in the same location for both experiments (~ 200 keV/ μm). The shift toward smaller y values for the " δ -peak" results in a smaller relative contribution to the dose distribution (Fig. 3B) compared to Figure 4B, even though the event distribution is greater.

Another difference which is noted is the much wider distribution of the main (primary beam) peak, extending to > 1000 keV/ μm in the present data. The reason is uncertain, but one may speculate that pile-up distortion may cause some of this. Although the machine beam current was lowered to give an instantaneous counting rate of < 1000 sec $^{-1}$, it was difficult to monitor continuously, and no electronic pile up rejection was employed. Instantaneous beam fluctuations could cause some pile up.

The other Bragg curve location is the ascending part of the curve, shown in Figure 5 and is to be compared with the distributions from last year's report in Figure 6. Again, the double peak characteristic is plainly evident. The peaks from this year's data are both shifted toward larger y values compared to last year. This may well be caused by a slight inaccuracy in determining the exact location on the Bragg curve. This is on the steeply rising portion of the curve, and an error of only a mm could make significant changes in the spectra.

Conclusions

Even though the most recent runs resulted in incomplete data, it can be inferred that: 1) The wall effect is substantial, and must be reckoned with to obtain accurate microdosimetric data. 2) The "V" effect from highly-forward-directed spallation products and very energetic rays forms an important contribution to the overall wall-effect. 3) Except near the Bragg peak, the event spectra are dominated by small-event sizes.

Further experiments must be performed with the tank set-up at the Bevalac. Learning from this experience, we can state that such experiments must be carried

Figure PM-3-5A:

Figure PM-3-5B:

Microdosimetric spectra of 450 MeV/amu ^{40}Ar at ascending portion of Bragg curve.

Figure PM-3-6: Microdosimetric spectrum of 450 MeV/amu ^{40}Ar at ascending portion of Bragg curve, to be compared with Fig. 5.

out over an adequate period of time so that analysis of preliminary data may be carried out as a guide to subsequent experiments. Careful attention must be paid to optimizing the parameters of gas gain and electronic gain so that the greatest dynamic range can be achieved without preamplifier saturation. In this regard, it may be advisable to investigate the possibility of changing the preamplifier sensitivity (by e.g., a selectable input capacitance) instead of changing gas gain. Attenuators preceding the log amplifier should be avoided. They unnecessarily complicate calibration procedures. Adequate pile-up protection must be employed. It is difficult to monitor the Bevalac pulses continuously, and they can change intensity drastically.

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The experiment described in last years' Annual Report which was designed to yield information on the deposition of energy by charged particles in regions of the order of a nanometer, is being continued.

A particular geometrical configuration for the avalanche grid and shield wires has been investigated. It consists of four stainless steel wires each 10 mils in diameter. One wire, the avalanche grid, is separated by 15 mils from the scintillator and then there are three shield wires between the grid and sensitive volume of the chamber. The actual configuration is shown in Figure 1. The purpose of the shield wires is to keep the high electric field at the grid wire from penetrating into the sensitive volume of the chamber, so as to keep the field there as uniform as possible for positive ion collection. A test chamber was designed for the purpose of investigating the best possible separation of the grid and shield wires from the grounded surface, which represents the scintillator. Measurements made with the chamber indicated that in order to maintain any reasonable voltage on the avalanche grid wire (i.e. -3000 v) the pressure must be 0.1mm Hg or below. Breakdown voltages for various separations ranging from 0.5 mil to 15 mils were measured. Data was obtained for shield voltages of -400 v, -540 v and -5 volts. Highest grid voltages were obtainable with the shield wires at -5 v, Figure 2, which would seem to indicate that the voltages on the grid and shield combine to cause breakdown to the grounded surface. Other configurations of shield and grid wires will be investigated along with the possibility of using smaller diameter wires for the shield.

Various methods of putting a thin conductive coating on the plastic scintillator have also been investigated. Thin coatings were made with various concentrations of aqua dag solution (a suspension of carbon in water) and also with Dag 154, a

Figure PM-4-1: Test chamber showing configuration of field shaping electrodes. The wires were potted in lucite and were made of 10 mil diameter stainless steel. The aluminum disc could be moved so that its separation from the grid wire could be varied. Note that the figure is not to actual scale.

AVALANCHE GRID TEST CHAMBER

Figure PM-4-2: Breakdown voltage measured at grid as a function of pressure for two shield voltages. The separation between the grid and ground plate (Aluminum disc) is 15 mils.

suspension of carbon in isopropyl alcohol. The coating needs to be as thin and as uniform as possible so that the electrons will have maximum energy when they enter the scintillator. Calculation of the range of 3 keV electrons in carbon yields a value 0.09 microns, using the best available mass absorption coefficients from ICRU Report Number 16, 1970. The actual thickness should be considerably less than this, so that the electrons are not significantly degraded in energy in passing through the carbon. It is estimated that any finite resistance reading ($< 10^{12}$ ohms) should be sufficient to prevent space charge buildup and maintain a constant electron field distribution in the region between the avalanche grid and the scintillator.

The spectrum from an aluminum x-ray source, 1.5 KeV, was measured with the 11 mil plastic NE102 scintillator and the EMI 9789QA photomultiplier tube (which was specially selected for low noise). The noise and background spectrum as well as the x-ray spectrum are shown in Figure 3. The noise is seen to be negligible compared to the signal from the 1.5 KeV x-rays.

In order to check the efficiency of the detector system for electrons, a monoenergetic electron source was designed and built. An ordinary flashlight bulb filament was used as the source of electrons. They were then accelerated toward the carbon coated scintillator by a high negative voltage 3-5 kilovolts (See Fig. 4). It is necessary to keep the filament current very low so that the filament does not glow and overload the photomultiplier tube. Preliminary measurements thus far have indicated that insufficient electron current is obtained at very low filament currents.

In order to determine if the electron generator was functioning properly, a thin foil of aluminum, coated with TiO_2 paint was used as a target. Since the aluminum foil protected the photomultiplier tube from light, the filament could be run at very high currents. Preliminary measurements show that the

Figure PM-4-3: Curve A is the 1.5 kev aluminum x-ray spectrum.
Curve B is the background and noise spectrum.
The x-ray spectrum is not as well resolved as
it might be, due to the geometry of the system.

Al

Insulator

Scale - 2x

Figure PM-4-4: Monoenergetic electron generator.

intensity of the spectrum obtained is a function of the filament current at an electron accelerating potential of 5.2 KV which is above the K edge of Titanium. For an accelerating voltage below the K edge, 3.2 KV, both the intensity and average pulse height of the spectrum are significantly reduced. Further investigations are in progress.

D. Srdoc, R.E. Mills and Stephen A. Marino

A series of microdosimetric spectra has been measured using tissue-equivalent proportional counters of various geometrics and constructions as described in detail in the following paragraphs. The counters were exposed to ^{60}Co gamma rays and 0.44 MeV neutrons while operating at pressures corresponding to tissue site sizes of 0.5, 1 and 2 μ . The aim of these measurements was to obtain reliable microdosimetric data for selected types of radiation and to assess the magnitude of the so-called wall effect and the influence of the geometry of the counter on the shape of spectra.

Experimental

The availability of several specially designed Rossi-type proportional counters built for previous measurements and the irradiation facilities at the RARAF greatly facilitated the task of measuring microdosimetric spectra of gamma and neutron radiations. The counters were selected in such a way that measurements were performed with a pair of walled and wall-less counters having the same geometry. Spherical and cylindrical volumes having the same mean chord length have also been compared. Propane-based tissue-equivalent gas was used throughout the measurements. Calibration of the counters was performed before and after taking the microdosimetric spectra by means of an x-ray tube emitting Aluminum K-lines ($\bar{E} = 1495 \text{ eV}$). Details on the calibration technique are presented elsewhere (1).

A special low noise FET preamplifier (RMS deviation $\approx 100 \text{ e}^-$) coupled to an amplifier with logarithmic response* enabled us to obtain in a

*The authors are indebted to Dr. V. Radeka, Mr. L. Rogers and Mr. D. Stephani, BNL, Instrumentation Division, for designing and making the electronics.

single measurement the entire $P(Y)$ spectrum which usually spans over 3 decades. The previous technique usually required 3 to 4 separate measurements to obtain a $P(Y)$ spectrum.

Gas handling and pressure measurement have also been modernized by replacing the old Manostat system with a Granville-Phillips capacitance-type pressure and flow regulating device.

On-line processing of data with the RARAF PDP8/e computer has been essential for efficient measurements. The data have been stored on magnetic tapes for further processing.

A 50 mCi ^{60}Co source was used as the gamma ray source. Neutrons having an energy of .44 MeV were produced by the $T(p,n)$ reaction using 2.3 MeV protons on a thin tritium target. The total dose and the gamma-ray dose were monitored using appropriate dosimeters. The gamma-ray dose is typically less than 2% of the total dose for these conditions.

Six different counters were used in the course of the measurement. Table PM-5-1 shows their principal features and gives references for details of the construction. The collection of counters contained a pair of spherical counters, walled and wall-less, a pair of cylindrical counters, also walled and wall-less, and two special counters: the parallel plate ("pancake") counter and the "Honeycomb" counter. The two special counters have been designed for dose-equivalent index measurements and will be discussed separately.

A typical set of data collection was performed in the following manner: After a thorough overnight pumping the counter and gas lines were

Table PM 5-1

Counter description	Geometry and dimensions	Detailed description, Ref. No.
1" Spherical counter, Single wire, solid wall	Sphere, 2.54 cm inner diameter	2
1" Spherical wall-less counter	Sphere, made of TE plastic grid; 2.54 cm inner dia. TE Helix	3
1" x 1/4" Cylindrical counter, solid wall	Cylinder, 2.54 x .635 cm	4
1" x 1/4" Cylindrical wall-less counter	Cylinder, 2.54 x .635 cm. Base: plastic insulator Wall: Metal wire helix	4
Dose-equivalent counter, parallel plate model	Array of 1 mil wires between TE plastic discs	5
Dose-equivalent counter, "Honeycomb" model	Cylindrical counters in TE plastic matrix	5

flushed with TE-gas for several hours. Meanwhile, the electronics was checked for logarithmic response with a pulse generator. The gas pressure was then adjusted to the value corresponding to the selected tissue site size and the counter calibration was made with the Al K-line. Fig. PM-5-1 shows the calibration spectrum. The calibration with the Al K-line was repeated after each P(Y) spectrum measurement to check the constancy of the electronic and gas gains.

Over 40 spectra have been measured using the described technique. Work is in progress to analyze the ^{60}Co gamma-ray and the 0.44 MeV neutron spectra. Figs. PM-5-2 and 3 show typical P(Y) vs Y spectra for ^{60}Co gamma rays and 0.44 MeV neutrons, respectively.

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Figure PM-5-1: Aluminum K-line calibration peak ($\bar{E} = 1495$ eV). Full logarithmic scale. 1" spherical single wire counter. Propane-based TE gas, 17.5 Torr-1 μ site size.

Figure PM-5-2: ^{60}Co gamma ray source. 1" spherical single wire counter.
Propane-based TE gas, 35 Torr-2 μ site size.

Figure PM-5-3: 0.44 MeV neutrons. 1" spherical single wire counter.
Propane-based TE gas, 35 Torr-2 μ site size.

BIOPHYSICS

BP-1 A rigorous formulation of the site model

Yuk-Ming P. Lam

This report attempts to demonstrate, through the use of an example, that it is possible to describe the competition between repair of sublesions and interaction between sublesions in a cell nucleus.

This formulation adheres to the following general concepts of the theory of dual radiation:

1. Radiation produces ionization energy in a cell nucleus, which is converted to non-lethal damage in DNA. Each nonlethal damage, called a sublesion, by itself has no effect on the cell.
2. The amount of ionization energy deposited is not a function of the dose of radiation but is a stochastic quantity whose distribution is dependent on dose.
3. A sublesion does not remain as such indefinitely in a cell. In time, it is eliminated either by some repair process or by combination with another to produce a lesion. The production of a lesion represents a definite change in the genetic content of the cell. Some lesions may not lead to any alterations in the behavior observable in the laboratory, others may result in cell transformation or loss of the capability to reproduce. It is also assumed that both the repair rate and the interaction of sublesions are sufficiently constant with respect to cell cycle changes, and that the cell cycle is sufficiently long that the sublesions will prior to mitosis usually be repaired or will have been one of the partners in lesion foundation.

For the purpose of illustration, it is not necessary to take into account all of the detailed intricacies of the cell, but it suffices to merely abstract

the pertinent features to make the description self-contained. Thus, the cell in the model has only one kind of sublesion and consequently only one kind of lesion. As soon as a lesion is formed, the cell is eliminated. The geometry of the cell nucleus is also disregarded; hence, the interaction between a pair of sublesions is the same, irrespective of where in the nucleus they occur, or the spatial separation between them. For this reason, this model is accordingly called the site model.

These considerations then lead to the following formulation of the model, in addition to Points 1, 2, and 3 of the theory of dual radiation above.

Model

The probability that a sublesion is repaired in the time interval Δt is $r\Delta t$, and the probability that a given pair of sublesions will produce a lesion in the time interval Δt is $v\Delta t$. Both r and v are constants, independent of the location of sublesions and the phase of the cell.

Solution of the Model

Let n sublesions be created in the nucleus of a cell by an instantaneous exposure to radiation. During a short time period Δt , the probability that one of the n sublesions will be repaired, thereby reducing the cell to one with $n-1$ sublesions, is

$$nr\Delta t.$$

In the same period, the probability that a lesion will be created from any of the $n(n-1)/2$ pairs of sublesions is:

$$\frac{n(n-1)}{2} v\Delta t$$

Since the ratio of these two probabilities is independent of time and since the fate of this cell is either that a lesion is formed before any sublesion is repaired, or that a sublesion is repaired before any lesion is formed, the probability that the latter occurs is:

$$P_n = \frac{nr}{nr + \frac{1}{2}(n-1)v} \quad (1)$$

when this happens, the cell originally with n sublesions and no lesions is reduced to one with $n-1$ sublesions and still no lesions. The only way a cell can survive is to have all of its sublesions repaired one by one. Hence, the survival probability of a cell with n sublesions is:

$$S_n = P_n P_{n-1} \dots P_1, \quad (2)$$

and

$$P_1 = 1, \quad (3)$$

since a cell with only one sublesion cannot incur a lesion. Therefore,

$$S_n = \prod_{j=2}^n \left(1 + \frac{j-1}{2} w \right)^{-1} \quad (4)$$

where

$$w = \frac{v}{r}. \quad (5)$$

S_n only depends on the ratio w of the interaction to repair rate.

Let $f_i(D)$ be the probability that i sublesions are produced in a nucleus by an instantaneous dose D of radiation. Then the survival probability as a function of dose D is:

$$S(D) = \sum_{i=2}^n f_i S_i \quad (6)$$

or

$$S(D) = \sum_{i=2}^n f_i \prod_{j=2}^i \left(1 + \frac{j-1}{2} w \right)^{-1} \quad (7)$$

It would be of interest to investigate how this simple minded model squares with real cell survival data. For this purpose the distributions $f_i(D)$ needs to be provided. For example, one may assume that an ionization energy q is needed for the production of each sublesion, and obtain from the distribution $f(z,D)$ of

z, for each dose D, the distribution $f_i(D)$:

$$f_i(D) = m \int_{iq}^{iq+q} dz f(z,D) \quad (8)$$

where m is the mass of the nucleus. Taking the point of view that the distribution $f(z,D)$ and the mass, m, of the nucleus are experimentally obtainable, then Equations (7) and (8) give a survival probability dependent only on two parameters, namely the ratio w of sublesion interaction rate to repair rate, and the ionization energy q required to produce a sublesion.

Future Enhancement

That this site model above is amenable to an exact solution is attributable to the simplifying assumptions. As they are replaced by more realistic axiomatizations, the solution can probably be obtained only through Monte-Carlo simulations on the computer. One way of approaching reality is to allow more than one type of sublesion capable of being produced in a nucleus, leading to more than one type of lesion. This will enable one to describe more than two end states of a cell, eg., transformed, killed or surviving and well. Upon abandoning the site model, one may allow the sublesions motion around a cell nucleus. If a sublesion succeeds in getting closer than a certain critical distance from another before it is repaired, then a lesion is formed from these two sublesions, whose nature determine which kind of lesion is formed. The case of finite dose rate can be treated by creating sublesions randomly in time during the period of exposure.

The variations are endless, and it is hoped that this kind of formulation will provide the framework for rigorous investigations in the future.

RADIOBIOLOGY

- RB-1 Oncogenic transformations with split doses of x-rays.
Richard C. Miller and Eric J. Hall
- RB-2 Neoplastic transformation following split doses of x-rays.
Carmia Borek
- RB-3 The intercomparison of relative biological effectiveness and oxygen enhancement ratio at clinically used neutron facilities.
Eric J. Hall (in collaboration with Dr. Albrecht M. Kellerer, Institut Für Med. Strahlenkunde, Würzburg, West Germany)
- RB-4 Sister chromatid exchanges: Interaction of a retinoid (Ro-10-9359) and a protease inhibitor (antipain) with x rays.
Melanie R. Freeman and Charles R. Geard
- RB-5 The effect of neutron induced recoil protons of restricted energy and range on chromosome aberration formation.
Charles R. Geard
- RB-6 Effects of 1.91 MeV monoenergetic neutrons on *Vicia faba* chromosomes: Microdosimetric considerations.
Charles R. Geard
- RB-7 Sequential exposures with high and low-LET radiations.
R.P. Bird
- RB-8 The associated ion experiment.
Richard P. Bird
- RB-9 Irradiations with the track segment facility and the analysis of data.
Richard P. Bird and Paul J. Kliauga
- RB-10 The modulating effects of the protease inhibitor Antipain on x-ray induced transformation.
Carmia Borek and Richard C. Miller
- RB-11 A comparison of two nitroimidazoles as hypoxic sensitizers, radiosensitizers and cytotoxic agents.
Eric J. Hall and Myles Astor (in collaboration with Drs. Philip Shapiro and Leon August, Naval Research Laboratory, Washington, D.C.)
- RB-12 MTDQ as a hypoxic cell radiosensitizer alone and in combination with misonidazole.
Myles Astor, Eric J. Hall, and Robert S. Osmak

RADIOBIOLOGY (continued)

- RB-13 Combination studies with Bleomycin, Misonidazole and x-rays.
Laurie Roizin-Towle and Eric J. Hall
- RB-14 The effect hyperthermia alone, or combined with x-rays, on the
incidence of oncogenic transformations *in vitro*.
R.C. Miller, L. Harisiadis and E.J. Hall
- RB-15 An investigation *in Vitro* of the clastogenic effects of Misonidazole.
Louis E. Schwartz and Charles R. Geard
- RB-16 Regulation of Retinol-Binding Protein Metabolism in Cultured Rat
Liver Cell Lines.
Carmia Borek

RB-1 Oncogenic transformations with split doses of x-rays.

Richard C. Miller and Eric J. Hall

In a recent paper, we reported that at dose levels below 1.5 groups splitting an x-ray dose into two equal fractions enhanced transformation frequency compared with the same total dose delivered in a single exposure, whereas at higher dose levels fractionation produced the more conventional sparing effect. In these experiments a 5 hour time interval was used between dose fractions. The increased effectiveness of split doses of x-rays has been reported using an assortment of experimental systems. Borek and Hall (2), using an *in vitro* hamster embryo system, reported enhancement of transformation if the low x-ray dose was split by 5 hours. Kaplan and Brown (3) found that four exposures at 4 or 8 day intervals were more effective in tumor production in mice than a single exposure. Little (4) recently reported that splitting low doses of x-rays was more effective in inducing transformation of the 3T3 cell than a single exposure.

The effect on transformation of fractionating low x-ray doses is of fundamental and practical importance that we considered it imperative to accumulate more data, subject them to a rigorous statistical analysis in order to allow unequivocal conclusions to be drawn, and to extend the scope of the experiments to even lower dose levels.

MATERIALS AND METHODS

The cells used in these experiments were the mouse fibroblast cell line, designated C3H/10T $\frac{1}{2}$. The methodology of using these cells for transformation studies has been described previously (5). Appropriate numbers of cells were plated 18 hours prior to irradiation into Corning 50 cm² Petri dishes such that approximately 400 reproductively viable cells survived the plating and

irradiation treatment. At the termination of the experiments, cells were fixed in formalin and stained with Giemsa. Transformed type II and III foci were scored using the criteria described by Reznikoff et al (6). Cells were irradiated using a Siemens Stabilipan constant potential x-ray unit, operating at 300 kVp, 12 mA and having an added filtration of 0.2 mm Cu. The dose rate was in the range of 32 to 180 rad min⁻¹.

Due to the relatively low frequency of transformation induced with low doses, physical limitations on the number of Petri dishes used prevented some x-ray experiments from producing large numbers of transformed clones. Consequently, data from experiments were pooled and error limits calculated assumed a Poisson distribution. Standard errors reported were determined by;

$$\frac{\sqrt{N}}{N} \times \text{frequency of transformation}$$

where N = the observed number of transformed clones.

RESULTS AND DISCUSSION

The effects of either a single exposure or a split dose exposure on cell survival can be seen in Figure RB-1-1. At doses less than 1 Gray, it is not possible to detect the differences in cell survival for single versus split doses. However, from 2 Gy to 10 Gy a substantial amount of sublethal damage has been repaired during the 5 hour interval between the split doses. This has resulted in increased cell survival of the split dose treated cells compared to cells receiving the same x-ray dose given in a single exposure.

With cellular transformation as an endpoint of radiation damage Figure RB-1-2 illustrates the effects of dose fractionation compared to single dose exposures. Splitting low doses of x-rays in the range of 0.3 Gy to 1 Gy into two equal fractions separated by 5 hours enhances the frequency of transformation compared to a single exposure to the same total dose. At x-ray doses above

Figure RB-1-1: Fraction of cells surviving increasing doses of x-rays given in a single exposure or split into two equal fractions separated by 5 hours.

Figure RB-1-2: Transformation frequency plotted as a function of x-ray dose given as a single exposure or split into two equal fractions separated by 5 hours.

2 Gy, a reduction in the frequency of transformation is observed when splitting the dose by a five hour interval compared to cells that received a single exposure. The time interval necessary for a split dose totaling 50 rad to express its enhancing effect of transformation compared to a single exposure is shown in Figure RB-1-3. From the relatively low transformation frequency at zero time interval, the frequency rose to a maximum after a 2 hour time interval.

DISCUSSION

The importance of the observations reported here lies in their implication to the development of human cancer risk estimates at low doses, by extrapolation from available epidemiological data relating to high dose levels. Both major reports to appear in recent years, the UNSCEAR report of the United Nations as well as the BEIR report of the U.S. National Academy of Sciences, use a linear extrapolation. The risk estimates assumed to apply at low doses are calculated from the slope of a straight line drawn from the origin through the data points for excess cancer incidence for higher doses, usually in excess of 100 rem. Furthermore, it is assumed in both reports cited above that the linear extrapolation leads to an upper limit for the risk estimation at low doses that is "conservative" and "prudent", since most high dose data in the human relate to single acute exposures, while the low dose exposure of the public from man-made radiations is the result of multiple small exposure which is assumed to be less effective.

The *in vitro* transformation data reported here must cast doubt on the validity of the linear extrapolation, and the expectation that it is necessarily conservative.

It must be admitted that morphologically identified transformed clones in a Petri dish are a far cry from leukemia or solid tumors in the human; as a

Figure RB-1-3: Transformation frequency as a function of time between split doses of x-rays. The cells were exposed to a total dose of 0.50 Gy.

model system it clearly has its limitations. However, the exquisite sensitivity of the system that is possible in large experiments makes it possible to obtain a dose-response relationship over a wide range of doses that is unlikely ever to be equalled in the human. It is not prudent or conservative to ignore the shape of this dose response curve; indeed we ignore it at our peril. Referring to Figure RB-1-2, it is evident that using a linear extrapolation from high doses may substantially overestimate or underestimate the low dose risk, depending upon the dose range involved. Furthermore, there is a broad and important range of doses over which fractionation enhances transformation incidence, so that estimates from data relating to a single prompt exposure do not necessarily constitute an upper limit to the cancer risk that could accrue from multiple small doses.

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RB-2 Neoplastic transformation following split doses of x-rays.

Carmia Borek

Most data for radiation induced malignancies in the human relate to a large single dose of radiation received in an acute exposure. An extrapolation is required in order to arrive at cancer risk estimates for low doses, and/or low dose-rates, which are pertinent to human medical or environmental exposure. Both the BEIR and UNSCEAR Committees have used a linear extrapolation from high to low doses, which assumes that the risk per rad is independent of dose or dose-rate. Risk estimates calculated in this way are assumed to be "conservative", since it has been reported that the biological consequences of a given dose are reduced when the dose is divided into a number of fractions and delivered over a prolonged period of time. Our experiments indicate that this is not always true in the case of neoplastic transformations produced *in vitro*.

The experiments involved fresh explants of diploid hamster embryo cells. The details of the methodology have been published previously (Annual Report 1978). Briefly, cells derived from freshly dissociated midterm hamster embryos were seeded at low density to allow for clone formation. Following overnight attachment, cells were irradiated with 210 kV x-rays, either in a single exposure or in two equal fractions separated by 5 hr. Cultures were incubated at 37°C for nine to ten days before being fixed and stained. Transformed clones were readily identified by their appearance, since they consist of piled up cells, randomly arranged. The relationship between the morphological appearance of these clones and their tumorigenicity has been amply documented.

Doses between 150 and 600 rad were used, delivered as single or split exposures. The transformation frequencies obtained are summarized in Table RB-2-1 and plotted in Figure RB-2-1; included are our earlier data for the low dose

range of 50 and 75 rad. In Figure RB-2-1, the quantity plotted on the ordinate is the ratio of the transformation frequencies relating to split versus single doses. For the three lower doses, this ratio is clearly in excess of unity, indicating that split doses produce more transformation than a single exposure to the same total dose. At the higher doses the opposite is true; fractionation leads to a reduction in the transformation frequency.

There have been several previous reports in the literature concerning the effect of split doses on *in vitro* transformation. Using fresh explants of diploid hamster cells, demonstrated that an x-ray dose of either 50 or 75 rad, split into two equal fractions, increased the transformation frequency by more than 70% compared with the same total dose given in a single exposure. Using higher doses and a heteroploid established mouse cell line (10T $\frac{1}{2}$), Little and Terzaghi reported a decreased transformation frequency following dose fractionation. More recently, Miller and Hall employing the same mouse 10T $\frac{1}{2}$ cell line, confirmed the results of both previous reports. Because of the pertinence of this area of research to the estimate of human cancer risks due to the accumulation of multiple small increments of radiation dose, the present series of experiments were designed to investigate the effect of fractionation over a wide range of doses using fresh explants of diploid cells.

The evidence provided by our cellular studies with fresh explants of diploid cells is relevant to the problem of cancer risk estimates in the human: Since splitting a dose of 50 - 150 rads into two equal fractions separated by an interval of five hours can enhance the frequency of transformation, the use of a linear extrapolation from high to low dose levels may not lead to risk estimates that are either conservative or prudent, depending on the distribution of the dose in time.

Figure RB-2-1: Dose response relationship for cell transformation following split and single doses of x-rays.

Table RB-2-1 Neoplastic transformation of hamster embryo cells following single and split dose of x rays.

Dose (rad)	Total No. Clones Counted	No. of Experiments	Mean Survival Fraction	Total Transformed Clones Counted	Mean & Cell Transformation ± 1 Standard Error	Split Dose/Single Dose ± 1 Standard Error
Control	51,699	4	1.0	0	0	0
25 + 25	15,582	4	0.96	55	0.35 $\pm$ 0.05	1.84 $\pm$ 0.24
50	14,272	4	0.92	27	0.19 $\pm$ 0.04	
37.5 + 37.5	23,762	5	0.92	255	1.07 $\pm$ 0.07	1.72 $\pm$ 0.17
75	25,882	5	0.89	160	0.62 $\pm$ 0.05	
75 + 75	13,118	3	0.73	110	0.83 $\pm$ 0.08	1.30 $\pm$ 0.18
150	15,348	3	0.65	98	0.64 $\pm$ 0.06	
150 + 150	11,925	3	0.58	48	0.40 $\pm$ 0.06	0.67 $\pm$ 0.13
300	11,450	3	0.47	69	0.60 $\pm$ 0.07	
300 + 300	11,037	3	0.15	48	0.43 $\pm$ 0.06	0.74 $\pm$ 0.14
600	11,818	3	0.10	69	0.58 $\pm$ 0.07	

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RB-3 The intercomparison of relative biological effectiveness and oxygen enhancement ratio at clinically used neutron facilities.

Eric J. Hall (in collaboration with Dr. Albrecht M. Kellerer, Institut Für Med. Strahlenkunde, Würzburg, West Germany)

The trial use of neutrons in clinics all over the world involves a substantial cost and investment of effort. Consequently, the need for full cooperation between the few centers using these particles has been recognized from the outset. Each of the neutron therapy beams in clinical use is characterized by a different energy spectrum, and as a consequence there are significant variations in their biological effectiveness. To facilitate the pooling of experience and the comparison of clinical results, a number of investigators have compared the various neutron beams using a variety of biological systems, and early results have already been published (Hall, 1977; Hall et al, 1978). The present report summarizes the results of a new series of biological intercomparisons using mammalian cells cultured *in vitro*.

The design of these experiments was based upon two important considerations.

(a) It is a characteristic of cell culture experiments that variations within an experiment are much smaller than those between experiments. Consequently, to exploit the precision of which the *in vitro* technique is capable, neutron facilities were intercompared in pairs, within a given experiment, using cells from a common culture irradiated on the same day.

(b) A standardized treatment fixture was used at all facilities, constructed of lucite, with space for 6 tissue culture flasks, and provision for an ionization chamber to be inserted into the jig to determine the dose received at the position occupied by the cells. Full build-up was ensured because the cells were overlaid with 2 cm of tissue culture medium. A substantial international

effort to achieve compatible dosimetry was mounted by the physicists at the various installations engaged in neutron therapy, as a consequence of which there is agreement to within $\pm 1.5\%$ for dose measurements in air. The use of the standard treatment fixture was an attempt to extend the compatible dosimetry to a practical set-up for the irradiation of cell cultures.

RBE INTERCOMPARISON

The neutron facilities visited, together with their principal characteristics, are listed in Table RB-3-1. For the most part, this paper will be concerned with our own experiments in the United States, Japan, Britain and Continental Europe, in which Chinese hamster V79 cells were used for RBE inter-comparisons. In a typical experiment, appropriate numbers of cells were plated into Falcon tissue culture flasks and allowed to attach by overnight incubation at 37.5°C . The flasks were then filled brimful with medium, sealed, and the temperature lowered to 17°C . For each experiment, half of the flasks were transported to the Naval Research Laboratory (NRL) cyclotron in Washington, D.C., and half to one of the other facilities listed in Table RB-3-1. The cells were transported in insulated water-jacketed carriers, with the temperature maintained at 17°C . It was found by trial and error that this temperature prevents cell division and progression through the cycle, while maintaining a high plating efficiency (which was characteristically in excess of 80%) for a period of 24 hours. Irradiations were performed simultaneously at the two neutron facilities to be compared, and the cells returned to an incubator for 8 days to assess the proportion able to form colonies.

The data from each experiment were analysed by a new non-parametric method, which evokes no form for the dose response relationship. The survival data for the two neutron beams to be compared were fitted by curves of the same shape, the only constraint being that the curve must be convex upwards, and the

Figure RB-3-1: The relative potency, or RBE relative to the Naval Research Laboratory, as a function of mean neutron energy, for various facilities in clinical use.

Figure RB-3-2: Values of the oxygen enhancement ratio (OER) for various neutron beams produced by the $p^+ \rightarrow Be$, $d^+ \rightarrow Be$, or $d^+ \rightarrow T$ reactions. Experiments at the University of Maryland at 80 MeV were repeated with a thick beryllium target and with a thin Be and Al target; the latter resulted in a neutron spectrum with a higher mean energy.

dose factor necessary to allow a common fit computed. This factor is then the best estimate of the relative potency or RBE difference between the two beams, based on the data from all dose levels studied. The results are shown in Figure RB-3-1; the potency of each beam relative to NRL is plotted as a function of the mean neutron energy. The data points fall close to a common line indicating a gradually increasing RBE with decreasing mean neutron energy. The data are summarized in a way that is more widely useful in Table RB-3-2 which consists of factors relating the RBE's between pairs of machines. In all cases, comparisons have been made on the basis of TOTAL DOSE, i.e. neutron and gamma-ray dose. For the European machines, consisting of a d^+T generator and two lower energy cyclotrons, the gamma-ray contribution is both larger and more variable than for the higher energy installation in the United States.

This report is restricted to *in vitro* data. However, recent reports (Hall et al, 1978) have summarized RBE intercomparisons performed with *in vivo* systems, such as skin and jejunal crypt cells in mice, as well as for various cultured mammalian cells. The closeness of the agreement observed bears out the suggestion made in the Part II meeting (Hall, 1977) that for intercomparing two neutron beams that differ in energy by only a modest amount, it matters little which biological system is chosen. Systems and endpoints which result in widely different values for the RBE of neutrons relative to x-rays, give similar values for the RBE difference between two closely related neutron energies. Consequently, the choice of a biological system for intercomparisons should be governed largely by its portability, repeatability and convenience.

OER SURVEY

During the past two years a standard biological technique has been used to determine the OER for neutron beams generated by the d^+Be or p^+Be processes, where the energy of the accelerated charged particle has ranged from 15 to

TABLE RB-3-1 Clinical Neutron Facilities Intercompared.

	Facility	Location	Production Process	Energy of Accelerated Particle (MeV)	Mean Neutron Energy (MeV)
USA	Fermilab	Batavia, Illinois	$p^+ \rightarrow Be$	66	25
	TAMVEC	College Station, Texas	$d^+ \rightarrow Be$	50	19.3
	NRL/MANTA	Washington, D.C.	$d^+ \rightarrow Be$	35	14.3
	NASA/GLANTA	Cleveland, Ohio	$d^+ \rightarrow Be$	25	10
	Univ. Wash.	Seattle, Washington	$d^+ \rightarrow Be$	22	8
U.K.	MRC, Hammersmith	London	$d^+ \rightarrow Be$	16	7
	MRC/Edinburgh	Edinburgh	$d^+ \rightarrow Be$	15	6
Netherlands	Antoni van Leuwenhoek Hospital	Amsterdam	$d^+ \rightarrow T$	--	14
Japan	NIRS	Chiba	$d^+ \rightarrow Be$	30	12
	IMS	Tokyo	$d^+ \rightarrow Be$	15	6

TABLE RB 3-2 Relative Potency of Neutron Beams.

	EDIN	HAM	AMS	U.W.	CLEV.	NIRS	NRL	TAM	FERMI
EDIN	**	1.04	1.01	0.95	0.95	0.91	0.90	0.86	0.82
HAM	0.97	**	0.97	0.91	0.92	0.87	0.87	0.83	0.79
AMST	0.99	1.03	**	0.94	0.95	0.90	0.89	0.85	0.81
U.W.	1.06	1.10	1.07	**	1.01	0.96	0.95	0.90	0.87
CLEV.	1.05	1.08	1.06	0.99	**	0.95	0.94	0.90	0.86
NIRS	1.10	1.14	1.11	1.04	1.05	**	0.99	0.95	0.91
NRL	1.11	1.15	1.12	1.05	1.06	1.00	**	0.95	0.91
TAM	1.17	1.21	1.18	1.11	1.12	1.06	1.05	**	0.96
FERMI	1.22	1.26	1.23	1.15	1.16	1.10	1.10	1.04	**

101 MeV. For these experiments hamster V79 cells were used and hypoxia produced by crowding a large number of cells into a small volume so that oxygen was consumed by respiration (Hall, Lehnert, and Roizin-Towle, 1974). The results are shown in Figure RB-3-2. There appears to be a continuous increase in OER with mean neutron energy, and our experience does not confirm the lower OER at high neutron energies previously reported (Harrison, Kubiczek, and Robinson, 1975, 1976). Using the same biological system, it has been shown also that a collimated beam of neutrons from the d^+T generator at Amsterdam is characterized by an OER equal to that of the clinically used cyclotrons in the United States. The value obtained, however, is significantly lower than the OER previously reported for 14 MeV d^+T neutrons in a scatter free environment.

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RB- 4 Sister chromatid exchanges: Interaction of a retinoid (Ro-10-9359) and a protease inhibitor (antipain) with x rays.

Melanie R. Freeman and Charles R. Geard

The induction of sister chromatid exchanges (SCE) was studied at the first post-irradiation mitosis of mouse C3H/10T $\frac{1}{2}$ cells and Syrian hamster embryo cells following 300 rad of x rays and incubation with a protease inhibitor (antipain) or a vitamin A analog (Ro-10-9359).

As seen in Figure RB-4 -1 and Table RB-4 -1, antipain alone and 300 rad x rays alone produce the same level of SCEs in 10T $\frac{1}{2}$ cells. When antipain is present during x-irradiation, or added after x-irradiation, the SCE frequency does not increase above the level of antipain alone. This indicates that antipain may prevent or reduce x-ray-induced SCEs in 10T $\frac{1}{2}$ cells, and does not need to be present during x-irradiation to be effective.

A similar effect is seen with the retinoid Ro-10-9359 in 10T $\frac{1}{2}$ cells. Ro-10-9359 slightly increases the SCE frequency over the control value, while x rays induce an even higher SCE frequency (factor = 1.8). When Ro-10-9359 is present during x-irradiation, the SCE frequency is reduced to nearly the level characteristic of Ro-10-9359 alone, and implies that Ro-10-9359 may also prevent or reduce x ray-induced SCEs in 10T $\frac{1}{2}$ cells.

The effect of antipain on hamster embryo cells is somewhat different. Antipain alone induces some SCE's (factor = 1.2), while x rays induce a much higher level (factor = 2.0 and 2.3 for A2 and A3, respectively). When antipain is present during x-irradiation, the SCE frequency is reduced (factor = 1.6 and 1.8 for A2 and A3), but does not reach the antipain alone level. In contrast, when antipain is added after irradiation, the SCE frequency was reduced slightly in one experiment (factor = 1.7 for A2) and increased slightly in another

Figure RB-4-1: Histograms are shown indicating the proportion of 1st, 2nd, and 3rd division cells, of mitoses in the sampled population and sister chromatid exchanges (S.C.E.'s) per chromosome. Mouse C3H/10T $\frac{1}{2}$ cells and Syrian hamster embryo (S.H.E.) cells were treated with antipain before and after irradiation with 300 rads 300 kVp x-rays.

Table RB- 4 -1 Sister Chromatid Exchanges Per Chromosome (S.e. $\bar{x}$)

<u>Treatment</u>	<u>(A1) C3H10T$\frac{1}{2}$</u>	<u>(A2) Hamster</u>	<u>(A3) Hamster</u>	<u>(R1) C3H10T$\frac{1}{2}$</u>
BUDR alone	0.26(0.01)	0.34(0.02)	0.38(0.01)	0.24(0.01)
BUDR + AP	0.47(0.02)	0.41(0.01)	0.47(0.02)	---
BUDR + Ro-10-9359	---	---	---	0.29(0.01)
BUDR + 300 rad	0.44(0.02)	0.69(0.02)	0.88(0.02)	0.42(0.02)
BUDR + AP + 300 rad	0.47(0.02)	0.53(0.02)	0.67(0.02)	---
BUDR + Ro-10- 9359 + 300 rad	---	---	---	0.33(0.02)
BUDR + 300 rad + AP(post-irrad)	0.44(0.02)	0.58(0.02)	0.98(0.02)	---

experiment (factor = 2.6 for A3) when compared to the x ray alone level. This implies that antipain must be present during x-irradiation to be most effective in hamster embryo cells.

The effect of antipain and x rays on the mitotic indices and the appearance of 1st, 2nd, and 3rd division mitotic cells is shown in Figure RB-4.-1 for $10T\frac{1}{2}$ cells collected at 30 hours and hamster embryo cells collected at 6 hours post-irradiation. In hamster embryo cells, antipain and x rays alone or combined delayed some of the cells causing an increase in 1st division cells and a corresponding decrease in 2nd division cells. This effect is paralleled by a decrease in mitotic indices. In contrast, $10T\frac{1}{2}$ cells show little effect from antipain or x rays alone, and a slight delay when antipain is added after irradiation. However, when antipain is present during irradiation, a pronounced decrease in 1st division mitoses and an increase in 2nd division mitoses is seen with a corresponding decrease in mitotic index. The present results suggest that antipain and the retinoid Ro-10-9359 act as radioprotectants in $10T\frac{1}{2}$ and hamster embryo cells by reducing the level of radiation-induced SCEs. That these results bear a positive application to malignant transformation requires further investigation.

RB-5 The effect of neutron induced recoil protons of restricted energy and range on chromosome aberration formation.

Charles R. Geard

The material presented in last years Annual Report (RB-12) has been expanded and updated by the inclusion of data obtained from assessing the effects of 430 keV monoenergetic neutrons on *Vicia faba* chromosomes. Further, a more precise determination of microdosimetric quantities of relevance has resulted in some alterations. Essentially, physical energy deposition events (as determined by microdosimetry) have been related to chromosomal changes (as determined by light microscopy) in nuclei irradiated in the latter part of the cell cycle of *Vicia faba*.

Monoenergetic neutrons of 230, 320, 430 and 1910 keV were produced via the $T(p,n)^3\text{He}$ reaction with the van de Graaff accelerator at RARAF. Since these neutrons release their energy almost exclusively into recoil protons, which then form a rectangular frequency distribution with energy, the effects of recoil protons over defined ranges of energy and track length can be determined by overlapping the results from the different neutrons at different doses (Fig. RB-5-1).

Microdosimetric spectra allow for the determination of the frequency of energy deposition events per rad from measurements with proportional counters filled with tissue equivalent gas at partial pressures. Altering the pressure simulates increasing or decreasing a normal density volume, so that frequencies can be contrasted against simulated diameters which can be thought of as diameters of cell nuclei. A plot of these quantities for the four monoenergetic neutrons under investigation is shown in Figure RB-5-2. Here, for the determined diameter of the cells under study, estimates of the frequency of energy deposition events per rad can be made. The properties of the four sets of recoil protons are given in Table RB-5-1.

Figure RB-5-1: Distribution of recoil proton energies after irradiation with unit doses of 230,320,430 and 1910 keV monoenergetic neutrons. The percentage of recoil protons from each range to the total distribution for 430 and 1910 keV monoenergetic neutrons are indicated.

Figure RB-5-2: Frequency of energy deposition events per rad versus simulated nuclear diameter as determined by microdosimetry for 230,340,430 and 1900 keV monoenergetic neutrons. Open symbols are derived from simulation by Caswell while solid symbols refer to measurements taken in this laboratory.

Table RB-5-1

Properties of Recoil Protons Initiated by Monoenergetic Neutrons

		Recoil Proton Energy (keV)									
		1500	1000	500	400	300	200	150	100	50	20
Range	(microns)	42	22.5	8.0	5.8	4.0	2.4	1.8	1.2	0.7	0.4
Mean	Linear energy										
	transfer										
	(keV/ μ)	36	44	62	69	75	83	83	83	71	50
Cumulative	1910	<21	<48	<74	<79	<84	<90	<92	<95	<97	<99
% of	keV										
given											
energy	430				<7	<30	<53	<65	<77	<88	<95
	keV										
	320					<6	<38	<53	<69	<84	<94
	keV										
	230						<9	<35	<56	<78	<91
	keV										

Primary roots of *Vicia faba* (var. Prolific Long Pod) were grown to about 10 cm in aerated culture solution. Root meristems were irradiated in specially constructed lucite jigs in aerated culture solution in groups of 12. After irradiation 3 or 4 roots were fixed in cold 3 parts ethanol: 1 part glacial acetic acid at 4, 6, 8 and 10 hours following accumulation of mitoses for 2 hours with 0.05% colchicine. Meristems were hydrolysed in 1 N HCL for 6 mins., stained with Feulgen, excised meristems squashed in 45% acetic acid and slides made permanent after cover slip removal. Estimates of nuclear dimensions were made by photographing nuclei, projecting negatives at an actual magnification of 4,300 x, then measuring the axes. Also traced nuclei were cut out, weighed and areas determined using a pre-standardised formula. Nuclear diameters for sphere-equivalents are shown in Figure RB-5-3. On this probit plot of cumulative frequency versus log nuclear diameter, a straight line indicates that nuclear diameters are log-normally distributed.

It can be seen at the 50% level that the mean nuclear diameter is 12.5 microns. Growing meristems were also pulse labeled with tritiated thymidine, then with C^{14} labeled thymidine, thus allowing assessments of nuclear diameters of early versus late S, and early mid G_2 cells. The sampling period utilised here excludes the large late G_2 and early prophase cells and the small G_1 and early S phase cells, and a mean sampled nuclear diameter of 13 microns is probably the most reasonable.

All cytologically visible alterations to the chromosomes were recorded, along with the associations (if any) between them. Results from individual accumulation periods were somewhat variable (Fig. RB-5-4) possibly indicating the effects of differential mitotic delay. The combined results, however, clearly indicate a linear dose-response relation (Fig. RB-5-5) where it can be seen that 320 keV neutrons are the most efficient at aberration production, followed by 230 and 430 keV neutrons and then 1910 keV neutrons. Values of the Relative Biological Effectiveness

Figure RB-5-3: Cumulative frequency of *Vicia faba* root tip nuclei on a probit plot versus nuclear diameter on a log scale. The straight line relationship indicates that nuclear diameters follow a log-normal distribution.

Figure RB-5-4: Aberrations per cell versus absorbed dose in rads for metaphase cells colchicine accumulated over 2 hourly periods after irradiation. Data points are joined on a log-log plot.

Figure RB-5-5: Accumulated aberrations per cell versus absorbed dose (rad) on a log-log plot. R.B.E.'s for the various neutron energies are indicated. Data were fitted to the relation $y = \alpha D$ where y = yield, D = dose, and α a constant.

(RBE) for equivalent aberration levels are also given in Figure RB-5-5. That is, the source of radiation producing the greatest proportion of high LET recoil protons is the most effective biologically.

In analysing the chromosomal aberrations, which were all of the chromatid type, i.e., induced post DNA replication, the most frequent were isochromatid deletions which result from breakage of sister chromatids at isologous points. Sister chromatids are expected to be intimately associated since they coil about each other. Chromatid interchanges involve physical exchange of parts of chromatids two chromosomes or chromosome arms. Chromatid intrachanges are intercalary deletions along the length of a chromatid usually resulting in a minute deletion. The frequencies of these three categories of aberrations are shown in Figure RB-5-6. The frequency of simple chromatid deletions was less than 5% of total aberrations for all three radiations. Thus of all aberrations seen, the frequency of those which necessitate the induction of two or more physical discontinuities is greater than 95%.

In all cases (Fig. RB-5-6) the basic response is linear with dose, however RBE relations differ for individual aberration types between the three neutron energies. Thus, a single determination of RBE for total aberrations does not necessarily reflect those for individual aberrations where the spatial relations between the initiating lesions are likely to differ.

The curves of Figure RB-5-2 allow estimates of the frequency of energy deposition events per rad per nucleus. For these monoenergetic neutrons the vast majority of physical events will be due to recoil protons and thus for the mean nuclear diameter plots can be constructed of aberrations per cell against recoil protons per cell. Such is shown in Figure RB-5-7 where as before there is a linear relation between aberrations and recoil protons per nucleus. On a log-log plot where the relation $y = \alpha P$ (y -yield, P -recoil proton with α a constant) the linear

term is obtained from the intercept. Data were fitted by least squares computer fit to the linear relation and the α terms which are also shown as intercepts are given in Figure RB-5-7. Here it can be seen that the order in terms of effectiveness has changed. Rather than 320 keV neutrons being the most effective as they were in terms of dosage, now 430 keV neutrons are the most effective. The α term essentially gives the proportion of effective proton recoils. Thus, for 430 keV monoenergetic neutrons, 21% of recoil protons incident in 13 μ diameter *Vicia faba* nuclei, with 18.5, 14.5 and 13.5% for 320, 1910 and 230 keV monoenergetic neutrons respectively.

For the three major classes of aberrations, plots of the frequencies of isochromatid deletions, chromatid interchanges and chromatid intrachanges versus recoil proton incidence are shown in Figure RB-5-8. Obviously the relationship for total aberrations shown in Figure RB-5-7 does not hold for all aberration types. For isochromatid deletions and chromatid interchanges the same order is maintained. However, for the chromatid intrachanges the frequency response relations are more scattered and overlapping. The same constraints apply as described earlier. Thus a recoil proton from a 430 keV neutron has about a 10% chance of producing an isochromatid deletion.

The frequency of recoil protons per nucleus for varying nuclear diameters are given in Table RB-5-2, along with the aberrations per recoil proton for a 13 μ nucleus. For the population of nuclei sampled it is most likely that there has been about a two fold difference in the frequencies of incident recoil protons.

Since the distribution of recoil protons from these neutrons is rectangular, recoil protons from 0 to 230 keV will comprise 12% of those from 1910 keV neutrons, while recoil protons from 230 to 320 keV will comprise 4.7%, those from 320 to 430 keV 5.8%, and recoil protons from 430 to 1910 keV the remaining 77.5% (See Fig. RB-5-1). Since the relationship between aberrations and energy deposition events (recoil protons) is linear (Fig. RB-5-7) the effects of recoil protons over the above ranges

Figure RB-5-6: Aberrations per cell versus absorbed dose (rad) for three different aberration types on a log-log plot.

Figure RB-5-7: Aberrations per cell versus the number of recoil protons per *Vicia faba* nucleus (13 μ diameter). Data were fitted by the method of least squares to the relation $y = \alpha P$, thus giving the aberration yield per incident recoil proton as indicated by the α terms.

Figure RB-5-8: Aberrations per cell versus the number of recoil protons per *Vicia faba* 13 μ nucleus on a log-log plot. Data are shown for isochromatid deletions, chromatid interchanges and chromatid intrachanges.

Table RB-5-2

Effects of Recoil Protons

Neutron energy (keV)	Recoil protons per 11μ nucleus per rad	Recoil protons per 13μ nucleus per rad	Recoil protons per 15μ nucleus per rad	Aberrations per recoil proton (13μ nucleus)
230	0.43	0.69	1.10	0.135
320	0.38	0.58	0.80	0.185
430	0.28	0.44	0.66	0.21
1910	0.22	0.33	0.43	0.145

can be readily estimated.

This is shown in Table RB-5-3, where utilization of the fact that these are overlapping proton spectra allows the extraction of data pertinent to a particular energy range of recoil protons. While recoil protons from 230 to 320 keV only comprise 4.7% of the total generated by 1910 keV monoenergetic neutrons they appear to be capable of inducing 10.2% of the aberrations that might be expected to be produced by recoil protons from 1910 keV neutrons. This increased effectiveness, however, does not apply to all aberration types since it is apparent that chromatid intrachanges are not enhanced to the same extent. Chromatid intrachanges appear to be induced most efficiently by recoil protons from 0 to 230 keV. These are the shorter range recoil protons and the fact that chromatid intrachanges result from two breaks, presumably in very close juxtaposition along a chromatid arm, (possibly a loop) may be of some relevance.

Overall, of the spectrum of recoil protons initiated by 1910 keV neutrons, the recoil protons with energies about 200 to 300 keV, travelling 2.5 to 4 microns and depositing energy at about 80 keV per micron (See Table RB-5-1) are more efficient at aberration induction than those recoil protons of lesser range though near equivalent LET, and those of greater range though lesser LET. Hence, it appears as if aberration production in these cells is not strictly dependent on linear energy transfer but that there is an influence of track length, which is probably related to the organization of chromosomes (the target material) in the interphase nucleus.

Table RB-5-3

Recoil Proton Induced Aberrations

Recoil proton energies (keV)	% from 1910 keV neutrons	Total aberrations %	Isochromatid deletions %	Chromatid interchanges %	Chromatid intrachanges %
0 - 230	12.0	11.2	10.4	10.9	13.0
230 - 320	4.7	10.2	13.1	10.7	5.2
320 - 430	5.8	11.2	12.1	11.6	9.8
430 - 1910	77.5	67.4	64.4	66.8	72.0

Microdosimetric considerations

Charles R. Geard

Neutron irradiation of living tissues characteristically results in the production of charged particles, the nature and frequency thereof being dependent upon both dose and neutron energy. For neutrons below 2 MeV the charged particles produced are almost exclusively recoil protons, which for a monoenergetic source of neutrons form a rectangular frequency distribution with energy. Thus, for a 2 MeV monoenergetic neutron beam 50% of recoil protons will have energies from 1 to 2 MeV, and their ranges, initial and mean energy deposition levels in cells, will be constrained by this initial energy. Recoil protons release energy gradually until they approach their Bragg peak when a greater fraction of energy is deposited per unit distance travelled until the proton stops and is captured.

The properties of recoil protons initiated by 1.91 MeV monoenergetic neutrons are shown in Table 1, where it can be seen that the majority of recoil protons travel distances in excess of 20 μm , whilst depositing energy at about 40 keV per μm . Obviously, if energy release into living matter from ionizing radiation is in the form of singular dissimilar energy deposition events, the concept of absorbed dose in terms of ergs per gram has little relevance at the level of the cell nucleus.

Microdosimetry utilizes the measurements of ionizations in tissue equivalent gas at low pressures in order to simulate microscopic volumes. By controlling gas pressure in a spherical proportional counter tissue spheres of varying volumes are simulated and local energy density distributions from single event spectra can be determined along with estimates of the mean number of energy deposition events per rad.

For monoenergetic neutrons below 2 MeV energy deposition events will be recoil

Table 1

Properties of Recoil Protons Initiated by 1.91 MeV Monoenergetic Neutrons

Recoil Proton Energy (keV)

	1900	1700	1500	1200	1000	800	500	200	100
Range (μm) in water	62	52	42	29.5	22.5	15.5	8.0	2.4	1.3
Initial linear energy (keV/ μm)	17	19	21	24	27	32	43	75	91
Mean linear energy (keV/ μm)	31	32	36	41	44	52	62	83	82
Cumulative % of given energy	<1	<11	<21	<37	<48	<58	<74	<90	<95

protons and a plot of frequency versus simulated nuclear volume is shown in Figure 1 on a logarithmic scale. In order to relate these data to a biological situation, the nuclear volumes in which the dose is delivered need to be determined along with an assessment of effects at a sub-nuclear level. Such is the case in this study, where chromosomal alterations in *Vicia faba* meristematic nuclei have been assessed after irradiation with 1.91 MeV monoenergetic neutrons.

Materials and Methods

Primary roots of *Vicia faba* (var. Prolific Long Pod) were grown to about 10 cm. in aerated culture solution. Root meristems were irradiated in specially constructed lucite jigs in aerated culture solution in groups of 12. After irradiation, three roots were fixed in cold 3 parts ethanol: 1 part glacial acetic acid at 4, 6, 7, 10 and 12 hours following accumulation of mitoses for 2 hours with 0.05% colchicine. Controls were similarly handled. Permanent Fielgen squashes were prepared and chromosome aberrations scored at metaphase.

For assessment of nuclear volumes non-colchicine treated roots were similarly handled except that following hydrolysis and Fielgen staining meristems were macerated in 45% acetic acid on slides until largely single cell suspensions were produced. Cover slips were then placed on the slides and blotted without pressure to prevent distortion of the nuclei. Fields of nuclei were then photographed and negatives made into transparencies which were projected at a final actual magnification of 4,300 times. The major and minor axes of individual nuclei were then measured, with the vast majority of meristematic nuclei being near circular or ovoid. Notation was also made as to the nature of the cell, whether it was early, mid or late prophase, late telophase or early G1. Volumes of individual nuclei were then determined.

Neutrons were produced via the $T(p,n)^3\text{He}$ reaction with incident particles of 2.82 MeV generated by the RARAF* Van de Graaf accelerator. The energy spread

Figure RB-6-1: Frequency of energy deposition events per rad as determined by microdosimetry versus simulated nuclear volume. Nuclear volumes were simulated by maintaining tissue equivalent gas at partial pressure.

around 1.91 MeV was +6.5, -7.5%, while the dose spread through the beam roots due to emission angle (0^0) was +2.5, -3.5% and due to depth of field $\pm 2\%$. The gamma ray contribution to total dose was 0.8% and the dose rate 3.5 rad per minute.

Absorbed dose determinations by means of a tissue-equivalent parallel plate ionization chamber were checked against a monitor ionization used during irradiations. Gamma ray absorbed doses were measured with an energy compensated Geiger-Mueller dosimeter.

Results

Nuclear volume measurements

All nuclei within randomly selected fields of view were measured and the volumes of 471 nuclei ranked into a cumulative distribution. It was determined that nuclear volumes were not normally distributed and that they followed more closely a log-normal distribution. This is indicated in Figure 2 where percent cumulative frequencies are given as a probit plot against log nuclear volume. On a probit plot a normally distributed population of values yields a straight line. Mean nuclear volume was 1060 cubic microns.

Mitotic index determinations

The frequency of cells in mitosis was determined for each dose-time regime in samples of 3600 cells from each of 3 meristems. The mean percentage of cells in mitosis for the 2 hour colchicine accumulation periods are shown at the mid-point of each period in Figure 3. It is apparent that there is an initial dose-dependent decline in the percentage of cells in mitosis with a subsequent recovery and overshoot of control levels. The roots from the 10-12 hour accumulation period after 80 rad of neutrons were mishandled, thus there is no data for this dose-time period.

Achromatic lesions

Achromatic lesions or gaps represent a Feulgen stain-less region along the length of a chromatid and not necessarily a physical discontinuity. Since they are

Figure RB-6-2: Percent cumulative frequencies as a probit plot against log nuclear volume of *Vicia faba* nuclei derived from root meristems. A straight line on a probit plot indicates a normal distribution hence nuclear volumes are log-normally distributed.

Figure RB-6-3: Percentage of cells in mitosis (mitotic index) from *Vicia faba* root meristems after irradiation with 1.9 MeV monoenergetic neutrons. Points shown are for the mid-points of two hourly colchicine accumulation periods.

scored on the basis of there being no physical displacement of the chromatid they are prone to some subjectivity in scoring, since chromatid deletions are scored as such if there is a physical displacement of the chromatid by at least half a chromatid width. Achromatic lesions may occur as twins in sister chromatids (isochromatid gaps) and the frequencies of single gaps and total gaps (chromatid plus isochromatid) are shown in Figure 4. In this plot of log frequency versus log absorbed dose for each time period a linear relationship would be a line of 45° slope. Whilst essentially linear, there is no consistent dose-response relationship although the frequency of achromatic lesions seem to be more enhanced in the 2-4 and 4-6 hour periods.

Chromosome aberrations-frequencies and dose-response relations

Chromosome aberrations involve physical alterations of chromosomal order and were scored in accordance with the scheme outlined by Savage. The frequencies of aberrations from each time period are shown in Figure 5 where it can be seen that aberrations per cell are related to absorbed dose in a linear manner. Data have been fitted to the form $y = \alpha D$ where y is yield of aberrations, D is absorbed dose and α a constant. While there are indications that there is a greater yield of aberrations from the 4-6 hour time period, basically there is no significant variation between yields over time. This is perhaps surprising in light of the substantial variation in cells in mitosis between doses over time. That is cells reaching mitosis during either the declining or recovery phases of the mitotic index curves show little variation in aberrations per cell. This would indicate that those cells that are delayed in progression through the cell cycle are not necessarily those carrying the highest level of aberrations.

When the yield of individual aberration types is considered, it can readily be seen that isochromatid deletions form the most frequent class, followed by chromatid interchanges, chromatid intrachanges and chromatid deletions. This

Figure RB-6-4: Frequencies of achromatic lesions (gaps) per cell for 2 hour colchicine accumulation periods after irradiation with 1.9 MeV monoenergetic neutrons. Data are expressed on a log-log plot.

order is maintained over all collection periods although the relative paucity of data for the less frequent aberration classes results in considerable scatter. Overall, isochromatid deletions comprised 42.2% and chromatid deletions comprised 11.2% of all aberrations. That is, aberrations which result from interaction between two damaged sites made up 89% of the total.

Figure RB-6-5: Frequencies of aberrations per cell for 2 hourly colchicine accumulation periods after irradiation with 1.9 MeV monoenergetic neutrons. Data are expressed on log-log plots for total aberrations (circles), isochromatid deletions (squares); chromatid interchanges (triangles); chromatid intrachanges (inverted triangles) and chromatid deletions (diamonds). Standard error bars are indicated for total aberrations while all data have been fitted by the method of least squares to the simple relationship y (yield) = α (a constant), D (absorbed dose in rad).

RB-7 Sequential exposures with high and low-LET radiations.

R.P. Bird

A series of cell survival measurements has been initiated using a first dose of high-LET charged particles to irradiate cells followed by a second dose of low-LET x-rays. These experiments are planned to determine the extent to which high-LET sublesions interact with low-LET sublesions as a function of time between the two exposures. The RARAF track segment facility provides beams of charged particles and it also has available a 50 kV x-ray unit. Irradiations are performed with the aid of computer-controlled positioning of samples in an irradiation fixture. A recent modification to the computer program will permit moving a sample dish from the charged-particle port to the x-ray port for sequential irradiations with time intervals as short as a minute. These measurements will be made using Chinese hamster V79 cells that are grown and synchronized using procedures described previously (1).

Measurements have been made with synchronized cells irradiated with split doses of x rays only. The cells were exposed at room temperature to 50 kV x rays with the total dose delivered either in one exposure or in two equal exposures separated by a few minutes or as long as 30 minutes. Dishes of irradiated cells, as well as control dishes, were simply kept on a lab bench at room temperature between irradiations. For the longest interval used (30 minutes) the pH of the cell medium increased slightly. Synchronized cells, either at the G_1/S transition or in late S phase, were irradiated with single doses to about the 5% survival level. Figure RB-7-1, panels A and B, show the results of these experiments, and indicate that splitting the radiation dose over these time intervals has very little effect on the cell surviving fraction. Enhancement of mammalian cell survival for split doses separated by minutes has been reported (2), for

Figure RB-7-1: The effect on synchronized V79 cells of two x-ray exposures separated by short time intervals. The different symbols denote separate experiments wherein the surviving fractions, with zero time interval, were about 5%. Relative survival is plotted here for direct comparison between experiments.

cells irradiated in plateau or stationary phase. There is no obvious trend of enhancement for the synchronized cells irradiated as described.

A preliminary determination of the effect of a high-LET dose followed by a low-LET dose has been made using deuterons (50 keV/ μ m) and the 250 kVp x-ray machine at RARAF. The time interval between the sequential exposures was 20 to 30 minutes. Two measurements using late S phase cells have been made, each including a deuteron survival curve and an x-ray survival curve as well as the sequential exposures in which a deuteron dose of 600 rad was followed by graded x-ray doses. The results are shown in Figure RB-7-2. The average of the surviving fractions for a deuteron dose of 600 rad is 6.8% and this survival level is indicated by the dashed line across Figure RB-7-2. A ten-fold reduction in survival below this 6.8% level is indicated for each survival curve. Deuterons produced the ten-fold decrease with 330 to 340 rad of additional dose delivered without a time delay. In contrast, the 20 to 30 minute delay followed by x-irradiation necessitated a 690 rad dose to produce the ten-fold decrease in surviving fraction. The x-ray survival curve shows this same reduction from the 6.8% survival is achieved by an additional x-ray dose of 580 to 590 rad delivered without interruption. The sequential exposures separated by about one-half hour clearly indicate that the 6.8% surviving fraction of cells following the deuteron dose of 600 rad have accumulated damage that interacts with additional low-LET damage. The additional low-LET dose delivered within one-half hour is not nearly as efficient as the uninterrupted deuteron irradiation.

References

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Figure RB-7-2: The results of two experiments where late S phase cells were irradiated with a beam of 50 keV/ μ m deuterons, or 250 kVp x-rays, or sequentially with a 600 rad dose of deuterons followed by graded x-ray doses within 20 to 30 minutes. The open and closed symbols denoted the two experiments.

RB- 8 The associated ion experiment

Richard P. Bird

Diatomic Ions

Diatomic deuterium ions were used as a source of correlated pairs of ions at RARAF in studies of the dependence on their separation for mammalian cell killing (1,2). However, the separations that could be attained, while holding constant the LET of the particles, are limited by the available RARAF energy. The RARAF acceleration voltage is adjusted so that the LET of the particles, as they subsequently traverse mammalian cells, is the same for each Mylar thickness. In order to extend the studies to greater separations, a change was made to diatomic hydrogen ions as the source of correlated pairs of ions. Table RB-8-1 lists the characteristic separations that result when diatomic hydrogen ions traverse different Mylar thicknesses. For comparison, the characteristic separation is approximately the same for paired deuterons traversing 10 μm Mylar plus a 3 μm cell thickness as it is for paired protons traversing 6 μm Mylar plus a 3 μm cell thickness.

The procedures for growing, synchronizing, and irradiating Chinese hamster V79 cells remain as previously reported (1,2). The measure of the effectiveness of correlated ions in these studies is the comparison of the surviving fractions obtained when irradiating cells with the same dose delivered either as single (uncorrelated) ions or as paired (correlated) ions. The surviving fractions are measured in one experimental setup for direct comparison. Results obtained from irradiating late S phase cells with protons are shown in Figure RB-8-1 as survival ratios for different doses where the ratio is the surviving fraction for single protons relative to that for paired protons. The broken lines indicate the previously obtained results using deuterons (1,2). Figure RB-8-1 indicates that the survival ratios are reduced by using protons and thicker Mylar foils to achieve greater

Figure RB-8-1: Survival ratios comparing paired and single protons delivered as the same dose of 32 keV/ μm particles. Two Mylar thicknesses (10 μm and 19 μm) were used. The dashed lines show the trend of results obtained previously with paired and single deuterons (1,2), also of 32 keV/ μm .

Table RB-8-1

Paired protons of 32 keV/ μm linear energy transfer

Mylar thickness (μm)	Characteristic separation*	
	Mylar exit	6 μm cell depth
6	140 nm	390 nm
10	285 nm	525 nm
19	595 nm	920 nm
23	770 nm	1110 nm

*Values determined by Dr. N. Rohrig, Brookhaven National Laboratory.

separation of the correlated pairs of ions.

Triatomic ions

Further studies to measure cell killing with larger particle separations will use triatomic hydrogen ions. The triple particle irradiation set-up has been used by Dr. Geard for studies on chromosome aberrations (3) and one test has been made for cell killing. In this test, a triplet proton irradiation was compared with a paired proton irradiation that followed it the same day. For each irradiation, cells were synchronized to be in the late S phase, the Mylar thickness was 6 μm , and the particle LET was 32 keV/ μm . For a dose of 600 rad, the survival ratio for the paired protons was 1.36, as expected (like that of paired deuterons and 10 μm Mylar), and was 1.81 for the triplet protons.

Radical scavenger and diatomic ions

Cysteamine at a concentration of 75 mM has been shown to be an effective radiation protection agent for high LET charged particles (4). A trial experiment was done to test its effect on the survival ratio obtained with paired deuterons. A dose of 800 rad was delivered to late S phase cells and the protective effect of

cysteamine altered the surviving fraction obtained with single (uncorrelated) ions from 0.6% to 30%. The effect on the survival ratio, for cells grown on 6 μ m Mylar, was inconsequential with values of about 1.8 for untreated cells and about 1.7 for cysteamine-protected cells. The effect of the radical scavenger in this initial test was to protect against lethality although the differential killing effect of correlated ions was unaltered.

References

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RB-9 Irradiations with the track segment facility and the analysis of data

Richard P. Bird and Paul J. Kliuga

Data Analysis

Results from track segment irradiations of synchronized mammalian cells were reported previously (1) for a range of LET values. Table RB-9-1 lists the ions that were used and their LET values. The highest LET values resulted from helium track segments with a considerable change in LET across cellular dimensions.

The survival curves obtained for two phases of the cell cycle, namely G_1/S and late-S, were fitted by computer analysis to the form:

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

where D is the dose and α and β are constants. The best-fit values of the parameters, α and β , are listed in Table RB-9-2 with 95% confidence limits indicated.

Figures RB-9-1 and RB-9-2 indicate the LET trends of the survival curve parameters for late-S and G_1/S cells, respectively. The values of $\alpha \cdot L$ are proportional to the cell inactivation probabilities per charged particle and show an increase with LET over the lower range of LET values. The high LET values approach a plateau or saturation value. The values of $\sqrt{\beta} \cdot L$ are a measure of the probability per particle track that sublesions are produced for two-track inactivation. These values also increase with LET but may show a saturation at lower LET values. However, determination of the values of β for high LET survival curves is limited by the restriction of the dose range that can be used.

Additional Track Segment Data

Further track segment results have been obtained using a line of V79 cells with an altered radiation response. The procedures for growing, synchronizing, and irradiating cells have been the same as previously reported (1). This "subline" is indistinguishable from the V79 cells used previously except for its radio-

Table RB-9-1

Physical characteristics of the charged particles

<u>Ion</u>	<u>Accelerated energy (MeV)</u>	<u>LET (keV/μm)</u>			
		<u>Track average</u>	<u>Entrance - 6 μm depth</u>		
Proton	4.0	10.2	10.1	-	10.3
Deuteron	3.3	20.4	20.2	-	20.6
	2.2	31	30	-	32
	1.9	40	37	-	43
Helium-3	7.9	67	65	-	69
	6.3	91	87	-	96
	5.0	127	116	-	143
	4.6	170	142	-	219

Table RB-9-2

Survival curve parameters for different radiations

<u>LET (keV/μm)</u>	<u>late-S cells</u>		<u>G₁/S cells</u>	
	<u>α (krad⁻¹)</u>	<u>β (krad⁻²)</u>	<u>α (krad⁻¹)</u>	<u>β (krad⁻²)</u>
10	1.22+0.18	1.49+0.16	3.99+0.69	2.72+1.06
20	2.08+0.42	2.39+0.42	4.97+0.88	5.23+1.40
31	2.72+0.51	2.54+0.60	7.73+1.46	4.61+2.45
40	3.64+1.23	3.69+2.15	8.54+1.12	11.53+3.47
67	-	-	11.65+2.32	6.30+8.16
91	10.14+1.45	(-2.39+2.87)	15.37+1.12	(0.634+3.46)
	6.53+1.16	(1.03+2.30)		
127	9.64+1.10	(-1.91+2.37)	12.76+1.93	(4.39+6.07)
170	6.61+0.42	(-0.044+0.56)	14.88+2.13	(-5.82+7.13)

The β values in parentheses are not significantly different from zero.

Figure RB-9-1: The trends in cell survival parameters, as a function of LET, for late S cells.

Figure RB-9-2: The trends in cell survival parameters, as a function of LET, for G₁/S cells.

sensitivity: the G_1/S phase is more radiosensitive and the late-S phase is more radioresistant. Figures RB-9-3 show a series of survival curves for the two synchronized cell-cycle phases irradiated with charged particles of different LET. An obvious feature of the late-S cell survival curves is the curvature for all LET values. These more resistant cells allow a determination of the trend of two-track events over a broader LET range. Table RB-9-3 lists the computer-fitted values of α and β from the survival curves. Figures RB-9-4 and RB-9-5, for late-S cells and G_1/S cells respectively, show the corresponding trends of $\alpha \cdot L$ and $\sqrt{\beta} \cdot L$ with the previously obtained trends indicated by the broken lines.

Reference

1. R.P. Bird, "Track segment experiments: cell survival data", in Progress Report, 1978, C00-4733-1 (H.H. Rossi and E.J. Hall, eds).

Figure RB-9-3: Cell survival curves for different LET values for late S cells and for G₁/S cells (panels A and B, respectively).

Table RB-9-3

Survival curve parameters for different radiations

LET (keV/ μ m)	late-S cells		G_1 /S cells	
	(krad^{-1})	(krad^{-2})	(krad^{-1})	(krad^{-2})
10.4	0.387 $\pm$ 0.224	1.45 $\pm$ 0.17	4.74 $\pm$ 0.39	3.07 $\pm$ 0.50
10.7	0.624 $\pm$ 0.224	1.49 $\pm$ 0.15	---	---
39	2.63 $\pm$ 0.24	1.83 $\pm$ 0.38	9.71 $\pm$ 0.92	4.48 $\pm$ 2.57
40	2.36 $\pm$ 0.32	2.51 $\pm$ 0.35	11.12 $\pm$ 0.74	3.83 $\pm$ 1.97
50	2.63 $\pm$ 0.33	2.55 $\pm$ 0.35	13.01 $\pm$ 1.08	(0.647 $\pm$ 2.89)
91	4.35 $\pm$ 0.76	2.47 $\pm$ 1.14	13.21 $\pm$ 1.67	(3.67 $\pm$ 5.26)
162	4.03 $\pm$ 0.38	4.07 $\pm$ 0.60	---	---
169	5.38 $\pm$ 1.12	(0.97 $\pm$ 1.16)	14.11 $\pm$ 1.31	(-0.65 $\pm$ 3.82)

The β values in parentheses are not significantly different from zero.

Figure RB-9-4: The trends in cell survival parameters, as a function of LET, for late S cells. The corresponding trends obtained previously and shown in Figure RB-9-1 are indicated by dashed lines.

Figure RB-9-5: The trends in cell survival parameters, as a function of LET for G₁/S cells. The previous trends (Figure RB-9-2) are given by dashed lines.

RB-10 The modulating effects of the protease inhibitor Antipain on x-ray induced transformation

Carmia Borek and Richard C. Miller

Protease inhibitors have been shown to inhibit the expression of mutations in bacteria and to inhibit the tumor-promoting effect of phorbol esters in mice.

We have investigated the effect of the protease inhibitor Antipain on cell transformation by x-irradiation in two *in vitro* systems; namely short-term cultures of freshly explanted hamster embryo cells and in the 10T $\frac{1}{2}$ cell line derived and cloned from C3H mouse embryo.

Materials and Methods

Cell Cultures

Hamster embryo cells. Minced midterm whole embryos from golden hamsters were used as the source of normal cells. Primary cultures were established by progressive dissociation of the minced fresh tissue. For transformation experiments the cells in 3-day-old primary cultures were suspended by trypsinization and 10⁴ cells were cloned into 100-mm petri dishes (Falcon) on x-irradiated (4000 rad) feeder cells of the same type in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/ml) and streptomycin (50 µg/ml).

C3H/10T $\frac{1}{2}$ clone 8 cell line. Cell cultures were maintained in Eagle's basal medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (50 units/ml), and streptomycin (50 µg/ml). For transformation experiments, 6000 cells were plated onto 100-mm petri dishes, allowing for the survival of 400-500 cells after radiation.

X-irradiation. Cells were irradiated at room temperature, 24 hours after plating, with 300 rad of x-rays at a dose rate of 32.2 rad/min and incubated at 37°C with 5% CO₂ in air, with weekly medium changes. The source of x-rays was a Siemens

300 kVp constant potential generator with an added filter of 0.2 mm copper. Controls consisting of unirradiated cultures with or without added Antipain were maintained under the same conditions as the experimental cultures.

After the incubation period, 2 weeks for the hamster cells to allow for colony growth and 6 weeks for the 10T $\frac{1}{2}$ cells to allow for focus formation, cells were fixed and stained with Giemsa and assayed for transformation.

Antipain. The water-soluble protease inhibitor Antipain, ((S)-1-carboxy-2-phenylethyl) carbamyl-L-arginyl-L-valyl-L-gininal, was obtained from the U.S.-Japan Cancer Program. Antipain was added to the cell cultures in complete medium at a final concentration of 6 μ g/ml. Figure 1 describes the protocol employed. Either Antipain was added at plating time and kept on during irradiation and throughout the period of incubation, or, alternately, Antipain was added at 10 minutes, 24 hours, or 48 hours after irradiation. After the initial addition, Antipain was maintained in the medium throughout the period of incubation.

Transformation Assay

Hamster Embryo Cells. After the appropriate period of incubation, medium was removed and cells were fixed and stained with Giemsa. A differential count was made of normal and transformed colonies, the latter being identified by their morphological appearance from among the surviving colonies. The number of normal and transformed colonies was scored so that both transformation frequency and cell survival could be assessed within the same experiment. Transformed colonies were identified by their irregular growth pattern and their tendency to form multilayers as compared to controls (Fig. 2). The relationship between this morphology and the malignant nature of the cells has been documented.

10T $\frac{1}{2}$ Cells. After the six week incubation period, dense foci of transformed cells growing as a multilayer were easily distinguishable morphologically from the flat confluent sheets of untransformed cells (Fig. 2). The guidelines described

Protocol:

Figure RB-10-1: General design of the experiments with antipain and the observed results on its effects on x-ray induced transformation frequency at scoring time. Cells were plated at time 0 and irradiated 24 hr later. Antipain was added at plating time or 10 min., 24 hr., or 48 hr., after irradiation. Transformed colonies were scored after 2 weeks for hamster embryo cells or 6 weeks for the 10T $\frac{1}{2}$ mouse cells.

Figure RB-10-2: (A) A 12 day-old normal colony of hamster embryo cells. (Giemsa, x25) (B) A 12 day-old colony of hamster cells transformed by 300 rad of x-irradiation. Note the dense multilayers within the colony compared to the normal and the criss-cross irregular pattern at the periphery of the colony. (Giemsa, x25) (C) A monolayer of untransformed 10T½ mouse cells 6 weeks in culture. Note the flat sheet of cells. (Giemsa, x25) (D) A type III focus of 10T½ cells transformed by 300 rad of x-irradiation and 6 weeks in culture. Note the dense multilayering within the focus. (Giemsa, x25)

by Reznikoff et al., for assessing cell survival and evaluating cell transformation were strictly followed. Both type II and type III transformed foci were scored, type III being the more dense and irregular of the two.

Results

A summary of the experimental protocol employed and the overall observations obtained are presented in Figure 1.

Cell survival and transformation data after treatment with 300 rad of x-irradiation and Antipain are presented in Table 1, for the hamster embryo cells and in Table 2 for the mouse 10T $\frac{1}{2}$ cells. While Antipain treatment before or after exposure to irradiation did not alter plating efficiency or cell survival in any significant way as compared to cells treated with x-rays alone, the effects of Antipain in modifying x-ray induced neoplastic transformation are most striking. These effects are similar qualitatively in the two cell systems. When Antipain is added to the culture medium prior to radiation treatment, the result is enhanced transformation frequency, yet the addition of Antipain within 10 minutes after radiation results in decreased transformation frequency. The protective effect of Antipain is diminished in the hamster cells when the protease inhibitor is added 24 hours after irradiation and is virtually eliminated when added at 48 hours after radiation (Table 1). In the 10T $\frac{1}{2}$ mouse cells this loss of protection is observed when Antipain is added 24 hours after irradiation (Table 2).

Discussion

We have shown that the protease inhibitor Antipain has two opposing modes of action in modulating the frequency of cell transformation *in vitro* by x-rays. In both diploid short term hamster embryo cell cultures and the heteroploid 10T $\frac{1}{2}$ mouse cell line the addition of Antipain to the cultures prior to irradiation resulted in enhanced cell transformation. In sharp contrast, when added soon after irradiation, Antipain had a protective effect, resulting in decreased cell transformation.

Table 1

Cell transformation *in vitro* after treatment of hamster embryo cells with x-rays and antipain

Treatment	Surviving fraction	Total colonies (A)	No.colonies transformed (B)	Transformation incidence (B/A x 10 ³)	Yield with antipain + x-ray Yield with x-ray
Antipain added before x-irradiation with 300 rad					
24 hr					
Control	1.00	3,500	0	0	
Antipain	0.94	3,400	0	0	
X-irradiation	0.60	6,500	43	6.6 ± 1.0	
X-irradiation and antipain	0.55	8,700	106	12.3 ± 1.2	1.9 ± 0.3
Antipain added after x-irradiation with 300 rad					
10 min					
Control	1.00	20,000	0	0	
Antipain	0.96	16,000	0	0	
X-irradiation	0.53	15,000	129	8.4 ± 0.7	
X-irradiation and antipain	0.44	17,000	62	3.7 ± 0.5	0.4 ± 0.1
24 hr					
Control	1.00	14,000	0	0	
Antipain	0.92	14,000	0	0	
X-irradiation	0.60	13,000	85	6.5 ± 0.7	
X-irradiation and antipain	0.59	15,000	75	5.0 ± 0.6	0.8 ± 0.1
48 hr					
Control	1.00	22,000	0	0	
Antipain	0.89	32,000	0	0	
X-irradiation	0.65	39,000	25	6.4 ± 1.3	
X-irradiation and antipain	0.62	42,000	25	5.9 ± 1.2	0.9 ± 0.3

Table 2

Cell transformation *in vitro* after treatment of C3H/10T $\frac{1}{2}$ cells with x-rays and antipain

Treatment	Surviving fraction	Total colonies (A)	No. Colonies transformed (B)	Transformation incidence (B/A x 10 ⁴)	Yield with antipain + x-ray Yield with x-ray
Antipain added before x-irradiation with 300 rad					
24 hr					
Control	1.00	18,000	0	0	
Antipain	0.98	17,000	0	0	
X-irradiation	0.41	16,000	78	4.9 $\pm$ 0.6	1.5 $\pm$ 0.3
X-irradiation and antipain	0.38	65,000	48	7.4 $\pm$ 1.1	
Antipain added after x-irradiation with 300 rad					
10 min					
Control	1.00	38,000	0	0	
Antipain	0.94	38,000	0	0	
X-irradiation	0.38	170,000	52	3.1 $\pm$ 0.4	0.5 $\pm$ 0.1
X-irradiation and antipain	0.35	110,000	17	1.5 $\pm$ 0.4	
24 hr					
Control	1.00	5,600	0	0	
Antipain	0.96	2,700	0	0	
X-irradiation	0.40	30,000	32	1.1 $\pm$ 0.1	0.9 $\pm$ 0.1
X-irradiation and antipain	0.41	100,000	10	1.0 $\pm$ 0.3	

Under both sets of conditions in which transformation rate was modified the presence of Antipain did not influence cell survival in any significant way, suggesting, as in earlier work, that x-ray-induced cellular damage responsible for cell transformation may differ from that responsible for cell survival.

The two diametrically opposed effects of Antipain strongly suggest that the protease inhibitor Antipain has more than one mechanism of action in regulating cellular or molecular events involved in the fixation and expression of cell transformation by x-irradiation. Some of these may be involved in the modification of DNA repair.

The protective effect of Antipain added after exposure of the cells to x-rays is a temporal phenomenon and is most efficient when Antipain is added within minutes of irradiation. Its addition, to the mouse cells after exposure to x-rays and to the hamster cells, 48 hours after irradiation had no significant effect on the radiation-induced frequency of transformation.

While the molecular mechanisms of this protective effect are not understood and are yet to be explored, it is of interest to speculate on the possibilities. It is at once clear that the effect is mediated by the inhibition of early events required for the fixation of transformation by x-rays (the fixation has been shown to be a temporal event) and a subsequent phenotypic expression of this fixation. Antipain has been shown to inhibit the induction of postreplication error-prone DNA repair (SOS systems) in bacteria exposed to UV irradiation, thus the decreased yield of transformation found when Antipain is added after irradiation could be interpreted as a result of the inhibition of postreplication DNA repair mechanisms.

While there is no direct molecular evidence for these DNA repair processes in mammalian cells exposed to x-irradiation, an induction of this type of repair is suggested by our transformation studies with fractionated doses of x-rays. We have reported that both hamster and 10T $\frac{1}{2}$ cells exposed to low doses of x-rays split into

two fractions separated by 5 hours have enhanced cell transformation frequency as compared to the frequency observed after exposure to a single acute dose.

An alternate mechanism for the protective effect of Antipain, or perhaps one that acts in addition to or as part of the above mode of action, may be mediated by the inhibition of cellular proteases associated with the expression of neoplastic transformation.

The enhancement of transformation frequency of Antipain when the protease inhibitor is added prior to irradiation could be due to a direct modification of DNA by x-irradiation and an inhibition by Antipain of some rapid error-free DNA repair, yet to be defined, involved in removing x-ray-damaged regions from DNA. This could result in a selective disturbance of DNA repair, different from that responsible for cell survival, and result in an enhanced rate of transformation.

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RB-11 A comparison of two nitroimidazoles as hypoxic sensitizers,
radiosensitizers and cytotoxic agents.

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In recent years there has been an explosion of interest in the identification of compounds which selectively sensitize hypoxic cells. For practical purposes, hypoxic cells are confined to tumors, so that this class of sensitizer enjoys the enormous advantage of specifically sensitizing tumor cells, while not affecting the radiation response of normal tissues. If hypoxic cells exist at all in human tumors (8,21), they constitute a barrier to successful treatment with x-rays since they are relatively resistant to killing by sparsely ionizing radiations (13,17). The rationale of hypoxic cell radiosensitizers is that they diffuse into poorly vascularized areas of a tumor and achieve the desired effect by chemical means (1). A vital property of these drugs upon which their success depends, is that the sensitizers are not rapidly metabolized by the cells in the tumor through which they diffuse. Because of this, they can penetrate further than oxygen and reach all of the hypoxic cells in the tumor, including those most remote from the blood supply.

The search for clinically useful radiosensitizers was guided by the hypothesis, now known to be correct, that the efficiency of sensitization is directly related to the electron affinity of the compound (1-4, 16). The Gray Laboratory in London first identified the 2-nitroimidazole misonidazole as a radiosensitizer, but it has since been studied widely with *in vitro* cell culture systems as well as transplanted tumors in experimental animals (5,7,12, 15, 18, 19, 20,22), and it has already been introduced into clinical practice.

By contrast Ro-07-0741 has received relatively little attention. The

chemical structure of Ro-07-0741 is compared with misonidazole as shown in Figure RB-11-1. Early studies were reported by Chapman (6), and Hall et al (11) and more recently the Radiation Oncology Coordination Committee of the National Cancer Institute has expressed interest in this drug as an alternative to misonidazole.

The present communication describes experiments designed to compare the two 2-nitroimidazoles Ro-07-0582 and Ro-07-0741 in terms of their radiosensitizing and cytotoxic properties. A standardized *in vitro* cell culture test system was used that has previously been described.

METHODS AND MATERIALS

Culture of the cells.

Chinese hamster cells (V79) were used throughout these investigations, cultured by standard techniques, and grown in Hams F10 medium (GIBCO) supplemented with 10% fetal bovine serum and antibiotics. Cells received their treatments with radiation and/or the appropriate drug, while held in suspension in small sealed glass ampules.

For each experiment, cells were harvested by trypsinization from actively growing, partly confluent, stock bottles, spun down and washed to remove excess trypsin, the concentration of cells was determined by counting with a Coulter electronic cell counter. This cell suspension was then divided into several parts and the cell concentration in each adjusted to 2×10^6 cells/ml, adding one or another of the drugs at an appropriate concentration according to the plan of the particular experiment. From each of the cell suspensions, containing various concentrations of drug or no drug at all, series of glass ampules were filled by pipetting 1 ml of the suspension into each. These ampules then were handled in the following way in order to render the cells hypoxic. The ampules were flushed with high purity nitrogen, plus 5% CO₂, in

Ro-07-0582

Ro-07-0741

Figure RB-11-1: Illustrating the chemical structures of Ro-07-0582 and Ro-07-0741. Both are 2-nitro-imidazoles; the only difference being a replacement of the methoxy side chain group by a fluorine.

order to displace the air above the cell suspension. Each ampule was heat sealed before it was transferred to a water bath at 37.5°C where it was continuously shaken and tumbled end to end to keep the cells in suspension. This procedure was maintained for a period of 1 hour to allow the cells to "scavenge" the oxygen dissolved in the medium by normal respiration and metabolism. This method of producing hypoxia has previously been described in detail (10).

A parallel series of ampules were filled with cells with a concentration of $10^4/\text{ml}$; these were gassed with a mixture of air and 5% CO_2 before being heat sealed. Because of the lower number of cells these ampules remained aerated throughout.

After the sealing of all of the ampules they were irradiated with gamma-rays or neutrons, according to the plan of the particular experiment. To investigate and compare the cytotoxicity of the drugs at various concentrations, ampules containing cells were maintained at 37.5°C for various periods of time, during which they were continually shaken and tumbled end for end to prevent cells from attaching to the glass walls. Five replicate ampules were used for each treatment condition.

At the conclusion of all treatment protocols, each ampule was agitated on a vortex mixer to resuspend the cells, after which it was opened and various aliquots of the cell suspension plated in Falcon tissue culture flasks containing fresh growth medium. The cells were then incubated for 8 days before they were fixed and stained. The number of colonies per flask was counted using a projection technique. By comparison with appropriate controls, the fraction of cells surviving each treatment schedule could be calculated readily.

Methods of irradiation.

The source of gamma rays was a ^{60}Co Cobalt teletherapy unit, directed

vertically upwards so that the cells were irradiated through the bottom of the glass ampules. At a treatment distance of 40 cm the dose rate was computed to be 2.1 Gy/min. For irradiation with neutrons, cells were transported to the Naval Research Laboratory in Washington, D.C. where a neutron beam, currently used for radiotherapy, is generated by bombarding a beryllium target with 35 MeV deuterons. The cells were irradiated at a treatment distance of 125 cm where the dose rate was computed to be 0.40 Gy/min with a gamma contamination of less than 3%.

Cells were irradiated at room temperature, but to avoid cell killing as a result of drug toxicity during the transportation of the cells to and from the radiation facilities, the ampules were stored in melting ice at 4°C. At this temperature it is possible to store hypoxic cells for up to 24 hours without noticeably reducing the plating efficiency which was characteristically in excess of 80%. In the experiments reported here the storage time was no more than 3 to 4 hours between preparation of the cells and irradiation of the cells in Washington, D.C. Preliminary experiments indicated that these storage procedures do not affect the radiation sensitivity nor the enhancement ratio produced by the drug.

RESULTS

Figure RB-11-2 shows the results of an experiment designed to compare the sensitizing properties of Ro-07-0582 and Ro-07-0741 using ⁶⁰Cobalt gamma rays. The protocol was to compare the two drugs at a given concentration within the same experiment. A range of concentrations was investigated in a series of experiments of which the data shown in Figure RB-11-2 constitutes a representative example. From these data, the enhancement ratio (ER) was calculated corresponding to each drug concentration. The ER is defined to be the ratio of radiation doses in the absence and presence of the drug which produce equal

Figure RB-11-2: Survival data for hamster V79 cells irradiated with graded doses of ^{60}Co gamma rays under aerated and hypoxic conditions, in the presence or absence of the nitroimidazoles Ro-07-0741 or Ro-07-0582 at a concentration of 5 mM. The survival curves were fitted to the data by eye.

biological effects in hypoxic cells. In our experiments, the ER was calculated at a cell surviving fraction of 10^{-2} , but since both oxygen and all three radiosensitizers tested in this study appear to be dose modifiers, the survival level at which calculations are performed is of little consequence. The ER is plotted as a function of drug concentration in Figure RB-11-3.

Figure RB-11-4 shows survival data for cells exposed to graded doses of high energy fast neutrons (35 MeV d^+Be) under aerated and hypoxic conditions and in the presence of various concentrations of Ro-07-0582. A parallel experiment was performed with Ro-07-0741. The enhancement ratios corresponding to these neutron experiments are also plotted in Figure RB-11-3.

The results of an experiment to compare the cytotoxic properties of the two 2-nitroimidazoles are shown in Figure RB-11-5; the fraction of hypoxic cells surviving is plotted as a function of the time of exposure to the drug at $37.5^{\circ}C$. A range of concentrations of each drug was tested. In Figure RB-11-6 the time needed to kill 90% of the hypoxic cell population is plotted as a function of the drug concentration, based on the data in Figure RB-11-5.

DISCUSSION

What emerges from this study is clear evidence that both of the nitroimidazoles tested are good radiosensitizers and both are extremely cytotoxic at high concentrations. In regard to both properties they are specific for hypoxic cells. Over the whole range of concentrations used and times of exposure, they had no effect on euoxic cells. The radiosensitizing properties of the two drugs are compared in Figure RB-11-3 in terms of their enhancement ratios. A number of conclusions are possible.

First, at all drug concentrations tested for both gamma-ray and neutrons, Ro-07-0741 is a slightly more effective sensitizer than Ro-07-0582. This finding further confirms the proposal by Adams et al (4) that the sensitizing

Figure RB-11-3: The Enhancement Ratio (ER) as a function of drug concentration for the three compounds MTDQ, Ro-07-0582 and Ro-07-0741. The Enhancement Ratio is defined to be the ratio of radiation doses in the absence or presence of the drug which produce equal biological effects at a cell survival level of 10^{-2} .

Figure RB-11-4: Survival data for hamster V79 cells irradiated with graded doses of fast neutrons (35 MeV $d^+ \rightarrow Be$) under aerated and hypoxic conditions. Hypoxic cells were treated in the presence of various concentrations of the radiosensitizer Ro-07-0582. The curves were fitted to the data by eye.

Figure RB-11-5: Survival data for hamster V79 cells under hypoxic conditions exposed for various time periods at 37.5°C to three concentrations of Ro-07-0741 or Ro-07-0582. The curves were fitted to the data by eye.

efficiency is determined by electron affinity, since Ro-07-0741 is slightly more electron affinic (-383 mV) than is misonidazole (-389 mV). The magnitude of the enhancement ratio for these drugs for x or γ -rays agrees closely with the results obtained by Chapman et al (6).

Second, these drugs sensitize hypoxic cells to the effects of neutrons as well as for gamma-rays. This has previously been reported for metronidazole using Vicia faba seedlings and cells in culture (9), and for misonidazole in the case of animal tumors (7). It should be emphasized, therefore, that electron affinic drugs must be regarded as an adjunct to high linear energy transfer (LET) radiotherapy, since the lowered oxygen enhanced ratio (OER) associated with neutrons can be further reduced by the addition of a radio-sensitizing drug.

It is also evident that Ro-07-0741 is more cytotoxic than Ro-07-0582. The results of a typical cytotoxicity experiment at high concentrations, as shown in Figure RB-11-5, can be replotted in many ways. In Figure RB-11-6 the drug concentration is plotted as a function of exposure time at 37.5°C that is required to kill 90% of the hypoxic cell population. On this double logarithmic plot, the data approximate to a straight line with a slope of approximately one-half. This implies that the concentration of drug required to produce this given level of cell population is inversely proportional to the square of the exposure time at 37.5°C. This simple relationship was suggested at the Gray Conference in 1977 as a convenient "rule-of-thumb" (12). At that time it has been shown to be true for misonidazole, but it now appears to be true also for Ro-07-0741. This finding helps to explain why a cytotoxic effect of these drugs on hypoxic cells might be important in humans, although it is not observed clearly in the mouse. The half-life for the clearance of the drug from the serum is 1 to 1½ hours in the mouse, but is about ten times longer in the human, with values

Figure RB-11-6: An intercomparison of the cytotoxic effect on hypoxic hamster V79 cells of the two 2-nitroimidazoles, Ro-07-0741 and Ro-07-0582. The drug concentration is plotted as a function of the exposure time to the drug at 37.5°C required to reduce the fraction of cells surviving to 0.1. For both compounds, the drug concentration required is inversely proportional to the square of the exposure.

quoted in the range 8 to 18 hours (14). Because of the inverse square law relating drug concentration and exposure time for a given degree of cell killing, the drug concentration in the mouse would need to be about a hundred times larger to produce the same cytotoxic effect as might be expected in hypoxic cells in human tumors.

This study has focussed on the two 2-nitroimidazoles Ro-07-0582 and Ro-07-0741. Ro-07-0741 has been shown to be a more effective radiosensitizer of hypoxic cells than is Ro-07-0582 but this may be offset by the increased toxicity shown by this drug. Whether this increased toxicity is seen in animals and presents a problem remains to be seen, but from first reports (Brown, personal communication) *in vivo*, Ro-07-0741 is indeed more toxic to animals than Ro-07-0582. Because of *in vivo* toxicity the doses of Ro-07-0741 administered to mice have been reduced approximately by a factor of two in respect to Ro-07-0582.

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RB-12 MTDQ as a hypoxic cell radiosensitizer alone and in combination with
misonidazole

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Misonidazole is an electron affinic compound that selectively sensitizes mammalian cells to the lethal effects of x and gamma rays, and is already in use in clinical trials as an adjunct to radiotherapy (Adams, 1973; Asquith et al., 1974; Brown, 1975; Fowler, et al., 1976). MTDQ (6,6-methylene-bis-2,2,4 trimethyl-1, 2-dihydroquinoline) is an antioxidant that was initially developed as a food additive but has recently been the subject of preliminary clinical investigations in Hungary and limited *in vitro* experimentation in the United States (Bar, et al., 1975, 1977; Hall et al., 1979).

It has been shown in numerous biological systems that hypoxic cells are relatively resistant to killing by sparsely ionizing radiations such as x or gamma rays. It is not known with certainty whether human tumors contain viable hypoxic cells that limit their curability by ionizing radiations, but from histological evidence and by analogy with animal tumors it is likely that they do (Thomlinson and Gray, 1955; Evans and Naylor, 1963). Various methods have been proposed to counter the problem of hypoxic cells; these include the use of high pressure oxygen chambers, hyperthermia, radiations with a high linear energy transfer and most recently the introduction of chemical radiosensitizers that interact with radiation to specifically increase the sensitivity of hypoxic cells while not affecting the response of normal cells (Fowler et al., 1976; Churchill-Davidson, 1966). Numerous compounds have been tested as radiosensitizers *in vitro* and *in vivo*. The result of this extensive research has shown the nitroimidazoles to possess the necessary properties and have emerged as leading candidates for clinical use. Currently, two of these compounds, misonidazole and metronidazole, are undergoing clinical

trials in a number of different countries (Urtasun et al., 1976; Thomlinson et al., 1976; Dische et al., 1976).

MTDQ (6,6'-methylene-bis-2,2,4-trimethyl-1,2-dihydroquinoline) was synthesized as an alternative to the antioxidant compounds BHT⁺ and ETQ[‡] for use as preservatives in foodstuffs. The chemical structure of MTDQ is illustrated in Figure 1. The demand for new antioxidants was precipitated by the less than optimal toxicity of BHT and ETQ to living organisms which is defined by Korber and later modified by Cornfield as a lethal dose (LD₅₀) greater than 5 g/kg body weight (Bar et al., 1977). In tests with rats MTDQ showed no toxicity following administration of a single dose of 5 g/kg body weight or a daily dosage of 0.25 g for three weeks. Furthermore, one group of rats with tumors were prefed MTDQ mixed into their food at graded dosages of 0.03 to 0.3 g/kg body weight and then exposed to a dose of 700 rad. Tumors treated with MTDQ showed greater sensitivity than the control group, which received radiation but no drug (Erdelyi et al., 1979). This finding is surprising in the light of other research which indicates that compounds that function as antioxidants tend to act as radioprotectors, rather than sensitizers (Chapman et al., 1973). The present communication describes experiments involving the testing of MTDQ as a hypoxic cell radiosensitizer alone and in combination with misonidazole in order to determine the sensitizing properties of the two widely different agents.

Materials and Methods

Standard culture techniques were used to grow Chinese hamster V79 cells in GIBCO F-10 culture medium, supplemented with 10% fetal calf serum, and antibiotics (Ham and Puck, 1962).

For the radiation or cytotoxicity experiments under hypoxia, cells were treated in suspension in glass ampules. To induce hypoxia, a large number of cells were crowded into a small volume of medium so that oxygen was reduced to a low level by

⁺BHT - 2,6-di-tertbutyl-4 hydroxytolulene

[‡]ETQ - 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline

Figure RB-12-1: Illustrating the chemical structure of MTDQ (6,6'-methylene-bis-2,2,4-trimethyl-1,2-dihydroquinoline). The drug tested consisted of $60 \pm 3\%$ of the monomer form, together with $39 \pm 3\%$ of a combination of dimer and trimer.

cell metabolism and respiration. This method has been widely used and described in detail elsewhere (Hall, Lehnert and Roizin-Towle, 1974). The essential steps are as follows: cells from a number of actively growing, partially confluent, stock flasks were harvested by trypsinization, washed to remove excess trypsin, and the concentration of cells determined by counting with a Coulter electronic cell counter. This cell suspension was then divided into several parts and the cell concentration in each adjusted to 2×10^6 cells/ml, adding one or another drugs at an appropriate concentration according to the plan of the particular experiment. Misonidazole dissolved readily in the cell culture medium, but in the case of MTDQ, the drug had first to be dissolved in a solvent such as TWEEN-80. This solution was then diluted with culture medium until the concentration of the TWEEN-80 represented no more than 0.1% of the tissue culture medium; this was necessary because the solvent per se was found to be cytotoxic. From each of the cell suspensions, containing various concentrations of one drug, two drugs, or no drug at all, series of glass ampules were filled by pipetting 1 ml of the cell suspension into each. These ampules were then handled in the following way in order to render the cells hypoxic. The ampules were flushed with high purity nitrogen plus 5% CO₂ in order to displace the air above the cell suspension. Each ampule was heat-sealed before it was transferred to a water bath at 37.5°C where it was continuously shaken and tumbled end to end to keep the cells in suspension. This procedure was maintained for a period of one hour to allow the cells to scavenge the oxygen dissolved in the medium by normal respiration and metabolism. Proof that this system produces adequate levels of hypoxia is evidenced by an oxygen enhancement ratio (OER) in excess of 3 when aerated and hypoxic cells are exposed to acute doses of ⁶⁰Cobalt gamma rays. A parallel series of ampules were filled with cells at a concentration of 10⁴ cells/ml; these were gassed with a mixture of air and 5% CO₂ before being heat-sealed. Because of the lower number of cells, these

ampules remained aerated throughout. For the periods of time used in these experiments, the plating efficiency for both groups of cells, aerated and hypoxic, remained high (typically 70%) and unaffected by the experimental conditions used.

Following this procedure the cells were irradiated with gamma rays, or subjected to various time periods in the water bath at 37.5°C to assess cytotoxicity. The source of gamma rays was a ⁶⁰Cobalt teletherapy unit, directed vertically upwards so that the cells were irradiated through the bottom of the glass ampules. At a treatment distance of 40 cm the dose rate was computed to be 2.05 grays per minute. Heat treatments were carried out by immersing the ampules in a water bath at 37.5°C controlled to $\pm 0.1^\circ\text{C}$; the ampules were continuously tumbled and shaken during this heat treatment to prevent attachment of the cells to the glass surface.

At the conclusion of all treatments, each ampule was agitated on a vortex mixer to resuspend the cells, after which it was opened and various aliquots of the cell suspension plated into Falcon tissue culture flasks containing fresh growth medium. Following an incubation period of 8 days at 37.5°C, cells were fixed and stained, and the number of macroscopic colonies counted by a projection technique.

Results

The initial experiments were focussed on comparing the radiosensitizing properties of MTDQ with Ro-07-0582, using a lower range of drug concentrations dictated by the limited solubility of the dihydroquinoline. Figure 2 summarizes the results of a typical experiment in which the radiosensitizing properties of MTDQ were investigated over a range of concentrations from 0.08 to 0.4 mM. Parallel experiments were also performed with Ro-07-0582 over a comparable range of concentrations. The enhancement ratios, calculated from those data are plotted as a function of drug concentration in Figure 3.

Figures 4 and 5 show the results of experiments in which Chinese hamster V79 cells were exposed to graded doses of ⁶⁰Cobalt gamma rays under aerated and hypoxic

Figure RB-12-2: Survival data for hamster V79 cells irradiated with graded dose of ^{60}Co gamma rays under aerated and hypoxic conditions in the presence or absence of various concentrations of MTDQ. The curves were fitted to the data by eye.

Figure RB-12-3: The Enhancement Ratio (ER) plotted as a function of the concentration of MTDQ or Misonidazole.

conditions, in the presence of various concentrations of misonidazole or MTDQ or a combination of both drugs. In Figure 6, the enhancement ratio is plotted as a function of drug concentration for MTDQ alone, misonidazole alone, or both drugs in combination. The enhancement ratio is defined as the ratio of doses in the absence and presence of the drug required to produce the same biological effect; the values plotted in Figure 6 were taken from the experimental data in Figures 4 and 5.

Figure 7 describes an experiment performed to resolve two important questions. Firstly, whether the increase of radiosensitizing efficiency that is seen with misonidazole over a 5 hour period is duplicated with MTDQ and secondly, to determine the effect of MTDQ on the increase in sensitization exhibited by misonidazole during a contact time of 5 hours. V79 cells under hypoxic or aerated conditions with either MTDQ alone, misonidazole alone or a combination of the two drugs were irradiated at 0 and 5 hours with $^{60}\text{Cobalt}$ gamma rays.

Discussion

The data presented in this paper show that MTDQ is a radiosensitizer equal, or slightly superior, to misonidazole at the same concentration. The preliminary *in vitro* results with MTDQ are limited by the difficulties associated with dissolving the drug in a solvent miscible with water, so that it can be added to tissue culture medium. At low drug concentrations between 10 and 100 μM , the enhancement ratios for MTDQ at least equal to and probably are larger than for misonidazole (See Fig. 3). There appears to be more of a "tail" to the curve relating ER to drug concentration for MTDQ than for misonidazole.

Used alone MTDQ is not a serious rival, competitor or alternative to misonidazole because the concentrations that can be obtained *in vivo* are limited by the solubility of the drug. By contrast, the combination of MTDQ and misonidazole yield results that are very interesting, and there may be considerable promise in simultaneous use of the two drugs.

Figure RB-12-4: Survival data for hamster V79 cells with graded doses of ^{60}Co gamma rays under hypoxic or aerated conditions with Misonidazole alone, MTDQ alone or a combination of MTDQ and Misonidazole.

Figure RB-12-5: Same as Figure RB-12-4.

Figure RB-12-6: The Enhancement Ratio (ER) plotted as a function of the concentrations of MTDQ alone, Misonidazole alone or a combination of the two.

Figure RB-12-7: Survival data for hamster V79 cells irradiated with graded doses of ^{60}Co gamma rays under hypoxic and aerated conditions. The cells were treated with Misonidazole alone, MTDQ alone, or a combination of Misonidazole and MTDQ and irradiated at the 0 and 5 hours treatment time.

First, the enhancement ratios obtained with the two drugs are additive even when the concentrations of the two drugs differ by a factor of 50. This is not the case when two electron affinic drugs, PNAP and misonidazole are added, or when oxygen is added to either agent. In this situation, partial additivity is seen only if the combined ER's of the two compounds is less than 1.6. When either (or both) of the two compounds alone has an ER greater than 1.6, the ER of the combination is determined solely by the compound with the higher ER (McNally et al., 1978). For example, if 5 mM misonidazole (ER = 2.4) and 0.4 mM PNAP (ER = 1.6) are combined, the resulting ER is only 2.4. The fact that the effects of MTDQ and misonidazole are additive, even when present in very different quantities, strongly suggests that the sites of action of the two drugs are different.

Secondly, there is no increase in the enhancement ratio (ER) for MTDQ over a 5 hour maturation period at 37.5°C as seen in Figure 7. In the case of misonidazole, the ER increases from 2.1 at 0 hours to a factor that equals the oxygen effect at 5 hours. This is very important in view of the apparent lack of toxicity exhibited by MTDQ. The lack of increase or decrease in the sensitizing effect and the structure of MTDQ indicates that little metabolism of MTDQ occurs over a 5 hour period. In fact, in the cases of animals or human the halflife is 2 and 4 days, respectively (Bar et al., 1977).

Third, when MTDQ is combined with misonidazole, additivity of the ER's is seen. When the two drugs are combined and cells irradiated after a 5 hour treatment period, there is little increase in the ER while for cells treated with misonidazole alone for 5 hours the sensitivity of hypoxic cells is equivalent to that of aerated cells. This has been postulated to be a result of the metabolism of misonidazole to intermediate metabolites (Gillete, 1971; et al., 1978; Fouts and Brodie, 1957; Mason and Holtzman, 1975; Willson, et al., 1974) that interact with and deplete the cellular reducing compounds (sulfhydryl etc.) present which

protect the cell (Chapman et al., 1973; Willson et al., 1974; Hall and Biaglow, 1977). When the intracellular sulfhydryl pool is increased by the addition of cysteamine which is a free radical scavenger, both the sensitizing and cytotoxic properties of misonidazole are reversed (Hall, et al., 1979).

Since the increase in radiosensitization with misonidazole over 5 hours is almost completely inhibited while there is no decrease in sensitization at the 0 hour irradiation, points to the alteration of the metabolism of misonidazole by MTDQ.

This could be a result of an increase in detoxification, an increase in metabolism, a direct reaction with the activated misonidazole, or a competitive inhibition for reduction with misonidazole (Wattenberg, 1976). It does not seem reasonable to attribute it to an increase in metabolism since this would still decrease the intracellular reducing equivalent pool, direct reaction (as is seen with cysteamine) a decrease in the ER at the 0 hour would be expected. Therefore, it appears that MTDQ is blocking the reduction of misonidazole by competitively binding to the same site (the reversal of toxicity is dependent on concentration of MTDQ) or works by increasing the detoxificate rate (i.e., increasing rate of reduction at which $R-CH_2CH(OH)CH_2OCH_3 \rightarrow R-CH_2CH(OH)CH_2OH$ relative to $R-NO_2 \rightarrow RNH_2$) (Wattenberg, 1976; Mannering, 1971; Yang et al., 1974).

For *in vivo* application in laboratory animals or in man, MTDQ can be dissolved in non-toxic solvents such as peanut oil or sunflower seed oil and administered orally. There are reports in the Hungarian literature of patients receiving a total dose of 132 g of the drug over a period of 100 days which produced no side effects other than occasional nausea (Erdelyi et al., 1979). Serum levels corresponded to about 13 μM with concentrations in solid tumors claimed to be about three times as high. Thus, it might be expected that a small but useful radiosensitizing effect might be obtained in humans with MTDQ that could be

maintained throughout a prolonged multi-fraction treatment regimen with no worrisome side effects. In addition a possibility of MTDQ combined with misonidazole to give a greater degree of sensitization of hypoxic cells with a possible decrease in toxicity (RB3) opens up many other intriguing possibilities. MTDQ merits extensive testing in a range of animal systems, and it would be a great advantage if a more water soluble derivative could be produced.

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Laurie Roizin-Towle and Eric J. Hall

Bleomycin (BLM) is an antineoplastic agent which was discovered in 1966 (Umezawa et al., 1966) and introduced into clinical use in the United States in 1970. It has been found to be useful in the treatment of solid tumors such as squamous cell carcinomas of the head and neck as well as lymphomas (Blum et al., 1973; Takeuchi, 1976). Although BLM is usually said to be cell cycle phase non-specific because it causes some cell killing at all phases of the cell cycle, cells in M and G₂ are more sensitive to the drug, and at low doses it reversibly inhibits cell progression at the S-G₂ boundary (Barranco et al., 1973).

It is well documented that the curability of some solid tumors by x-rays is limited by the presence of hypoxic cells that are resistant to killing by sparsely ionizing radiations (Powers and Tolmach, 1963; Thomlinson and Gray, 1955). The possibility that hypoxic cells are equally refractory to killing by chemotherapeutic agents has received little attention until recently (Roizin-Towle and Hall, 1978). Two factors make this possibility likely:

- (a) Hypoxic cells are situated in poorly vascularized areas of tumors, to which drugs may not penetrate adequately.
- (b) Hypoxic cells are essentially non-cycling, which may be of particular importance in the case of drugs that are cycle specific.

The experiments described here were designed to investigate the effect of bleomycin (BLM) on aerated and hypoxic cells in culture, and to study the interaction between BLM and x-rays. In addition, the combination of BLM and misonidazole has been studied to investigate the extent to which the electron affinic compound kills the hypoxic cells that are resistant to BLM. Since the majority of antineoplastic drugs kill actively dividing cells, they probably are not as

effective in killing cells in solid tumors which are deficient in molecular oxygen and unlikely to be in active cell cycle. Misonidazole was used with BLM for this reason, that it is selectively cytotoxic towards hypoxic cells, and would avoid further damage to normal aerated cells in tissues already treated with other antineoplastic agents. Although misonidazole is used primarily as an hypoxic cell sensitizer, its use as chemotherapy agent warrants consideration.

MATERIALS AND METHODS

Chinese hamster V79 cells were used for these experiments. Standard culture techniques were utilized, with the cells grown in GIBCO F10 culture medium, supplemented with 10% foetal calf serum and antibiotics. Under these conditions routine growth curves indicated a population doubling time of about 10 hours.

Experiments involving split dose studies with BLM, or the interaction of x-rays with the drug, were carried out using attached cells. Known numbers of cells were inoculated into Falcon tissue culture flasks (25 cm^2) and allowed to attach by overnight incubation at 37.5°C before being treated with x-rays or BLM. The source of radiation was a Siemen's x-ray therapy unit, operated at 300 kV, 12 mA, with added filtration of 0.2 mm Cu. The dose-rate at a treatment distance of 50 cm was computed to be 180 rad/min. For treatments with BLM, the drug was dissolved in complete culture medium and added to the flasks containing cells. At the conclusion of the treatment period, the medium containing drug was removed, the cells were rinsed, and fresh culture medium added.

For drug experiments designed to compare aerated and hypoxic conditions, cells were treated in suspension in glass ampules. The method used to induce hypoxia has been described in detail elsewhere (Hall, Lehnert and Roizin-Towle, 1974). Briefly, cells were harvested by trypsinization from stock flasks, counted and prepared into a suspension containing 2×10^6 cells/ml. At this

point BLM or a mixture of BLM and misonidazole was added to achieve the drug concentration required by the plan of the particular experiment. A series of glass ampules were filled from this cell suspension, flushed with pure nitrogen containing 5% CO₂ to remove the air from the space above the cells, and then sealed. The ampules were shaken and tumbled to keep the cells in suspension, and the temperature raised to 37.5°C for 1 hour to allow the oxygen remaining in the medium to be consumed by respiration. A parallel series of ampules were filled with cells at a concentration of 10⁴ cells/ml, and flushed with a mixture of air and 5% CO₂ before being sealed. These ampules remained aerated because of the smaller number of cells. After the sealing of all of the ampules, they were maintained at 17.5, 37.5 or 42.5°C for various "treatment" times according to the plan of the particular experiment. At the conclusion of all treatments, each ampule was opened and various aliquots of the cell suspension inoculated into tissue culture flasks containing fresh growth medium. Following an 8-day incubation period at 37.5°C, the cells were fixed and stained and the number of colonies per flask counted. The fraction of cells surviving a given treatment could be computed by comparing the number of colonies from treated and control ampules.

RESULTS

Figure RB-13-1 shows the results of experiments in which aerated and hypoxic cells were treated for 4 hours with graded concentration of BLM. It is evident that hypoxic cells are protected to a significant extent compared with those that are aerated. The results of experiments in which a combination of BLM and misonidazole was used are shown in Figure RB-13-2. Both aerated and hypoxic cells were treated for 4 hours with graded concentrations of BLM, or the same concentration of BLM with the addition of 5 mM misonidazole. While the nitroimidazole has no effect on the response of the aerated cells treated with BLM,

Figure RB-13-1: Survival data for V79 hamster cells treated for four hours with graded concentrations of bleomycin at 37.5°C under aerated and hypoxic conditions.

Figure RB-13-2: Survival data for V79 hamster cells treated for 4 hours at 37.5°C under aerated and hypoxic conditions with graded concentrations of bleomycin, with or without the addition of misonidazole.

it greatly increased the cell killing observed in hypoxic cells.

The effect of temperature on the response of hamster cells to BLM is illustrated in Figure RB-13-3. There is a dramatic reduction in the fraction of cells surviving a given "integral dose" of bleomycin as the temperature is raised. Integral dose is defined as the product of exposure time and concentration and was used here to show the effect of cytotoxicity at 17.5, 37.5 and 42.5°C.

The results of fractionation experiments with x-rays and BLM are shown in Figure RB-13-4. The upper curve shows the effect of varying the time interval between two equal treatments with BLM (15 µg/ml for 30 min.). The lower curve shows the effect of various time intervals between a 500 rad dose of x-rays followed by a BLM treatment of 30 min. at 15 µg/ml. In either case, an interval of 4 hours between treatments leads to substantially greater cell killing.

The effect of sequencing on the combination of BLM and x-rays is shown in Figure RB-13-5. A 500 rad dose of x-rays was delivered up to 6 hours before or after a one hour treatment with BLM at a concentration of 15 µg/ml. The fraction of cells surviving a single dose of x-rays alone, or a one hour treatment with BLM alone, was also determined.

DISCUSSION

The experiments reported here indicate clearly that hypoxic cells treated at 37.5°C are less sensitive to BLM than aerated cells. The most likely explanation for this difference is that aerated cells continue to progress through the cell cycle and can enter a more drug-sensitive phase, while hypoxic cells are essentially non-cycling. However, this may not be the whole story. Hypoxic cells may be resistant to killing by BLM as a result of other factors, including a reduced rate of metabolism, pH effects or rate of drug absorption. What is clear is that misonidazole preferentially kills hypoxic cells: as a

Figure RB-13-3: Survival data for V79 hamster cells, treated with bleomycin under aerated conditions, at three different temperatures. The fraction of cells surviving is plotted as a function of the integral dose of drug, defined to be the product of the exposure time and the concentration.

Figure RB-13-4: The effect of split dose experiments with bleomycin. The fraction of cells surviving is plotted as a function of the time interval between two treatments with BLM (15 µg/ml for 30 min.). Lower Curve: Effect of varying the interval between a single dose of x-rays (500 rad) and a 30 minute exposure to bleomycin (15 µg/ml) with a synchronous hamster cells.

Figure RB-13-5: The effect of the sequence of delivery of x-rays and bleomycin. A dose of 500 rad of x-rays was delivered at various time intervals up to 6 hours before or after a one hour treatment with bleomycin (15 µg/ml).

consequence, hypoxic cells are substantially more sensitive to the combination of BLM + misonidazole than are aerated cells. The suggestion has been made more than once that an electron affinic compound, such as misonidazole, would be a logical addition to a chemotherapy protocol (Roizin-Towle and Hall, 1978; Stratford and Adams, 1977; Sutherland et al., 1976) and could be of significant value in killing those cells that escape the action of other antineoplastic agents. The present study supports this notion.

The data shown in Figure RB-13-3 illustrate the dramatic way in which BLM cytotoxicity varies with temperature, lending support to several previous reports (Hahn, Brown and Har-Kedar, 1975). The combination of chemotherapy and hyperthermia is a large, potentially fruitful, but largely unexplored field.

The studies reported here of the interaction of x-rays and BLM on asynchronous cells indicate that the effects are simply additive when the two modalities are applied together. The cell killing potential of a combination of given doses of x-rays and BLM can be greatly enhanced by allowing a time interval between the two treatments of sufficient duration for the resistant survivors of the first treatment to progress into a sensitive phase of the cell cycle prior to the second. Modest doses of x-rays and BLM have this much in common, that they both produce their greatest effect on cells in M and G₂. Consequently, the order in which the two modalities are applied makes little difference; what is far more important is the time interval. Needless to say, such considerations apply only to cycling cells, and in a practical situation non-cycling hypoxic cells may constitute the limitation to the effectiveness of either modality.

Future planning for the treatment of malignant disease should involve a multidisciplinary approach incorporating the most expedient use of radiation, chemotherapeutic agents, and possibly hyperthermia as well. Within this context, misonidazole and BLM would seem to be complementary, the one making

good the weaknesses of the other.

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RB-14 The effect of hyperthermia alone, or combined with x-rays, on the incidence of oncogenic transformations *in vitro*.

R.C. Miller, L. Harisiadis and E.J. Hall

Hyperthermia is evolving as a promising addition to the anticancer armamentarium. Preliminary clinical applications show encouraging results and a systematic evaluation of hyperthermia in the management of patients with cancer is underway. Current major therapeutic modalities in oncology include radiotherapy and chemotherapy, both of which have clear carcinogenic potential. It was considered pertinent to investigate whether hyperthermia, alone or in combination with ionizing radiation was also prone to be carcinogenic.

The *in vitro* assay for oncogenicity that was used, is based on the observation that mouse embryo C3H/10T $\frac{1}{2}$ clone 8 cells when exposed to oncogenic polycyclic hydrocarbons *in vitro* produced malignantly transformed foci of characteristic morphology, with a dose dependent frequency. A high percentage of transformed foci, but not untreated cells, when inoculated into recipient mice, produced sarcomas (1). Similar transformations, again with dose response frequency, are obtained when those cells are exposed to x-rays or neutrons (2, 3).

This quantitative assay has been used extensively by us and others in investigations involving the study of radiation carcinogenesis (2,3,4), tumor promoting agents (5,6) and the induction and inhibition of oncogenic transformation by drugs (7,8).

In the present study it is described how elevated temperatures influenced the transformation rates of irradiated and non-irradiated cells.

MATERIALS AND METHODS

C3H/10T $\frac{1}{2}$ cells between passages 11 and 15 were grown in Eagle's basal

medium supplemented with 10% heat-inactivated fetal calf serum, penicillin and streptomycin. Appropriate numbers of cells were seeded into Corning 50 cm² Petri dishes, or into 25 cm² Falcon flasks, so that approximately 400 cells per dish or 200 cells per flask survived the heat and/or irradiation treatment which was carried out 18 hours after the cell inoculation. Following the treatment the dishes were incubated at 37°C (100% humidity, 5% CO₂, 95% air) for a period of 5 weeks; the medium was changed weekly.

At the completion of the incubation period, the cells were fixed and stained with Giemsa and transformed Type II and III foci were scored using the criteria described by Reznikoff et al. (9).

Irradiations were carried out immediately before the heat treatment in a Siemens 300 KVP orthovoltage unit (12 mA, 0.2 mm Cu filter added). The dose rate was calculated to be 33 rad/min.

Hyperthermia consisted of immersion of the flasks in a water bath with the temperature precisely controlled. For experiments involving prolonged exposures (i.e. 2 hours) an incubator preset at the desired temperature was also used. The temperature control with the incubator was not as precise as with the water bath. This method of heating was used primarily with heat exposures not involving cell killing; for such heat exposure some variation of the temperature did not have any appreciable consequence. Also, it would be stressed that the experimental design was such that for all conditions, large scale, self-contained experiments with appropriate controls were carried out.

RESULTS AND DISCUSSION

In accordance with our previous experience, no spontaneous transformation was observed in the non-irradiated control cells (Table RB-14-1). In addition, not a single transformed focus was observed among large numbers of cells exposed to temperatures from 40.0°C up to 45.0°C. Lack of transformation was

TABLE RB-14-1

DEMONSTRATING THE LACK OF ONCOGENIC TRANSFORMATION IN NON-IRRADIATED CONTROLS AND HEATED C3H CELLS.

	EXPERIMENT NUMBER	HEAT TREATMENT	PLATING EFFICIENCY	CELL SURVIVING FRACTION (S.F.)	NUMBER OF SURVIVING CELLS ($n \times 10^3$)	NUMBER OF TRANSFORMED FOCI	
						TYPE I	TYPE II
A.	II	none	0.33	--	1.90	0	0
		45.0°C x 20 min	--	0.045	1.90	0	0
	III	none	0.21	--	7.21	0	0
		45.0°C x 10 min	--	0.30	28.20	0	0
	IV	none	0.21	--	7.56	0	0
		42.2°C x 2 hrs	--	0.61	42.00	0	0
B.	I	none	0.18	--	5.37	0	0
		40.5°C x 2 hrs	--	0.95	5.40	0	0
	V	none	0.31	--	8.64	0	0
		40.0°C x 2 hrs	--	0.93	20.50	0	0
	VI	none	0.25	--	8.89	0	0
		41.5°C x 2 hrs	--	0.91	16.40	0	0
	VII	none	0.17	--	4.03	0	0
		41.5°C x 2 hrs	--	0.92	4.02	0	0
	VIII	none	0.23	--	13.10	0	0
		41.5°C x 2 hrs	--	0.91	11.30	0	0
	IX	none	0.16	--	7.68	0	0
		41.5°C x 2 hrs	--	1.06	17.3	0	0

noted both with heat treatments involving appreciable cell killing and in experiments where the surviving fraction was in excess of 0.9. (Table RB-14-1).

The transformation frequency following x-rays only was quantitatively very consistent and was dose related (Table RB-14-2). When hyperthermia was combined with x-irradiation a substantial reduction in the transformation frequency was observed.

In all the experiments, hyperthermia inhibited the transformation frequency to a greater extent than it enhanced the cell killing (Table RB-14-2 and Figure RB-14-1).

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TABLE RB-14-2

INHIBITION OF RADIATION INDUCED TRANSFORMATION BY HYPERTHERMIA.

EXPERIMENT NUMBER	X-RAY DOSE (GRAY)	HYPERTHERMIA	CELL SURVIVING FRACTION	THERMAL ENHANCEMENT OF CELL KILL	NUMBER OF TRANSFORMED FOCI	NUMBER OF SURVIVING CELLS ($n \times 10^3$)	TRANSFORMATION FREQUENCY ($n \times 10^{-4}$)	THERMAL INHIBITION OF TRANSFORMATION
V	4.0	none	0.31	= 0.97	20	22.4	8.93	= 2.27
	4.0	40.0°C x 2 hrs	0.32		20	50.7	3.94	
I	4.0	none	0.39	= 1.22	24	32.3	7.43	= 2.28
	4.0	40.5°C x 2 hrs	0.32		15	46.2	3.25	
VI	4.0	none	0.22	= 1.57	17	19.4	8.67	= 2.75
	4.0	41.5°C x 2 hrs	0.14		10	31.7	3.15	
IV	4.0	none	0.34	= 1.42	30	30.3	9.89	= 2.44
	4.0	42.2°C x 2 hrs	0.24		37	91.1	4.06	
VII	6.0	none	0.20	= 1.43	24	17.8	13.50	= 2.11
	6.0	41.5°C x 2 hrs	0.14		11	17.2	6.40	
VIII	2.0	none	0.76	= 1.33	20	79.7	2.51	= 1.79
	2.0	41.5°C x 2 hrs	0.57		9	64.1	1.40	
IX	2.0	none	0.78	= 1.18	9	38.5	2.34	= 1.18
	2.0	41.5°C x 2 hrs	0.66		8	40.3	1.99	
VIII	8.0	none	0.45	= 56.3	26	18.2	14.30	= 1.81
	8.0	41.5°C x 2 hrs	0.008		18	22.8	7.89	
IX	8.0	none	0.060	= 10.2	28	2.42	11.60	= 2.24
	8.0	41.5°C x 2 hrs	0.0059		15	2.89	5.19

Figure RB-14-1:

Top Panel: Fraction of cells surviving as a function of x-ray dose, with or without hyperthermia (41.5°C) immediately after irradiation.

Bottom Panel: Transformation frequency as a function of x-ray dose, with or without the addition of hyperthermia.

Louis E. Schwartz and Charles R. Geard

Introduction

The essential properties for an hypoxic cell radiosensitizer intended for use in clinical radiotherapy have been outlined by Adams¹. These include selective sensitization of hypoxic cells to radiation; rapid diffusability into tumors; slow metabolic breakdown; and non toxicity to normal tissues. The nitroimidazole class of drugs satisfy these criteria and the 5-nitroimidazole (Metronidazole) and the 2-nitroimidazole (Misonidazole) are currently under clinical investigation^{9, 19, 21}. A selective toxicity towards hypoxic cells due to Misonidazole alone has been established by a number of investigators including Hall and Roizin-Towle¹⁰ and Stratford and Adams¹⁸. Clinical toxicity as reported by Dische⁶ seems to be limited to neurotoxicity, and, is dose related.

In the forementioned studies, the focus has been on possible short term clinical effects. In view of the possible wider utilization of Misonidazole as an adjunct to clinical radiotherapy, it becomes important to establish the potential for more subtle effects on normal cells. The present study included an investigation of the effects of Misonidazole on aerated "normal" cells, *in vitro*, with respect to oncogenic transformations, sister chromatid exchange frequency, chromosomal aberrations, cell cycle progression, cell killing, and cell doubling time.

Materials and Methods

Cell Culture. The cell line used was the C3H 10T $\frac{1}{2}$ derived from the ventral prostate of C3H mouse embryos¹⁵. Cells between 12th and 16th passage

were used for the transformation assay, while cells from 12th to 19th passage were utilized for other assays. The cells were grown in Eagle's basal medium (BME) supplemented with 10% heat-inactivated fetal calf serum and penicillin and streptomycin. Cultures were grown in humidified incubators at 37°C in an atmosphere of 5% CO₂ in air. The cells were cultured in 100 mm Corning plastic petri dishes for all experiments except doubling time, where 60 mm dishes were used.

Transformation assay. The transformation experiments followed the format outlined by Reznikoff, et al.¹⁵. Briefly, cells were seeded into 100 mm Corning petri dishes 24 hours prior to treatment with Misonidazole; the number of cells plated were such that 350 - 450 viable cells per dish survived the subsequent treatment. The surviving fraction was determined for various concentrations and contact times with the drug. The cells were exposed to concentrations of 1 mM Misonidazole and contact times of 1, 3, 4, or 6 days. This concentration was chosen for intensive study because it represents a clinically feasible dose. At the termination of drug exposure, medium containing Misonidazole was removed and replaced with fresh growth medium which was subsequently changed every 2 weeks for 5-6 weeks until the dishes were confluent and transformed foci were large enough to be visible. Type II and Type III transformed foci were scored at that time. Type II foci are defined as having massive piling up of cells into opaque multi layers. The cells are only moderately polar; thus criss crossing is not pronounced. Type III foci are composed of highly polar, fibroblastic multi layered, criss crossed arrays of densely stained cells. These transformed foci produce sarcomas when large numbers of these cells (2×10^6) are inoculated into immunologically suppressed C3H mice¹⁵.

Chromosomal studies. Cells were studied for sister chromatid exchange

frequency, chromosomal aberrations and mitotic index.

The sister chromatid exchange studies were performed following Bromodeoxyuridine incorporation into the chromosomes as described by Alves and Jonasson². Prior to choosing a BrdU concentration for sister chromatid exchange analysis, effects on cell survival and doubling time were studied after 72 hours contact with BrdU concentrations of 1 micromolar up to 100 micromolar. Results indicated that concentrations less than 10 micromolar would be appropriate in order to render sufficient cells for chromosomal analysis.

10^6 cells were plated in 100 mm petri dishes. Eight dishes were plated for each concentration studied. After 24 hours of incubation BrdU and Misoniazole were added. To the control group, BrdU was added at concentrations of 3 and 5 micromolar while to the test group BrdU at 3 and 5 micromolar was added along with 1, 3 and 6 mM Misonidazole. Twenty hours later, Colcemid at 5 micromolar was added to half the dishes, and to the other half after 44 hours of exposure. The total mitotic cell accumulation period was 4 hours. Thus, cells were collected at 24 and 48 hours after initiation of treatment. The cells were trypsinized for 4 minutes and then spun down at 2000 rpm for 3 minutes. The supernatant was removed and 0.075 M KCl added to produce hypotonicity. After 8 minutes in KCl the cells were spun down again at 2000 rpm for 3 minutes. After removing the supernatant the cells were resuspended in cold fixer consisting of 3 parts methanol: 1 part glacial acetic acid. After fixing for 5 minutes, the cells were spun at 3000 rpm for 2 minutes. The supernatant was removed and the cells resuspended in 0.5 cc of fixer. Slides were prepared by wetting in distilled water. The cell solution was dropped on the wetted slide and dried by

air blower. The slides were stained with a solution of Giemsa and 0.2 - 0.4 M Na_2HPO_4 adjusted to pH 10.2 - 10.6 with 1 N NaOH as per Alves and Jonasson².

Sister chromatid exchanges (S.C.E.) were scored in 1000 chromosome samples from mitoses at the 2nd division after BrdU incorporation and 200 chromosome samples from chromosomes that had not undergone prior exchange in mitoses at the 3rd division post BrdU incorporation. By analyzing cells at these divisions sister chromatid exchanges were scored in chromosomes which had undergone two replications in BrdU and hence 2 cycles in which S.C.E. could be induced and in chromosomes which could demonstrate exchanges induced in one cycle only.

Mitotic indices were studied by a similar technique. 10^6 cells were plated, and after 24 hours of incubation Misonidazole was added at 1, 2, 3 and 6 mM concentrations. Colcemid was added at 2 hourly intervals from 0-8 hours post contact and at 24 and 48 hours post contact for 2 hours incubation. Slides were prepared as in the S.C.E. analysis study, but stained with Giemsa at pH 6.8. The indices were scored for mitotic cells containing approximately the full chromosome complement expected for C3H 10T $\frac{1}{2}$ cells.

Chromosomal observations were made on the slides prepared from the mitotic index experiment with either 50 or 100 cells being examined from each dose-time regimen.

Cell survival. Graded numbers of attached cells were exposed to 1 to 6 mM Misonidazole for 6, 24, 48, and 72 hours. At the termination of treatment, drug containing medium was removed and replaced with complete fresh medium. There were approximately 20 dishes per concentration and contact time. Survival was determined after 2 week incubation when colonies were large enough to be visibly seen after Giemsa staining.

Doubling time. Doubling times were determined by absolute cell counts

counts using a Coulter counter at daily intervals up to 6 days of contact with Misonidazole at concentrations up to 6 mM. Initially 5×10^4 cells were plated per dish. Ten dishes were counted for each contact time and concentration.

Results

Table I and Figure I show the results of transformation studies for 1 mM Misonidazole exposure and contact times varying from 1 through 6 days. While an increasing incidence of transformation with drug contact time is readily apparent the form of the dose-response relationship is uncertain. It is possible that there is a simple linear relationship between contact time and transformation; however, the result obtained after a 1 day exposure is somewhat higher than might be expected from simple linearity.

Sister chromatid exchange analysis revealed mean control frequencies of 0.24 to 0.36 S.C.E.'s for 2nd division chromosomes from 3 separate experiments. Third division chromosomes which had not undergone prior exchange for cells exposed to BrdU alone had 0.18 S.C.E.'s per chromosome. When concentrations of Misonidazole of 1, 3 and 6 mM were added to either 3 or 5 micromolar BrdU, sister chromatid exchange frequencies ranging from 1.05 times control at 1 mM Misonidazole up to 1.8 times control at 6 mM Misonidazole were observed. (Fig.2). 6 mM Misonidazole did not allow any cells to progress into the 3rd division (mitoses less than 1 in 10^5 cells) and very few into the 2nd division.

Chromosomal aberrations were scored from cells exposed to varying concentrations and contact times. Results are expressed in Table 2 as percentage of cells free of aberrations. Comparatively few aberrations were noted at exposures less than 6 mM and contact time less than 4 hours. As contact time and concentration increased the frequency of chromosomal aberrations increased.

RB-15 TABLE 1

Transformation Induction by Misonidazole in
C3H 10T½ Cells

Concentration	Contact time	Number of cells/dish	Number of dishes	Survival relative to control	Number of transformed clones versus cells surviving	Frequency of transformation per surviving cell $\times 10^{-4}$
0	0	355	80	1.00	0/2.8 $\times 10^4$	0
1 mM	1 day	206	180	1.01	7/3.71 $\times 10^4$	1.89 $\pm$.71
1 mM	3 days	382	71	.88	8/2.71 $\times 10^4$	2.95 $\pm$ 1.04
1 mM	4 days	148	120	.72	7/1.78 $\times 10^4$	3.94 $\pm$ 1.49
1 mM	6 days	405	26	.78	6/1.05 $\times 10^4$	5.71 $\pm$ 2.33

Figure RB-15-1: Oncogenic transformation frequency after exposure of C3H/10T $\frac{1}{2}$ cells to 1 mM Misonidazole for contact times of 1 through 6 days.

Figure RB-15-2: Sister chromatid exchange frequency/chromosome expressed as a ratio to control for C3H/10T $\frac{1}{2}$ cells exposed to concentrations of 0 through 6 mM of Misonidazole. Frequencies are plotted at 2nd and 3rd mitotic divisions post incorporation of BrdU and Misonidazole.

RB-15 TABLE 2

Percentage of Aberration-free C3H/10T $\frac{1}{2}$ Mouse Embryo Cells
After Treatment with Misonidazole

Accumulation Period (hrs) After Drug Addition	Concentration of Misonidazole (mM)			
	0	1	3	6
0 - 2	94	94	94	94
2 - 4	96	90	80	76
4 - 6	94	90	94	82
6 - 8	98	96	90	78
22 - 24	97	84	88	70*
46 - 48	94	94	78	-
70 - 72	95	94	84	-

* Only 30 cells from 3 experiments were scored at 22 - 24 hrs. after 6 mM Misonidazole treatment. There were no mitotic cells found at later times. 50 cells were scored for the 1 and 3 mM treatments, and 100 cells for the remainder.

Determination of mitotic index at 2 hourly intervals after applying Misonidazole revealed no change from control up until 8 hours of contact time for concentrations of 1, 2, and 4 mM. For 6 mM drug there was a continuing decrease in mitotic index after 4 hours on contact. Between 8 and 48 hours after application of Misonidazole the mitotic index remained similar to control for 1 and 2 mM concentrations but declined dramatically for the 4 mM concentration after 24 hours of exposure. For 6 mM drug the mitotic index was practically 0 by 24 hours. Fig. 3 expresses the proportion of mitotic index to control for varying concentrations and contact times. Control mitotic indices for the 2 hour accumulation periods varied inconsistently from 7.3 to 11.2 per cent and proportions of control are expressed relative to the control mean of $8.1 \pm 0.8\%$.

Cell survival was determined for contact times of 6, 24, 48 and 72 hours and drug concentrations up to 6 mM. Approximately 30 dishes were evaluated at each concentration and contact time. The effect of Misonidazole on cell survival was most pronounced after 24 hours on contact. When contact time for a 1 mM drug concentration is extended to 6 days, cell survival remains at or above 90% of control but growth of individual colonies is diminished (Fig. 4). From the findings it appears as if the low drug concentration does slow progression through the cell cycle to the extent that smaller colonies result without any substantial cytotoxicity. Survival was progressively diminished for increasing concentrations and contact times (Fig. 5).

Doubling times were estimated from absolute cell counts. Control doubling time was about 24 hours for this C3H/10T_{1/2} cell line. Doubling time remained at about 24 hours for concentrations of Misonidazole up to 2 mM.

Figure RB-15-3: Cell cycle progression of C3H/10T_{1/2} cells exposed to concentrations of 1 through 6 mM of Misonidazole for contact times of 0 through 24 hours expressed as a ratio of mitotic indices for cells exposed to drug vs. no drug exposure.

Figure RB-15-4: Comparison of colony size after exposure of C3H/10T $\frac{1}{2}$ cells to 1 mM Misonidazole for 6 days contact (right side), with cells exposed to no drug at all (left side).

Figure RB-15-5: Survival curves for C3H/10T½ cells exposed to 1 mM through 6 mM of Misonidazole for 6, 24, 48 and 72 hours contact time.

Above 2 mM the doubling time became prolonged and at 6 mM exposures it was so prolonged it could not be measured (Fig. 6). It should be noted that the finding of no difference in cell doubling time with a 1 mM drug concentration in contact with the cells for 6 days is not consistent with the observation that this treatment reduced colony size but not survival (Fig. 4). With the large numbers of cells used for cell doubling experiments confluence is reached in 4 days and small differences in cell cycle progression could not then express in this time period. In the cell survival experiments where small numbers of cells are plated and confluence does not play a limiting role, a 6 day drug exposure allows the expression of comparatively small differences as reduced size colonies. Further, it is possible that the larger cell population more readily metabolizes the drug thus modifying long term drug action at this marginally effective concentration.

The finding that a Misonidazole concentration of 6 mM prevents cell progression through the cell cycle (Fig. 3), results in a failure of cell doubling and induces significant levels of cell death along with comparatively low levels of chromosomal aberrations favors the view that Misonidazole at high concentrations kills aerated cells in interphase rather than via a postmitotic cell death. This conclusion is consistent with that reported by Geard et al.⁸, who found that hypoxic Chinese hamster V-79 cells also appeared to suffer an interphase cell death which was attributed to disruption of cell membranes.

Discussion

To interpret these data in a clinical context, the consequences of a concentration of Misonidazole of 1 mM and a contact time of 24 hours must be considered. This dosage and contact time is above the maximum safely achievable in clinical conditions as reported by Dische et al.⁷.

Figure RB-15-6: Growth curves for C3H/10T_{1/2} cells exposed to 0 through 6 mM of Misonidazole for contact times up to 6 days.

The transformation data *in vitro* indicate the potential ability of the agent tested to produce tumor *in vivo*. In order to determine the clinical significance of these data, transformation frequencies should be correlated with observed carcinogenesis *in vivo*. Such a comparison has been made by Benedict et al.⁴ for cancer chemotherapeutic agents. Using their data for transformation frequencies observed at clinically significant peak concentrations, Table 3 was prepared correlating transformation frequencies and carcinogenesis *in vivo*. Drugs with observed low levels of transformation frequencies such as Methotrexate, Vincristine, 6 Mercaptopurine are either equivocal or not associated with carcinogenesis *in vivo*. Misonidazole produces transformation frequencies similar to this group of drugs, implying that the carcinogenic potential *in vivo* at clinically achievable concentrations is equivocal.

The transformation frequencies following x ray exposures have been reported by Miller and Hall¹², Borek and Hall⁵. A transformation frequency of .0185%, as observed for 1 mM and 1 day contact time of Misonidazole correlates with a rate observed after mammalian or C3H cells are exposed, *in vitro*, to 80-100 rads of radiation. In view of the fact that administering Misonidazole *in vivo* provides a total body exposure to the drug, it may be concluded that the resulting transformation frequency is equivalent to a total body x ray exposure of 80-100 rads. The BEIR and UNSCEAR^{3,20} committees estimate that a total body exposure to this dose would result in a total cancer risk for the population exposed amounting to 1 in 100.

Sister chromatid exchange frequency should be analyzed in a similar manner. From experimental data by Popescu, Turnbull and Dipaolo¹⁴, analyzing sister chromatid exchange with the use of carcinogens and non carcinogens *in vitro* it is found that carcinogens produce a SCE frequency 3-5 x that

RB-15 TABLE 3

Comparison of Observed *in vivo* Carcinogenesis with
in vitro Transformation of C3H/10T $\frac{1}{2}$ Cells at
 Clinically Observed Peak Concentration

Agent*	Transformation Frequency (%)	Carcinogenesis Observed <i>in vivo</i>
Melphalan	2.94	+
Thiotepa	1.5	+
Actinomycin D	1.92	+
Bleomycin	0.50	+
Vincristine	0.09	-
Methotrexate	0.16	+
6-Mercaptopurine	0.00	-
5-azaCytidine	0.45	+
Misonidazole**	0.0185	?

*All data except that for Misonidazole are taken from Benedict et al.⁴

**Present study

found in control (BrD U alone). Similar findings of dramatic increases of SCE frequencies up to 10x control, by known mutagenic agents have been summarized by Wolff²³. Perry and Evans¹³ have also demonstrated SCE frequencies 2-8x control with cancer chemotherapeutic agents such as Adriamycin, Bleomycin, Nitrogen Mustard, Mitomycin C and Cyclophosphamide. From these studies it may be concluded that induced sister chromatid exchange frequencies above 2 x control may more readily be associated with carcinogenesis *in vivo*. Misonidazole does not produce a significant enough increase in SCE frequency, even at concentrations up to 6 mM, to be placed in the same category as the efficient SCE inducers, many of which have proven carcinogenic abilities.

Analysis of cell survival, doubling time and mitotic index seems to pinpoint the primary Misonidazole effect to be after 24 hours of contact with the drug and with concentrations above 2 mM. At 1 mM Misonidazole there is a small effect with regard to these parameters for contact times up to 3 days.

The carcinogenic potential of this compound was shown by Miller and Hall¹² who reported *in vitro* transformation of C3H/10T $\frac{1}{2}$ cells with drug contact times of 3 and 6 days and Misonidazole concentrations from 0.5 to 6 mM. The related 5-nitroimidazole Metronidazole, which is also undergoing clinical evaluation²¹, is a known carcinogen¹⁷ and mutagen^{11, 16, 22}, so it is hardly surprising that Misonidazole should demonstrate carcinogenic abilities. Nevertheless this study shows that at drug concentrations and contact times which would be relevant in a clinical situation the carcinogenic, clastogenic and cytotoxic effects of this drug are substantially less than that of many chemotherapeutic agents of common usage. When comparisons are made with radiation, the effect of Misonidazole appears to be equivalent to 80-100 rads total body radiation. The subsequent 1/100 cancer risk, although small, should be considered when

Misonidazole is used in patients with a long term prognosis.

Conclusion

In clinically useable concentrations (1 mM) and contact times (24 hours), Misonidazole exhibits the following properties for aerated cells *in vitro*:

1. A frequency of oncogenic transformations of .0185%
2. Sister chromatid exchange frequency 1.05 x control
3. Over 90% of cells free of chromosomal aberrations
4. Over 90% of cells survive (even with contact up to 6 days)
5. Doubling time indistinguishable from control (up to 4 days contact)
6. A similar rate of progression through the cell cycle as control

The frequency of oncogenic transformations following treatment with Misonidazole at clinically useable concentrations is small compared with a range of chemotherapeutic agents shown to be potent carcinogens. Based on a comparison with radiation, the cancer risk would appear to be about 1 in 100. This must be considered, if patients treated with Misonidazole are projected to survive for prolonged periods.

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RB-16 Regulation of Retinol-Binding Protein Metabolism in Cultured Rat Liver Cell Lines.

Carmia Borek

Retinol, a water-insoluble lipid alcohol, is the main form of vitamin A normally found in blood plasma. Vitamin A has been shown to be involved in differentiation processes and to have inhibitory effects in neoplastic transformation of cells by radiation and chemicals. Retinol circulates in plasma bound to a specific transport protein, retinol-binding protein (RBP) (Kanai et al., 1968). This protein, which has been purified from human (Kanai et al., 1968) and rat (Muto and Goodman, 1972, Peterson et al., 1973) plasma, and from several other species (Goodman, 1976), has a molecular weight of approximately 20,000, a single binding site for one molecule of retinol, and normally circulates as a protein complex with plasma prealbumin. RBP is synthesized in the liver and secreted into plasma mainly as the holo-protein (with bound retinol) (Muto et al., 1972, Smith et al., 1973).

The serum levels of RBP and of vitamin A, and the delivery of retinol to extrahepatic target tissues, appear to be regulated mainly by processes that regulate the rates of RBP production and secretion by the liver. Previous studies in the rat have shown that the secretion of RBP by the liver is strongly and specifically regulated by nutritional vitamin A status (Muto et al., 1972, Smith et al., 1973, Navab et al., 1977). These studies demonstrated that vitamin A deficiency leads to a specific block in the secretion of RBP by the liver, and that the deficient liver contains an expanded pool of apo-RBP that can be released rapidly into the serum

as holo-RBP when vitamin A is made available. The molecular and cellular mechanisms involved in the regulation of RBP secretion by vitamin A status remain to be defined.

Our work has involved the study of vitamin A binding protein RBP *in vitro* in liver cells and its regulation by retinol.

Studies were carried out on the synthesis and secretion of RBP in culture *in vitro* by two cell lines derived from well differentiated rat hepatomas, and the apparent specific regulation of these processes by retinol in the medium. These liver cell lines thus resemble the liver of the intact rat with regard to RBP metabolism and its regulation. Regulations of vitamin A binding protein may have some function in the inhibitory action of vitamin A on neoplastic development. It is well known that plasma vitamin A levels are normally maintained in a fairly narrow range, despite wide variations in dietary intake or in body stores (in the liver) of vitamin A (Lewis et al., 1942). This phenomenon is apparently achieved within the body by processes that regulate the production and secretion of RBP by the liver, and thus regulate the delivery of vitamin A from hepatic stores to peripheral target tissues (Goodman, 1976).

The present studies were undertaken in order to try to develop an effective model system for the more detailed study of the regulation of RBP metabolism in the liver cell. Two cell lines were found that synthesized detectable amounts of RBP *in vitro*. One of these, the MH₁C₁ cell line, was derived from a transplantable Morris hepatoma #7795 (Richardson et al., 1969). These cells have a doubling time of 5 days. The other cell line (H₄ II EC₃ ,

or H₄ cells) was initially established from a Reuber H-35 hepatoma (Pitot et al., 1964), and is a heteroploid cell line (Miller et al., 1972). The H₄ cells proliferate initially (in serum-containing media) as monolayers, but later pile up at high densities (Borek, 1978). In contrast to the slow growing MH₁C₁ cells, H₄ cells have a doubling time of about 24 hours.

Both of these cell lines have retained the ability to synthesize a variety of serum proteins. The MH₁C₁ cells have retained the ability to produce serum albumin (Richardson et al., 1969) and prothrombin (Munns et al., 1976). The H₄ cells have been shown to synthesize serum albumin, transferrin, C₃-component of complement (Stecher and Thorbecke, 1967; Ohanian, et al., 1969), and prothrombin (Munns et al., 1976).

In the present experiments, both cell lines synthesized RBP when incubated in serum-free NTS medium, while the MH₁C₁ cells also synthesized RBP when incubated in Ham's F-12 medium supplemented with serum. No cell proliferation was observed when cells were incubated in the NTS medium, as judged by labeled thymidine uptake. The net synthesis of RBP was a function of the number of cells initially plated in each dish, within the ranges studied. This relationship was apparent with the MH₁C₁ cells in both the NTS and F-12 (+serum) media, and the H₄ cells in the NTS medium.

When incubated in the NTS medium, many of the cells rounded up and detached from the dish. Evidence that the metabolism of RBP was not affected by the physical adherence of the cells to the plate was obtained following the addition of glutathione to the culture and the monitoring of the net synthesis and secretion of RBP by the cells. The addition of 15 µg of glutathione per ml to the medium caused most of the cells to adhere to the

dish; the cells, however, showed the same net synthesis and secretion of RBP as did the cells in the NTS medium alone which were partially in suspension. Further evidence that the net synthesis and secretion of RBP were not related to adhesion of the cells to the plate was obtained in the experiments involving the addition of retinol carrier solution alone to the dish. This addition caused some increase in cell adhesion to the plate, but did not affect the net synthesis or secretion of RBP. Addition of serum to cells in NTS medium resulted in both a higher net synthesis of RBP and an increased cell adhesion to the plate, compared to the cells incubated in the serum-free NTS medium alone. In view of the above results, these data suggest that the stimulation of the net synthesis of RBP by serum in NTS medium resulted from a direct effect of the serum on the cells, rather than from its effect on cell adhesion. Moreover, while the addition of serum to NTS medium at lower cell densities ($1 - 3 \times 10^6$ cells/dish) resulted in some stimulation of cell proliferation, at the higher cell densities (6×10^6 cells/dish) at which most experiments were carried out this effect was negligible.

Two effects of the addition of serum to NRS medium were observed. Serum both stimulated the release of RBP from the cells into the medium, and, at a later time period, stimulated the net synthesis of RBP. Both of these effects were graded, and related to the amount of serum added within the range 0.1 to 10%.

The amount of RBP present in the cells was considerably less than that found in normal (approximately 8 pmol/mg protein) or vitamin A deficient (25-30 pmol/mg protein) rat liver (Muto et al., 1972). The H_4 cells contained about 4.9 pmol of RBP/mg of protein within the cells when incubated in NTS medium alone, and 1.8 pmol of RBP/mg of protein after the

cells were incubated in NTS medium containing retinol. Other investigators have reported that the rate of albumin synthesis by H_4 cells does not compare favorably with that observed in perfused liver (Ohanian et al., 1969). H_4 cells have been reported to synthesize more albumin when they are in the log phase of growth (Ohanian et al., 1969), rather than in a stationary phase. Data are not yet available as to whether actively growing H_4 cells produce RBP.

The cells incubated in NTS medium were essentially vitamin A deficient liver cells. We anticipated that, if these cells behaved like normal hepatocytes *in vivo*, then they should accumulate an expanded pool of RBP intracellularly, that can be released when retinol is made available to the cell. In short (e.g., 1 day) incubations, both the H_4 and the MH_1C_1 cells retained a large proportion (average approximately 28%) of the RBP within the cells. Addition of retinol to the incubation medium resulted, within these short time periods, in the stimulation of RBP secretion from the cells into the medium. At longer time periods, a stimulation of the net synthesis of RBP, after addition of retinol, was also observed. The effects of the administration of retinol to the vitamin A-deficient intact rat. It is of interest that the effects of retinol seen here were also similar to the effects observed from the addition of serum. It is thus possible that the major factor responsible for the effects of serum on RBP metabolism by the cells was the vitamin A content of the serum added. Serum may, of course, have had other effects as well.

The levels of retinol used in the present experiments were not toxic to the cells, as established by cell count and viability studies. At higher levels of retinol, the cells have marked changes in their surface properties

(Borek et al., 1973). Further evidence that the stimulation of RBP release by addition of a low dose of retinol was not due simply to cell damage or to other nonspecific effects was obtained by studying the effects of retinol addition on the metabolism of serum albumin. In both types of cells, addition of retinol to the incubation did not affect either the net synthesis of albumin or the cell-to-medium distribution of the albumin. These findings suggest that the effects of retinol on RBP metabolism were specific for RBP, similar to the known effects of retinol on RBP metabolism in the liver of the intact rat. Taken together, the data reported here suggest that retinol has a specific regulatory effect on RBP metabolism by these two cell lines in culture *in vitro*, and provide evidence that the effects of retinol seen in the intact rat represent a direct effect of retinol itself on the liver cell. These cells provide a good model for the further detailed study of the mechanisms involved in the regulation of RBP synthesis and secretion by the liver cell. Ongoing studies show that some compounds which suppress RBP synthesis are effective promoters of neoplastic transformations.

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TEACHING

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

During the Fall, a course in radiobiology was given for residents in both therapy and radiodiagnosis; about 30 residents attended from Columbia-Presbyterian, affiliated hospitals and other institutions in the New York area. A third year radiotherapy resident spent a six-month period in the laboratory to train in research techniques.

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

INVITED SEMINARS AND PAPERS AT SCIENTIFIC MEETINGS

Dr. Carmia Borek

Radiation Research Society, Toronto, Canada, May 1978

Conference on aging

Inst. for Medical Research, Camden, N.J., May 1978

Seminar

Department of Pathology, College of Medicine and
Dentistry of N.J. Newark, N.J., June 1978

Cancer Biology Conference

Aspen, Colorado, July 1978

Conference on Differentiation and Carcinogenesis

Minneapolis, Minnesota, September 1978

National Cancer Inst. Workshop on

Human Cells in Culture. September 1978

Seminar, Oak Ridge National Labs., Oak Ridge, Tenn., October 1978

Am. Soc. Cell Biology Annual Meeting

San Antonio, Texas, November 1978

Seminar, Department of Radiology, Ohio State Univ., February 1979

Seminar, University of Saskatchewan School of

Medicine, Saskatoon, Canada, March 1979

Seminar, Atomic Energy of Canada, Whiteshell

Nuclear Establishment, Pinawa, Canada, March 1979

Seminar, N.Y. University, Department of Environmental

Medicine, Sterling Forest, N.Y., April 1979

Sixth International Congress of Radiation Research Society, Tokyo,
Japan, May 1979

Seminar, University of Tokyo, May 1979

Seminar, National Cancer Institute of Japan, Tokyo, May 1979

Lecture, University of Hiroshima, Japan, May 1979

Dr. Charles R. Geard

Sixth International Congress of Radiation Research Society, Tokyo,
Japan, May 1979

Dr. Eric H. Hall

Hammersmith Hospital, England. Seminar. August 22, 1978

International Meeting on the Fundamental and Practical Aspects of Neutron Therapy. The Hague, The Netherlands. September 13 & 14, 1978

Oliver Symposium. Cambridge, England. September 15, 1978

Harvard Medical School, Massachusetts General Hospital, Boston, Mass. Seminar. September 28, 1978

American Society of Therapeutic Radiologists, Los Angeles, California, October 31 to November 1, 1978

CROS Combined Modalities Meeting, Hilton Head, South Carolina. November 15 to 18, 1978

Radiological Society of North America, Chicago, Illinois. November 26 to 29, 1978

NCI Radiosensitizer Workshop, Washington, D.C. December 4, 1978

CALGB, New York, N.Y. Seminar. February 9, 1979

Harvard Medical School, Joint Radiotherapy Center, Boston, Mass. Seminar. February 14, 1979

Univ. of North Carolina, Chapel Hill, N.C. Seminar. February 20 & 21, 1979

M.D. Anderson Symposium on Fundamental Cancer Research, Houston, Texas. February 27 to March 2, 1979

N.Y. Academy of Medicine, New York, N.Y. Seminar. March 19, 1979

NCI Sensitizer Workshop, Banff, Canada. April 1 to 4, 1979

Memorial-Sloan-Kettering, New York, N.Y. Seminar. April 11, 1979

Radiation and Medical Physics Society, New York, N.Y. Seminar. April 18, 1979

Biology Dept., New York Univ., New York, N.Y. Seminar. April 20, 1979

Spring Meeting N.Y. Roentgen Society, New York, N.Y. April 28, 1979

UCLA, Los Angeles, California. Seminars. May 1 to 3, 1979

Program Directors' Meeting of Dept. of Energy, South Carolina. May 6-8, 1979

6th International Congress of the Radiation Research Society, Tokyo, Japan, May 13 to 17, 1979

U.S.-Japan Cooperative Cancer Res. Program. Kyoto, Japan, May 22 & 23, 1979

Dr. Richard C. Miller

University of Hawaii Cancer Center, Hawaii, May 1979

6th International Congress of the Radiation Research Society, Tokyo,
Japan, May 1979

Dr. Harald H. Rossi

Fourteenth Annual Meeting of the European Society of Radiation Biology
Jülich, Germany, October 9 to 14, 1978

Thirty-Second Annual Symposium on Fundamental Cancer Research, Radiation
Biology in Cancer, Houston, Texas, February 27 to March 2, 1979

Conference on Neutrons from Electron Medical Accelerators. Sponsored
by the Bureau of Radiological Health and the National Bureau of
Standards, Maryland, April 9 to 10, 1979

Mrs. Laurie Roizin-Towle

Combined Modalities Conference on Chemotherapy, Hilton Head Island,
South Carolina, November 1979

LOW LEVEL RADIATION GROUP

During the year Drs. Rossi, Hall, Bird, Borek, Geard, Kliauga, Goldhagen, Lam, Miller, and Rini, Mr. Goodman, and Mrs. Roizin-Towle have attended meetings of the Low Level Radiation Group at the Brookhaven National Laboratory. This group, which consists of scientists from the Safety and Environment Protection Division and the Medical Division of Brookhaven National Lab., as representatives from this laboratory, was set up to discuss the feasibility of extending radiobiological studies at RARAF to lower doses and lower dose-rates. The group serves the dual function of reviewing experiments that have been performed in the past, and also advising on the plan for future investigations, and coordinating the efforts of the different laboratories concerned. In particular, a number of potential outside users of the RARAF facility have been asked to attend meetings of the group to discuss their proposed research. As a result several projects are under way involving scientists from other universities.

OTHER ACTIVITIES

Members of the Laboratory were active on the following:

Dr. Carmia Borek

Pathology B Study Section

National Institute of Aging, Program Project, Review Section

Member of Council: Tissue Culture Association

Member of the New York Academy of Sciences Conference

Organizing Committee

Science and Society Committee

Women in Science Committee

Review Committee for the TRIGA reactor.

Assoc. Editor, J. of Environmental Pathology and Toxicology

Dr. Charles R. Geard

32 Annual Radiation Research Society Meeting, Houston, Texas

Dr. Eric J. Hall

Societies

British Institute of Radiology
Radiation Research Society
Association for Radiation Research
Hospital Physicists Association
Oxford Society

Awards and Honors

Fourteenth Douglas Lea Memorial Lecturer, Hospital Physicists Assoc. - 1975
The Roentgen Award of the British Institute of Radiology - 1976

Committees

Committee 40 NCRP
Chairman, Radiobiology Committee RTOG
National Board of Medical Examiners: Examination Committee for
Radiation Therapy
Radiation Study Section, National Institutes of Health
Awards Nomination Committee, Radiation Research Society (Chairperson)
Council: Radiation Research Society

Dr. Paul J. Kliauga

Member of: ICRU Report Committee on Microdosimetry

Dr. Harald H. Rossi

International Commission on Radiation Units and Measurements (ICRU)

Main Commission

Technical Areas:

Radiobiology (Co-sponsor)

Radiation Physics - Neutrons and Heavy Particles (Sponsor)

Report Committees:

High Energy and Space Radiation Dosimetry (Sponsor)
Fundamental Quantities and Units
Concepts and Principles of Radiation Protection Measurement (Cooperative effort with the ICRP) (Chairperson)
Intercomparison of Neutron Instruments (Sponsor, Member, Consultant)
Microdosimetry (Co-sponsor, Member)
Neutron Dosimetry for Biology and Medicine (Sponsor, Member)
Radiobiological Dosimetry (Co-sponsor)

National Council on Radiation Protection and Measurements (NCRP)

Main Council

Sc. Comm. 1: Basic Radiation Protection Criteria

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

Technical Advisory Committee on Radiation for the Commissioner of Health,
City of N.Y. (Chairperson)

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

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

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

Nuclear Regulatory Commission (Consultant)

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

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

Awards Nomination Committee, Radiation Research Society (Chairperson)

Dr. Richard C. Miller

Sigma Xi

Radiation Research Society

Dr. Michael L. Freeman

Radiation Research Society

BOOKS

Advances in Pathobiology 7

Biology of Aging. C. Borek, D.W. King (Eds.) Stratton
Intercontinental Medical Book Corp., N.Y. In Press 1979

Radiobiology for the Radiologist. 2nd edition. Eric J. Hall
Harper and Row, Hagerstown. 1978.

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0108012019040

080 Goodman, L.J.

090 W/sub n/ and neutron kerma for methane-based tissue-equivalent gas

110 Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150 C00--4733-2

370 Jul 1979 390 19-36 430 NTIS, PC A12/MF A01

530 EDB;ERA;INS

540 EDB-654003;

550 TIC 560 United States of America (USA) 570 United States of America (USA)

600 R 610 A31 700 187 2000 710 Columbia Univ., New York (USA). Radiological Research Lab.

AUG 620- NONE

801 NEUTRON DOSIMETRY;T2,Q1;TISSUE-EQUIVALENT DETECTORS;T1;METHANE;ENERGY DEPENDENCE;KERMA;Q2;NEUTRONS;IONIZATION
CHAMBERS;

T;Q1 TISSUE-EQUIVALENT DETECTORS;NEUTRON DOSIMETRY

T;Q2 NEUTRON DOSIMETRY;KERMA

950 The principal dosimeter used to determine the absorbed dose of neutrons employed in radiobiology and radiotherapy is the homogeneous tissue-equivalent (TE) ionization chamber. A methane-based TE gas mixture is most commonly used in the chamber cavity. This instrument is used to measure the charge liberated in the gas by the secondary charged particles set in motion by the neutrons. Conversion of this charge to absorbed dose requires knowledge of W/sub n/, the mean energy expended to form an ion pair in the gas by the initial spectra of secondary particles produced by the neutrons. The computation of W/sub n/ requires that secondary charged particle spectra calculated from neutron interaction cross sections and energetics be employed for each neutron energy used, as is done in this report. The computation of W/sub n/ also requires that experimental data on W for each type of secondary charged particle be available. Revision puts them in sufficient agreement with the other data that they are here given equal weight in the evaluations of W. Further, this report discusses the relative kermas contributed by the various types of charged particles. By combining computed charged particle spectra with the evaluations of W, the number of ion pairs produced in the gas by each type of charged particle is derived, from which W/sub n/ is

computed as the quotient of the total kerma by the total number of ion pairs.

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0108012019041

060Srdoc, D.

090W-value for electrons and photons in tissue-equivalent gases

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150COU--4733-2

370JUL 1979 39037-50 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-640304;654001;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610A12;A32 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801ION PAIRS;METHANE:T1;PROPANE:T2;TISSUE-EQUIVALENT DETECTORS;IONIZATION:Q3;ELECTRON-MOLECULE COLLISIONS:Q1,Q2;

PHOTON-MOLECULE COLLISIONS:Q1,Q2,T3;

T;Q1 METHANE;ELECTRON-MOLECULE COLLISIONS

T;Q1 METHANE;PHOTON-MOLECULE COLLISIONS

T;Q2 PROPANE;ELECTRON-MOLECULE COLLISIONS

T;Q2 PROPANE;PHOTON-MOLECULE COLLISIONS

T;Q3 PHOTON-MOLECULE COLLISIONS;IONIZATION

950The W-value for electrons and photons was measured in methane and propane based tissue-equivalent gas mixtures using the proportional counter technique. The results agree within statistical limits with some of the published values. Measured values are given. The measurements of energy dependence of W resulted in practical formulae describing W(E) as a function of incident electron or photon energy. (GHI)

0108012019042

060Mills, R.E.

090Radial energy deposition profile of proton tracks

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150C00--4733-2

370Jul 1979 39051-57 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-654001;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610A32 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801PROTON BEAMS:T1;BEAM PROFILES:Q1;ENERGY LOSSES:Q1;ENERGY DENSITY;TISSUE-EQUIVALENT DETECTORS:T2;PROTON

DOSIMETRY:Q2,T3;SPATIAL DOSE DISTRIBUTIONS:Q3;MEV RANGE 01-10;

T;Q1 PROTON BEAMS;BEAM PROFILES

T;Q1 PROTON BEAMS;ENERGY LOSSES

T;Q2 TISSUE-EQUIVALENT DETECTORS;PROTON DOSIMETRY

T;Q3 PROTON DOSIMETRY;SPATIAL DOSE DISTRIBUTIONS

950Measurements of the energy deposition distribution around a proton beam were continued using the ABC chamber.

The three dimensional translator was used to position the chamber center on the proton beam axis at distances from about 4 to 60 cm from the downstream collimating aperture, allowing virtually scatterfree measurements with gas pressures in excess of 100 torr. An experiment was performed to determine the variation of central fraction (the ratio of ionization collected from the inner cylindrical volume to that collected in the entire cylindrical chamber volume) as a function of axial position along the beam axis, holding the incident proton energy and the tank gas pressure constant. This was done for 3.5 MeV protons utilizing gas pressures from 0.2 to 100 torr and for 1.0 MeV protons with pressures from 0.1 to 9.1 torr, thus nearly spanning the range of capability of the RARAF Van de Graaff accelerator.

0108012019043

060Srdoc, D.

090Measurement of the Fano-factor in tissue-equivalent and other gases

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150C00--4733-2

370Jul 1979 39058-63 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-654001;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610A32 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801GASES;IONIZATION;PROPORTIONAL COUNTERS;ENERGY SPECTRA;ION PAIRS;SYNTHESIS;TISSUE-EQUIVALENT DETECTORS;FANO

FACTOR;T1;MEASURING METHODS:Q1;

T;Q1 FANO FACTOR;MEASURING METHODS

950Measurements of pulse height spectra of very low energy radiation in a proportional counter filled with a polyatomic gas or mixture of gases proved to be an ample source of valuable information on gas properties as well as on some basic physical processes. For example, it is possible to calculate the energy required to form an ion pair in a gas, to study the gas multiplication phenomena and to calculate the probability distribution function of primary ionization charges. This report also deals with a basic physical property of gases when irradiated with low energy radiation: the Fano factor which determines the width of the primary ionization charge distribution.

647170 PREV SN - 647169 RA HDRB EB121580 FILE-EB1212.E03

0108012019044

050Mills, R.E.

090Neutron dosimetry for rat irradiations

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150COO--4733-2

370Jul 1979 39064-71 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560152;440200;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C14;E43 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG820- NONE

801NEUTRON DOSIMETRY:T1;RATS;MICRODOSIMETRY;TISSUES;SAMPLE PREPARATION;PROPORTIONAL COUNTERS:T2,Q1;PERFORMANCE:Q2;

T:Q1 NEUTRON DOSIMETRY;PROPORTIONAL COUNTERS

T:Q2 PROPORTIONAL COUNTERS;PERFORMANCE

950Monoenergetic neutron irradiations of rats described in last year's Annual Report (COO-4733-1) were continued in collaboration with the Department of Radiology of Thomas Jefferson University. Three groups of 24 rats received absorbed doses of 0.43 MeV neutrons corresponding to 300, 400, or 560 mGy (30, 40, or 56 rad), respectively, of total tissue kerma in free air at the center of the animal positions. In all cases the absorbed dose contributed by gamma rays was about 3 percent of the total absorbed dose. In order to alleviate some of the traumatic effects of the experimental procedure upon the pregnant rats, extra air holes were drilled in the aluminum end caps of the rat holders, thus providing increased ventilation for respiration and cooling. It was also found to be desirable to postpone transporting them for one day after irradiation.

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060Kliauga, P.J.

090Influence of amplifier time constant on proportional counter calibration

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150COD--4733-2

370Jul 1979 39072-75 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-440200;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610E43 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801PROPORTIONAL COUNTERS:T1;CALIBRATION:Q1;AMPLIFIERS;TIME DEPENDENCE;MICRODOSIMETRY;NEUTRON DOSIMETRY;

T;Q1 PROPORTIONAL COUNTERS;CALIBRATION

950This note describes the observation of the effects of a particular electronic parameter on the calibration of proportional counters. The effect in question is well-known and in a sense obvious, but it is easily overlooked in some applications of proportional counters to microdosimetry. Therefore, it is thought worthwhile to recount an example where this problem did occur, with a view toward helping others avoid such difficulties in the future. The effect will be of greatest importance for large gas pressures and/or small electrode potentials (low gas gain), and for radiation modalities which exhibit a large dynamic range of energy deposition.

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0108012019046

060Kliuga, P.J.; Lam, Y.M.P.; Rossi, H.H.; Goodman, L.J.

090Microdosimetry at the Bevalac

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150COO--4733-2

370JUL 1979 39076-87 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-655003;440102;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610D15;E41 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801BEVALAC:T1;MICRODOSIMETRY:Q1;TISSUE-EQUIVALENT DETECTORS;WALL-LESS COUNTERS:T2;COMPARATIVE EVALUATIONS:Q2;WALL

EFFECTS;ARGON IONS;MEV RANGE 100-1000;

T;Q1 BEVALAC;MICRODOSIMETRY

T;Q2 WALL-LESS COUNTERS;COMPARATIVE EVALUATIONS

950Since the successful production of high intensity energetic heavy ion beams at the Bevalac a few years ago, this facility, now the only one in this country, has become a highly productive center of research in the biological and therapeutic uses of heavy ions. The potential usefulness of accurate microdosimetric data on this unique radiation modality is apparent, but has not been realized because of the scarcity of such data. This scarcity is due in part to the exceptionally severe constraints placed upon a microdosimetric experiment by these particles and in part to logistical constraints brought about by geography and the unique nature of this facility. Some initial measurements on carbon and argon ions were reported in the previous annual report, and at the Sixth Symposium on Microdosimetry. These data were taken with wall-less counters of the spherical-grid type, enclosed within tissue-equivalent plastic shells. Since two counters differing in diameter by a factor of four were used (enclosed in the same size spherical shells), comparison of data between the two counters for the same simulated tissue diameter gave an indication of differences due to the wall effect. As anticipated this effect is substantial, and confirmed the desirability of using a counter as wall-less as possible for obtaining accurate data on high-

energy ions.

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0108012019047

060Blavati, M.H.; Rossi, H.H.; Kliuga, P.J.; Goldhagen, P.E.

090Microdosimetry in the nanometer region

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150COU--4733-2

370Jul 1979 39088-94 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-655003;440102;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610D15;E41 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801FLOW COUNTERS;Q1,T2;SPECIFICATIONS;Q2;PERFORMANCE;MICRODOSIMETRY;T1;X RADIATION;KEV RANGE 01-10;ELECTRONS;

T;Q1 MICRODOSIMETRY;FLOW COUNTERS

T;Q2 FLOW COUNTERS;SPECIFICATIONS

950The experiment designed to yield information on the deposition of energy by charged particles in regions of the order of a nanometer, is being continued. A particular geometrical configuration for the avalanche grid and shield wires has been investigated. It consists of four stainless steel wires each 10 mils in diameter. One wire, the avalanche grid, is separated by 15 mils from the scintillator and then there are three shield wires between the grid and sensitive volume of the chamber. The actual configuration is shown. The purpose of the shield wires is to keep the high electric field at the grid wire from penetrating into the sensitive volume of the chamber, so as to keep the field there as uniform as possible for positive ion collection. A test chamber was designed for the purpose of investigating the best possible separation of the grid and shield wires from the grounded surface, which represents the scintillator. Measurements made with the chamber indicated that in order to maintain any reasonable voltage on the avalanche grid wire (i.e. -3000 v) the pressure must be 0.1mm Hg or below. Breakdown voltages for various separations ranging from 0.5 mil to 15 mils were measured. Data were obtained for shield voltages of -400 v, -540 v and -5 volts. Highest grid voltages were obtainable with the shield wires at -5 v, which would seem to indicate that the voltages on the grid and shield combine to cause breakdown to the grounded surface. Other

configurations of shield and grid wires will be investigated along with the possibility of using smaller diameter
wires for the shield.

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0108012019048

060Srdoc, D.; Mills, R.E.; Marino, S.A.

090Microdosimetry measurements at the RARAF

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1978-September 30, 1979

150C00--4733-2

390JUL 1979 39095-101 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-440200;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610E43 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

(2)

counters

detectors

801MICRODOSIMETRY;PROPORTIONAL DETECTORS;T1;TISSUE-EQUIVALENT COUNTERS;T2;GAMMA RADIATION;NEUTRON REACTIONS;

PERFORMANCE:Q1,Q2;

****T;Q1 PROPORTIONAL DETECTORSLCK KWDJJ;PERFORMANCE

****T;Q2 TISSUE-EQUIVALENT COUNTERSLCK KWDJJ;PERFORMANCE

950A series of microdosimetric spectra has been measured using tissue-equivalent proportional counters of various geometrics and construction as described in detail in the following paragraphs. The counters were exposed to ^{60}Co gamma rays and 0.44 MeV neutrons while operating at pressures corresponding to tissue site sizes of 0.5, 1 and 2 μ . The aim of these measurements was to obtain reliable microdosimetric data for selected types of radiation and to assess the magnitude of the so-called wall effect and the influence of the geometry of the counter on the shape of spectra.

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647175 PREV SN - 647174 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019049

060Lam, Y.M.P.

090Rigorous formulation of the site model

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390102-107 430NTIS, PC A12/MF A01

530EDB;ERA

540EDB-550200;

550TIC 560United States of America (USA) 570United States of America (USA)

700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801BIOLOGICAL MODELS:Q1,Q2;NEOPLASMS:T1;BIOCHEMICAL REACTION KINETICS;CYTOLOGY;BIOLOGICAL REPAIR:T2;

T:Q1 NEOPLASMS;BIOLOGICAL MODELS

T:Q2 BIOLOGICAL REPAIR;BIOLOGICAL MODELS

950This report attempts to demonstrate, through the use of an example, that it is possible to describe the competition between repair of sublesions and interaction between sublesions in a cell nucleus. This formulation adheres to the following general concepts of the theory of dual radiation: (1) radiation produces ionization energy in a cell nucleus, which is converted to non-lethal damage in DNA. Each nonlethal damage, called sublesion, by itself has no effect on the cell. (2) The amount of ionization energy deposited is not a function of the dose of radiation but is a stochastic quantity whose distribution is dependent on dose. (3) A sublesion does not remain as such indefinitely in a cell. In time, it is eliminated either by some repair process or by combination with another to produce a lesion. The production of a lesion represents a definite change in the genetic content of the cell. Some lesions may not lead to any alterations in the behavior observable in the laboratory, others may result in cell transformation or loss of the capability to reproduce. It is also assumed that both the repair rate and the interaction of sublesions are sufficiently constant with respect to cell cycle changes, and that the cell cycle is sufficiently long that the sublesions will prior to mitosis usually be repaired or will have been one of the partners in lesion foundation.

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0108012019050

060Miller, R.C.; Hall, E.J.

090Oncogenic transformations with split doses of x-rays

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390108-113 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560152;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801CARCINOGENESIS:T1;BIOLOGICAL MODELS:Q1;FRACTIONATED IRRADIATION:T2;X RADIATION;BIOLOGICAL RADIATION EFFECTS:Q2;

SURVIVAL CURVES;ANIMAL CELLS;

T:Q1 CARCINOGENESIS;BIOLOGICAL MODELS

T:Q2 FRACTIONATED IRRADIATION;BIOLOGICAL RADIATION EFFECTS

950At dose rates in the range of 32 to 180 rad min⁻¹, splitting low doses in the range of 0.3 Gy to 1.0 Gy into two equal fractions separated by 5 hours enhanced the frequency of transformation when compared to single exposure to the same dose. (PSB)

647177 PREV SN - 647176 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019051

060Borek, C.

090Neoplastic transformation following split doses of x-rays

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390115-119 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560120;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG820- NONE

801NEOPLASMS:T1;BIOLOGICAL MODELS:Q2;RADIOINDUCTION:Q1;CARCINOGENESIS:T2;FRACTIONATED IRRADIATION:T3;BIOLOGICAL RADIATION EFFECTS:Q3;

T:Q1 NEOPLASMS;RADIOINDUCTION

T:Q2 CARCINOGENESIS;BIOLOGICAL MODELS

T:Q3 FRACTIONATED IRRADIATION;BIOLOGICAL RADIATION EFFECTS

950Experiments with fractionated radiation doses, showed that cell transformation frequencies remained about the same for doses between 150 and 600 rad whether delivered by a single dose or by split exposures. At higher doses fractionation led to a reduction in the transformation frequency. (PSB)

0108012019052

060Hall, E.J.

090Intercomparison of relative biological effectiveness and oxygen enhancement ratio at clinically used neutron facilities

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390120-127 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-550603;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C63 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801NEUTRON THERAPY:T1;RBE;OXYGEN ENHANCEMENT RATIO;COMPARATIVE EVALUATIONS:Q1;ERRORS;

T;Q1 NEUTRON THERAPY;COMPARATIVE EVALUATIONS

950The present report summarizes the results of a new series of biological intercomparisons of neutron therapy

beams using mammalian cells cultured in vitro. Experiment design was based upon two important considerations: (a)

It is a characteristic of cell culture experiments that variations within an experiment are much smaller than those

between experiments. Consequently, neutron facilities were intercompared in pairs, within a given experiment, using

cells from a common culture irradiated on the same day. (b) A standardized treatment fixture was used at all

facilities, constructed of lucite, with space for 6 tissue culture flasks, and provision for an ionization chamber

to be inserted into the jig to determine the dose received at the position occupied by the cells. Full build-up was

ensured because the cells were overlaid with 2 cm of tissue culture medium. A substantial international effort to

achieve compatible dosimetry was mounted by the physicists at the various installations engaged in neutron therapy,

as a consequence of which there is agreement to within $\pm 1.5\%$ for dose measurements in air. The use of the standard

treatment fixture was an attempt to extend the compatible dosimetry to a practical set-up for the irradiation of

cell cultures.

647179 PREV SN - 647178 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019053 040N

060Freeman, M.R.; Geard, C.R.

090Sister chromatid exchanges: interaction of a retinoid (Ro-10-9359) and a protease inhibitor (antipain) with x rays

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390128-131 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560121;560114;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

801SISTER CHROMATID EXCHANGES:T1,D;GENETIC RADIATION EFFECTS:Q1,D;X RADIATION:T2,D;ENZYME INHIBITORS;ENZYMES;

VITAMIN A:T3,D;BIOCHEMICAL REACTION KINETICS:Q2,Q3,D;MITOSIS;RADIOSENSITIVITY:Q4,D;ANIMAL CELLS:T4,D;

RADIOPROTECTIVE SUBSTANCES;CARCINOGENESIS;MITOTIC INDEX;

T;Q1 SISTER CHROMATID EXCHANGES;GENETIC RADIATION EFFECTS

T;Q2 X RADIATION;BIOCHEMICAL REACTION KINETICS

T;Q3 VITAMIN A;BIOCHEMICAL REACTION KINETICS

T;Q4 ANIMAL CELLS;RADIOSENSITIVITY

**** CHECK DATA TAGGING

950The induction of sister chromatid exchanges (SCE) was studied at the first post-irradiation mitosis of mouse C3H/10T1/2 cells and Syrian hamster embryo cells following 300 rad of x rays and incubation with a protease inhibitor (antipain) or a vitamin A analog (Ro-10-9359).

64/180. PREV SN - 647179 RA HDRB EB121680 EB122280 FILE-EB1212.E03
0108012019054 040N
080Geard, C.R.
090Effect of neutron induced recoil protons of restricted energy and range on chromosome aberration formation
110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979
150C00--4733-2
370Jul 1979 390132-144 430NTIS, PC A12/MF A01
530EDB;ERA;INS
540EDB-560121;560153
550TIC 560United States of America (USA) 570United States of America (USA)
600R 610C12;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.
AUG620- NONE

801CHROMOSOMAL ABERRATIONS:T1,D;NEUTRON THERAPY;RECOILS;BIOLOGICAL RADIATION EFFECTS:Q2,D;ENERGY RANGE;KEV RANGE

100-1000;NEUTRONS:T2,D;RADIOINDUCTION:Q1,D;PROTONS;

T;Q1 CHROMOSOMAL ABERRATIONS;RADIOINDUCTION

T;Q2 NEUTRONS;BIOLOGICAL RADIATION EFFECTS

**** CHECK DATA TAGGING

950The material presented in last years Annual Report (R8-12) has been expanded and updated by the inclusion of data obtained from assessing the effects of 430 keV monoenergetic neutrons on Vicia faba chromosomes. Further, a more precise determination of microdosimetric quantities of relevance has resulted in some alterations. Essentially, physical energy deposition events (as determined by microdosimetry) have been related to chromosomal changes (as determined by light microscopy) in nuclei irradiated in the latter part of the cell cycle of Vicia faba.

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0108012019055 040N

060Geard, C.R.

090Effects of 1.91 MeV monoenergetic neutrons on Vicia faba chromosomes: microdosimetric considerations

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390145-153 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560141

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C13 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

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**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-
September 30, 179

801VICIA:D;CHROMOSOMAL ABERRATIONS:T2,D;NEUTRONS:T1,D;RECOILS;BIOLOGICAL RADIATION EFFECTS:Q1;RADIOINDUCTION:Q2;

MEV RANGE 01-10;

T;Q1 NEUTRONS;BIOLOGICAL RADIATION EFFECTS

T;Q2 CHROMOSOMAL ABERRATIONS;RADIOINDUCTION

**** CHECK DATA TAGGING

950Chromosomal alterations have been assessed after irradiation with 1.91 MeV monoenergetic neutrons. Results are
tabulated. (PSB)

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0108012019056

080Bird, R.P.

090Sequential exposures with high and low-LET radiations

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390154-157 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560120;560152

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 179

801ANIMAL CELLS:T1;HAMSTERS;X RADIATION:T2;BIOLOGICAL RADIATION EFFECTS:Q2;LET;CHARGED PARTICLES;FRACTIONATED
IRRADIATION;RADIOSENSITIVITY:Q1;

T:Q1 ANIMAL CELLS;RADIOSENSITIVITY

T:Q2 X RADIATION;BIOLOGICAL RADIATION EFFECTS

950Measurements have been made with synchronized Chinese hamster V79 cells irradiated with split doses of x rays only. The cells were exposed at room temperature to 50 kV x rays with the total dose delivered either in one exposure or in two equal exposures separated by a few minutes or as long as 30 minutes. Dishes of irradiated cells, as well as control dishes, were simply kept on a lab bench at room temperature between irradiations. For the longest interval used (30 minutes) the pH of the cell medium increased slightly. Synchronized cells, either at the G₁/S transition or in late S phase, were irradiated with single doses to about the 5% survival level. The results of these experiments indicate that splitting the radiation dose over these time intervals has very little effect on the cell surviving fraction. Enhancement of mammalian cell survival for split doses separated by minutes has been reported for cells irradiated in plateau or stationary phase. There is no obvious trend of enhancement for the

synchronized cells irradiated as described.

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0108012019057

060Bird, R.P.

090Associated ion experiment

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390158-161 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560121;560152;

550YIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG820- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-
September 30, 179

801DEUTERIUM IONS;ANIMAL CELLS;LET;TI;CYSTAMINE;RADIOPROTECTIVE SUBSTANCES;RESPONSE MODIFYING FACTORS;DOSE-

RESPONSE RELATIONSHIPS;BIOLOGICAL RADIATION EFFECTS;Q1;MOLECULAR IONS;CHROMOSOMAL ABERRATIONS;

T;Q1 LET;BIOLOGICAL RADIATION EFFECTS

**** DOSE RESPONSE RELATIONSHIPSLLCK KWDJJ

950Diatomic deuterium ions were used as a source of correlated pairs of ions at RARAF in studies of the dependence on their separation for mammalian cell killing. However, the separations that could be attained, while holding constant the LET of the particles, are limited by the available RARAF energy. The RARAF acceleration voltage is adjusted so that the LET of the particles, as they subsequently traverse mammalian cells, is the same for each Mylar thickness. In order to extend the studies to greater separations, a change was made to diatomic hydrogen ions as the source of correlated pairs of ions. The characteristic separations that result when diatomic hydrogen ions traverse different Mylar thicknesses are tabulated. For comparison, the characteristic separation is approximately the same for paired deuterons traversing 10 μm Mylar plus a 3 μm cell thickness as it is for paired protons traversing 6 μm Mylar plus a 3 μm cell thickness.

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0108012019058 040N

060Bird, R.P.; Kliauga, P.J.

090Irradiations with the track segment facility and the analysis of data

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390162-169 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560121;560152;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-
September 30, 179

801ANIMAL CELLS:T1,D;RADIOSENSITIVITY:Q1;SURVIVAL CURVES:D;LET:T2,D;BIOLOGICAL RADIATION EFFECTS:Q2,D;DOSE-

RESPONSE RELATIONSHIPS:D;SYNCHRONOUS CULTURES;

T;Q1 ANIMAL CELLS;RADIOSENSITIVITY

T;Q2 LET;BIOLOGICAL RADIATION EFFECTS

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950Results from track segment irradiations of synchronized mammalian cells are reported previously for a range of
LET values listed with the ions used and their LET values. The highest LET values resulted from helium track
segments with a considerable change in LET across cellular dimensions. The survival curves obtained for two phases
of the cell cycle, namely G₁/S and late-S, were fitted by computer analysis to the form: $S = e / \sup -\alpha D - \beta D^2$ where D
is the dose and α and β are constants. The best-fit values of the parameters, α and β , are tabulated with 95%
confidence limits indicated for protons, deuterons and helium -3 ions. The LET trends of the survival curve
parameters for late-S and G₁/S cells, respectively, are indicated.

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647185 PREV SN - 647184 RA HDRB EB121680 FILE-EB1212.E03

0108012019059 040N

060Borek, C.; Miller, R.C.

090Modulating effects of the protease inhibitor Antipain on x-ray induced transformations

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390170-178 430NTIS, PC A127MF A01

530EDB;ERA;INS

540EDB-560120;560114;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C14 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

NEW INIS DESC.900PHORBOL ESTERS;ONCOGENIC TRANSFORMATIONS;

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 179

801HAMSTERS;ANIMAL CELLS:T1,D;RADIOSENSITIVITY:T3,Q1;X RADIATION:T2,D;RADIOPROTECTIVE SUBSTANCES:Q2;RESPONSE

MODIFYING FACTORS:Q3,Q5;PHORBOL ESTERS:T4,D;ENZYME INHIBITORS;SURVIVAL CURVES;IN VITRO;DNA;BIOLOGICAL REPAIR;

BIOLOGICAL RADIATION EFFECTS:T5;RADIOSENSITIVITY EFFECTS:Q4,D;ONCOGENIC TRANSFORMATIONS:Q1,D;

T;Q1 ANIMAL CELLS;RADIOSENSITIVITY

T;Q1 ANIMAL CELLS;ONCOGENIC TRANSFORMATIONS

T;Q2 X RADIATION;RADIOPROTECTIVE SUBSTANCES

T;Q3 RADIOSENSITIVITY;RESPONSE MODIFYING FACTORS

T;Q4 PHORBOL ESTERS;RADIOSENSITIVITY EFFECTS

T;Q5 BIOLOGICAL RADIATION EFFECTS;RESPONSE MODIFYING FACTORS

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950Protease inhibitors have been shown to inhibit the expression of mutations in bacteria and to inhibit the tumor-promoting effect of phorbol esters in mice. We have investigated the effect of the protease inhibitor Antipain on

cell transformation by x-irradiation in two in vitro systems; namely short-term cultures of freshly explanted

hamster embryo cells and in the 10T1/2 cell line derived and cloned from C3H mouse embryo.

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0108012019060 040N

060Hall, E.J.; Astor, M.

090Comparison of two nitroimidazoles as hypoxic sensitizers, radiosensitizers and cytotoxic agents

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370JUL 1979 390179-192 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560120;560111;560305;

550TIC 580United States of America (USA) 570United States of America (USA)

600R 610C11 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

801RADIOSENSITIVITY:T2,D,Q1;ANOXIA;TUMOR CELLS:T1;CELL CULTURES;DRUGS:Q4;RESPONSE MODIFYING FACTORS:Q2,D;

RADIOPROTECTIVE SUBSTANCES:Q3,Q5;TOXICITY:Q3,Q5;HAMSTERS;GAMMA RADIATION:D;NEUTRON BEAMS;NEUTRONS:D;RADIOTHERAPY:T4;

ORGANIC NITROGEN COMPOUNDS:T3,D;DRUGS;OXYGEN ENHANCEMENT RATIO;BIOLOGICAL RADIATION EFFECTS;SURVIVAL CURVES:T,D;

IMIDAZOLES:T5,D;

T;Q1 TUMOR CELLS;RADIOSENSITIVITY

T;Q2 RADIOSENSITIVITY;RESPONSE MODIFYING FACTORS

T;Q3 ORGANIC NITROGEN COMPOUNDS;RADIOPROTECTIVE SUBSTANCES

T;Q3 ORGANIC NITROGEN COMPOUNDS;TOXICITY

T;Q4 RADIOTHERAPY;DRUGS

T;Q5 IMIDAZOLES;RADIOPROTECTIVE SUBSTANCES

T;Q5 IMIDAZOLES;TOXICITY

T SURVIVAL CURVES

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⁹⁵⁰Experiments designed to compare the two 2-nitroimidazoles Ro-07-0582 and Ro-07-0741 in terms of their radiosensitizing and cytotoxic properties are reported. A standardized in vitro cell culture test system was used. Ro-07-0741 has been shown to be a more effective radiosensitizer of hypoxic cells than is Ro-07-0582 but this may be offset by the increased toxicity shown by this drug. Whether this increased toxicity is seen in animals and presents a problem remains to be seen, but from first reports (Brown, personal communication) in vivo, Ro-07-0741 is indeed more toxic to animals than Ro-07-0582. Because of in vivo toxicity the doses of Ro-07-0741 administered to mice have been reduced approximately by a factor of two in respect to Ro-07-0582.

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647187 PREV SN - 647186 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019061

080Astor, M.; Hall, E.J.; Osmak, R.S.

090MTDQ as a hypoxic cell radiosensitizer alone and in combination with misonidazole

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150COO--4733-2

370Jul 1979 390193-206 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560122;560301;

550TIC 580United States of America (USA) 570United States of America (USA)

600R 610C21 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG820- 6,6-methylene-bis-2,2,4 trimethyl-1,2-dihydroquinoline

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 179

801ANIMAL CELLS:M1;RADIOSENSITIZERS:Q1,Q2;RADIOTHERAPY:M2;RESPONSE MODIFYING FACTORS;ANOXIA;FOOD;PRESERVATION;

TOXICITY:Q3;HEALTH HAZARDS;ORGANIC NITROGEN COMPOUNDS:M3;DRUGS;ANTIOXIDANTS;X RADIATION;GAMMA RADIATION;

M;Q1 ANIMAL CELLS;RADIOSENSITIZERS

M;Q2 RADIOTHERAPY;RADIOSENSITIZERS

M;Q3 ORGANIC NITROGEN COMPOUNDS;TOXICITY

950Misonidazole is an electron affinic compound that selectively sensitizes mammalian cells to the lethal effects of x and gamma rays, and is already in use in clinical trials as an adjunct to radiotherapy. MTDQ (6,6-methylene-bis-2,2,4 trimethyl-1,2-dihydroquinoline) is an antioxidant that was initially developed as a food additive but has recently been the subject of preliminary clinical investigations in Hungary and limited in vitro experimentation in the United States. The present communication describes experiments involving the testing of MTDQ as a hypoxic cell radiosensitizer alone and in combination with misonidazole in order to determine the sensitizing properties of the two widely different agents.

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647188 PREV SN - 647187 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019062

060Roizin-Towle, L.; Hall, E.J.

090Combination studies with bleomycin, misonidazole and x-rays

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390207-215 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560121;550603;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11;C63 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

801RADIO THERAPY;T1;OPTIMIZATION;Q1;X RADIATION;ANOXIA;CHEMOTHERAPY;CELL CULTURES;SYNERGISM;CELL CYCLE;TUMOR CELLS;T2;RADIOSENSITIVITY;Q2;RESPONSE MODIFYING FACTORS;RADIOSENSITIZERS;OXYGEN ENHANCEMENT RATIO;OXYGEN;NEOPLASMS; DRUGS;

T;Q1 RADIO THERAPY;OPTIMIZATION

T;Q2 TUMOR CELLS;RADIOSENSITIVITY

950The experiments investigated the effect of bleomycin (BLM) on aerated and hypoxic cells in culture, and studied the interaction between BLM and x-rays. In addition, the combination of BLM and misonidazole has been studied to investigate the extent to which the electron affinic compound kills the hypoxic cells that are resistant to BLM. Since the majority of antineoplastic drugs kill actively dividing cells, they probably are not as effective in killing cells in solid tumors which are deficient in molecular oxygen and unlikely to be in active cell cycle. Misonidazole was used with BLM for this reason, that it is selectively cytotoxic towards hypoxic cells, and would avoid further damage to normal aerated cells in tissues already treated with other antineoplastic agents. Although misonidazole is used primarily as an hypoxic cell sensitizer, its use as chemotherapy agent warrants consideration.

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060 Miller, R.C.; Harisiadis, L.; Hall, E.J.

090 Effect of hyperthermia alone, or combined with x-rays, on the incidence of oncogenic transformations in vitro

110 Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150 COO--4733-2

370 Jul 1979 390 216-221 430 NTIS, PC A12/MF A01

530 EDB;ERA;INS

540 EDB-560121;550603;

550 TIC 560 United States of America (USA) 570 United States of America (USA)

600 R 610 C11;C63 700 187 2000 710 Columbia Univ., New York (USA). Radiological Research Lab.

AUG 820- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 179

801 HYPERTHERMIA:T2;X RADIATION;ANIMAL CELLS;PATIENTS;RADIOTHERAPY;RADIOSENSITIVITY:Q1;TUMOR CELLS:T1;RESPONSE

MODIFYING FACTORS:Q2;CHEMOTHERAPY;CARCINOGENESIS;BIOLOGICAL MODELS;

T;Q1 TUMOR CELLS;RADIOSENSITIVITY

T;Q2 HYPERTHERMIA;RESPONSE MODIFYING FACTORS

950 This study describes how elevated temperatures influenced the transformation rates of irradiated and non-irradiated cells.

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647190 PREV SN - 647189 RA HDRB EB121680 EB122280 FILE-EB1212.E03

0108012019064

060Schwartz, L.E.; Geard, C.R.

090Investigation in vitro of the clastogenic effects of misonidazole

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390222-241 430NTIS, PC A12/MF A01

530EDB;ERA;INS

540EDB-560122;560301;

550TIC 560United States of America (USA) 570United States of America (USA)

600R 610C11 700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 179

801DRUGS;RADIOSENSITIZERS:T1,Q3;RADIOTHERAPY;ANOXIA;TOXICITY:Q1;CARCINOGENESIS:T2;BIOLOGICAL MODELS:Q2;CHROMOSOMAL

ABERRATIONS;SISTER CHROMATID EXCHANGES;ORGANIC NITROGEN COMPOUNDS;ANIMAL CELLS:T3;

T;Q1 RADIOSENSITIZERS;TOXICITY

T;Q2 CARCINOGENESIS;BIOLOGICAL MODELS

T;Q3 ANIMAL CELLS;RADIOSENSITIZERS

950In view of the possible wider utilization of misonidazole as an adjunct to clinical radiotherapy, it becomes important to establish the potential for more subtle effects on normal cells. The present study included an investigation of the effects of misonidazole on aerated normal cells, in vitro, with respect to oncogenic transformations, sister chromatid exchange frequency, chromosomal aberrations, cell cycle progression, cell killing, and cell doubling time.

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647191 PREV SN - 647190 RA HORB EBI21680 EBI22280 FILE-EBI212.E03

0108012019065

060Borek, C.

090Regulation of retinol-binding protein metabolism in cultured rat liver cell lines

110Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-September 30, 1979

150C00--4733-2

370Jul 1979 390242-248 430NTIS, PC A12/MF A01

530EDB;ERA

540EDB-551000;550300;

550TIC 560United States of America (USA) 570United States of America (USA)

700187 2000 710Columbia Univ., New York (USA). Radiological Research Lab.

AUG620- NONE

**** Check DE: 110 DUPLICATE DE NUMBER.

****DUPLICATE LINE,110-Radiation physics, biophysics and radiation biology. Progress report, October 1, 1979-
September 30, 179

801RATS;LIVER:T2;CELL CULTURES;PROTEINS:T1;METABOLISM:Q1;BIOSYNTHESIS;DYNAMIC FUNCTION STUDIES:Q2,Q3;LIPIDS;

ALCOHOLS;VITAMIN A:T3;NUTRITIONAL DEFICIENCY;NEOPLASMS;PREVENTIVE MEDICINE;EPIDEMIOLOGY;DIET;

T;Q1 PROTEINS;METABOLISM

T;Q2 LIVER;DYNAMIC FUNCTION STUDIES

T;Q3 VITAMIN A;DYNAMIC FUNCTION STUDIES

950The present studies seek to develop an effective model system for the more detailed study of the regulation of
retinol binding protein (RBP) metabolism in the liver cell. Two cell lines were found that synthesized detectable
amounts of RBP in vitro. One of these, the MH₁C₁ cell line, was derived from a transplantable Morris hepatoma No.

7795. These cells have a doubling time of 5 days. The other cell line (H₄ II EC₃, or H₄ cells) was initially
established from a Reuber H-35 hepatoma (Pitot et al., 1964), and is a heteroploid cell line (Miller et al., 1972).

The H₄ cells proliferate initially (in serum-containing media) as monolayers, but later pile up at high densities
(Borek, 1978). In contrast to the slow growing MH₁C₁ cells, H₄ cells have a doubling time of about 24 hours.

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