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THE ORNL SPACE BIOLOGY PROGRAM

ANNUAL REPORT

PERIOD ENDING JUNE 30, 1964

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BIOLOGY DIVISION

THE ORNL SPACE BIOLOGY PROGRAM

Annual Report

Period Ending June 30, 1964

Prepared by

G. E. Stapleton

The Basic Supporting Program, which involves estimation of the effectiveness of high-energy protons is one phase of research at ORNL, carried out under NASA Order Number R-77. The Biosatellite research is carried out at ORNL under NASA Order Number R-60.

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SUMMARY

This report describes work performed to July 1, 1964 in the ORNL Space Biology Program through an interagency agreement with the U.S. Atomic Energy Commission and the National Aeronautics and Space Administration. The Program is arbitrarily divided into (1) ground-based experiments, the Basic Supporting Program, and (2) the in-flight experiments and preparatory testing, the Biosatellite Program. This subdivision is more apparent than real, since the Basic Program supplies base-line data of importance for the Biosatellite and other in-flight experiments. The time table or schedule determined that the Basic Program would precede to a great extent the Biosatellite Program, the latter being on strict schedule determined by NASA.

As of July 1964, the Basic Program has proceeded to the investigation of the relative biological effectiveness (RBE) of protons of energies ranging from 22 to 750 Mev compared with 250 kvp x rays. The biological materials that can be made into very thin samples have been subjected to heavy ions as well as protons. Plans for a systematic assessment of long-term effects induced by protons in mice are now complete.

Three groups in the Biology Division will participate in two in-flight experiments on Biosatellite A and Gemini III. The former is a radiation-source-bearing satellite scheduled for late 1965, and the latter is a manned spaceflight scheduled for late 1964 carrying a self-contained source-bearing package. Progress in preparatory experiments will be discussed briefly.

I. INTRODUCTION

The Biology Division of ORNL serves two chief functions in the U.S. Space Sciences Program: (1) to evaluate the relative biological effectiveness (RBE) of space radiations, and (2) to investigate the possible synergistic or combined effects of low level irradiation and other physical or mechanical factors encountered in space flight.

The radiations of chief importance in space flight are protons, ranging from a few to hundreds of Mev depending on conditions of flight (i.e. if the Van Allen radiation belts are encountered or a solar flare occurs during the flight). A systematic survey of RBE versus linear energy transfer (LET) for protons of various energies from 22 to 750 Mev has been made. The survey was extended to heavy ions for those biological materials adaptable to irradiation as very thin layers. Data from previous neutron experiments are included for comparison.

The following biological materials and end points have been investigated for LET effects:

(a) Inactivation and mutation in *Escherichia coli*;

(b) Inactivation and mutation on conidia of *Neurospora crassa*;

(c) Killing of spermatogonia and oocytes of the mouse; and

(d) Production of chromosome aberrations in human leukocytes.

The following experiments were selected for measurement of the synergistic effect of radiation and other physical and mechanical factors that accompany launch, orbit, and reentry of space vehicles:

(a) Measurement of dominant and recessive lethality in the wasp *Habrobracon* (Biosatellite A);

(b) Inactivation and forward mutation in *Neurospora* (Biosatellite A); and

(c) Measurement of chromosome aberration frequency in human leukocytes (Gemini III).

The preparatory ground-based experiments are in various stages, and a short progress report is given.

II. PROTON-RBE PROJECT

A. SOURCES OF RADIATIONS

1. Description of Facilities

The following accelerators have been used to provide the various radiations:

Facility	Radiation
ORNL 86-in. Cyclotron	22-Mev protons
Harvard University	
160-in. Synchrocyclotron	50-, 100-, and 130-Mev protons
University of Chicago	
170-in. Synchrocyclotron	442- and 447-Mev protons
University of California	
184-in. Synchrocyclotron	750-Mev protons
Heavy-Ion Linear Accelerator (HILAC)	Helium, carbon, and neon ions at 10 Mev/nucleon
ORNL, Biology Division	
Cockcroft-Walton Accelerator	2.5- and 14.1-Mev neutrons

In addition to these facilities a biology facility has been completed at the ORNL Isochronous Cyclotron (ORIC), which will provide 40- to 80-Mev protons for whole animal exposures and heavy particles for partial body exposures.

2. Development of Biology Facility at the Oak Ridge Isochronous Cyclotron

E. B. Darden Jr., R. S. Bender,[1] and A. C. Upton

The Oak Ridge Isochronous Cyclotron, which was recently completed, is a variable-energy accelerator employing a radially increasing magnetic field to maintain isochronism. It is designed to accelerate protons and a variety of heavier ions over a wide range of energies up to 145 Mev. For biological studies the main beam is deflected and brought out into a separate experimental area by means of a beam optical system, which is designed to provide a uniform beam with low background over a 6-in. diameter circle (Figure 1). The dosimetry apparatus uses a calibrated thin-wall ionization chamber with a preset scaler, so that the cyclotron is turned off automatically when the desired dose is achieved. When the range of the particles exceeds the thickness of the specimens, an evacuated tube with a graphite plate is used to stop the beam at a distance of 10 ft to minimize background radiation. It is expected that a dose of 10 to 10^5 rads of 75-Mev protons can be delivered in a period of 10 sec to 5 min. All components were constructed and tested individually. The beam optics are being aligned and tests of uniformity and reliability made. The first experiments with mice are tentatively scheduled for the fall of 1964.

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[1]Electronuclear Division, ORNL

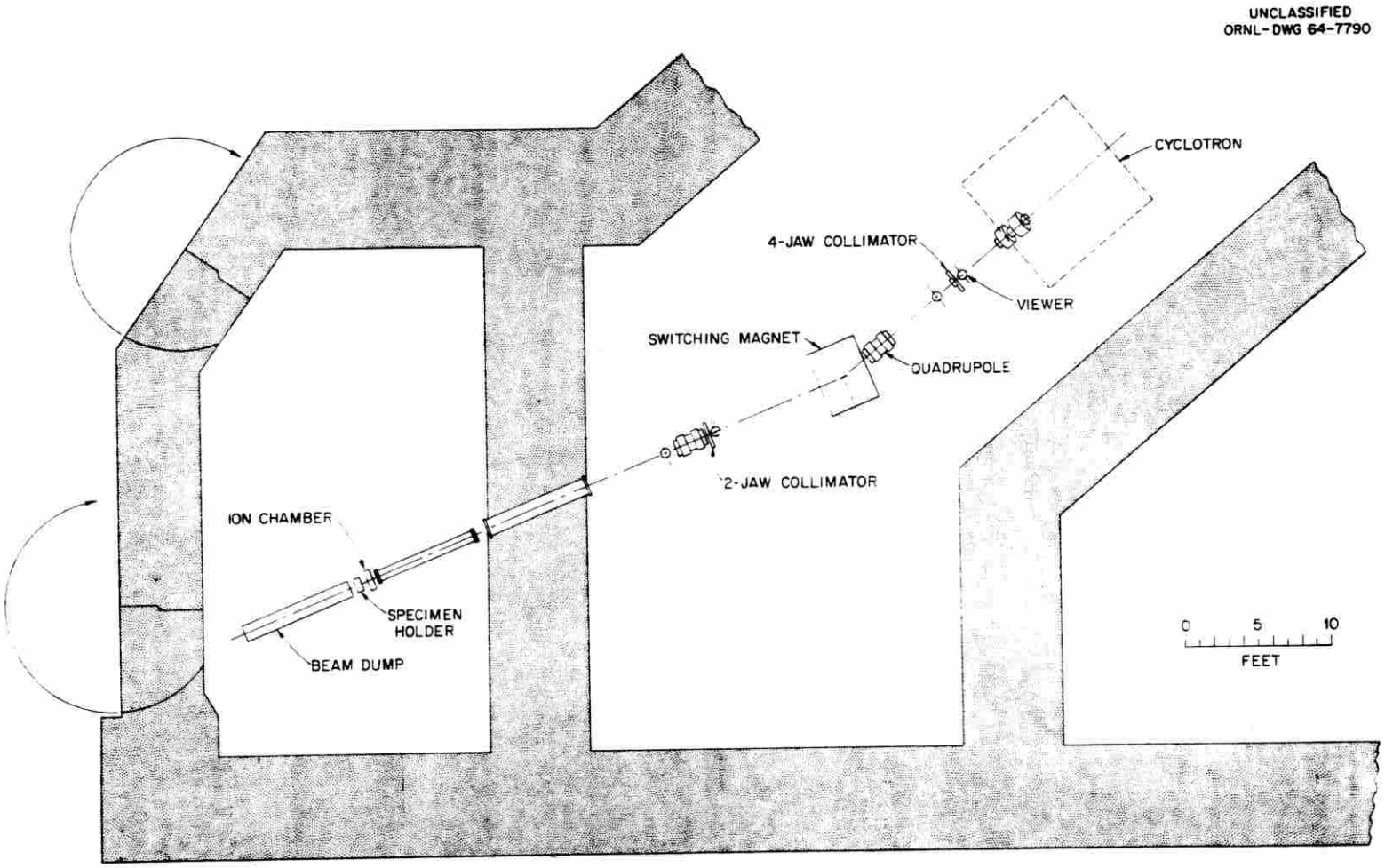

Fig. 1. Diagram of the ORIC Biology Irradiation Facility, Including Elements of the Beam Optical System.

B. PHYSICAL METHODS

1. Physical Methods for Irradiations

The systems for exposures of biological specimens at the ORNL 86-in. cyclotron and at the Harvard 160-in. synchrocyclotron were described in our previous annual report.[2] The physical setup at the University of Chicago 170-in. synchrocyclotron is described in detail in a report from that site.[3] The exposure system at the University of California is described by Tobias,[4] and the experimental arrangement and dosimetry for the University of California HILAC are described in detail by Brustad.[5]

The physical methods used at all accelerators for proton irradiations have much in common and consist generally of allignment and focusing of the external beam of particles to the exposure rooms and scattering of the focused beam in materials such as water, plastic, aluminum, or copper, depending on the energy of the particle. The scattering material is chosen to obtain a beam of sufficient size and of sufficiently uniform flux (particles per unit area) to permit the exposure of the biological specimen. The size of the scattered beam is determined roughly by darkening of x-ray or photographic film, and the flux is determined by proton activation of small foils or "buttons" of scintillating plastic distributed over the useful area of the beam.

Dosimetry was performed in several ways, (a) by critical measurement of the flux over a defined (collimated) beam and calculation of the adsorbed energy from

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[2] ORNL-Space Biology Program Annual Report, June 30, 1963, ORNL-TM-720. pp. 9-15.

[3] U.S. Air Force Radiation Laboratory Quarterly Report, April 15, 1963.

[4] C. A. Tobias, in Radiation Biology and Medicine Atoms for Peace, ed. by W. D. Claus, (1958).

[5] Tor Brustad, "Molecular and Cellular Effects of Fast Charged Particles," Rad. Res. 15, pp. 139-158 (1961).

the known stopping power of the biological material, (b) by direct ionization measurements with a uniform-beam in a gas-filled ion chamber and correction for the relative stopping powers of the gas and biological material, or (c) by use of tissue-equivalent chambers such as the Victoreen thimble chambers. Where the energy of the protons permitted, two or all methods were used and intercompared.

The beam current was continuously monitored during the exposures with accessory ion chambers in conjunction with a current integrater and scaler. The exposures could be determined in terms of accumulated counts.

The details of dosimetry for the exposures with 442-Mev protons are described in the following section.

2. Dosimetry in High Energy Proton Beams

W. A. Gibson,[6] G. E. Stapleton, and B. B. Webber

a. Introduction

A number of biological studies have been made by irradiating biological systems with a monoenergetic proton beam in the multihundred Mev region. The interpretation of these experiments often depends on accurate dosimetry. In measuring the dose, it is desirable to employ a method that involves little or no electronics, so that the experimenter need not familiarize himself with techniques far from his field of interest.

An ion chamber such as the Victoreen thimble dosimeter fits these requirements; however, it was designed primarily for x-ray dosimetry. The purpose of this note is: (a) to describe dose measurements in a 442-Mev proton beam with a ^{12}C activation method that, electronically, requires only a simple photomultiplier counting system; and (b) demonstrate quantitatively that the Victoreen dosimeter can be successfully used in high-energy proton beams.

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[6]Neutron Physics Division, ORNL

A technique employed to measure the time-integrated intensity of high-energy monoenergetic proton beams is to irradiate carbon to produce the following reaction:

$$^{12}C + p \rightarrow {}^{11}C + p + n . \qquad (1)$$

The ^{11}C produced undergoes beta decay with the release of a positron. The half life of the positron-decay reaction is 20.4 min. Thus with a knowledge of the cross section for reaction (1) and a measure of the positron decay rate of the resulting ^{11}C, one is able to measure the time-integrated intensity of the incident proton beam. A summary of the cross section for ^{11}C production and a description of the various techniques for determining the decay rate is given in a review article by Cumming.[7] Although this technique is applicable from 30 Mev to 30 Bev, the method is more adaptable to the energy region above several hundred Mev, because the cross section becomes fairly independent of energy in this region and, at the present, the cross-section measurements appear to be more precise.

b. Technique

Among the several methods available to measure the decay rate of ^{11}C, the one chosen was irradiation of an organic scintillator and direct observation of the scintillations produced by the positrons. The direct observation was made by optically coupling the proton-irradiated scintillator directly to the face of a 2-in. photomultiplier with opthalmological jelly and covering the top with an Al-foil light reflector. The pulses from the photomultiplier were amplified and counted on a scaler.

The external proton beam at the University of Chicago synchrocyclotron was used. Since the beam was asymmetric in shape and relatively sharply focused, it was spread out by placing a 1/8-in.-thick copper Coulomb scatterer several feet in front of the dosimeter position. This resulted in a beam with intensity variations no

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[7] J. B. Cumming, "Monitor Reactions for High Energy Proton Beams," Ann. Rev. Nucl. Sci. 13: pp. 261-286 (1963).

greater than 20% over a circle with a 1-in. diameter. From previous measurements, the energy of the beam at this position was 442 Mev.

The organic scintillator was machined into cylinders 1 cm in diameter and 1 cm long, and all surfaces were polished. The mass of the resulting cylinders was approximately 0.9 gm. The scintillator was placed in position, and the beam was turned on long enough to produce adequate activation (10 sec in these measurements). The scintillator was then placed on the photomultiplier, and the counting was started 3 min after the beginning of the irradiation and continued for 1 min. Using the accumulated counts and the reaction cross section (taken to be 32.5 ± 1.3 mb at 442 Mev) the number of protons/cm^2 passing through the scintillator was calculated. A typical accumulated count over a 1-min interval was 1.4×10^5, resulting in a calculated beam of 4×10^9 protons/cm^2 during the 10-sec irradiation.

The positron energy spectrum extends from zero to 0.98 Mev. Thus there will be some positrons with insufficient energy to be counted, and some will pass out the side of the scintillator before producing enough pulses to be counted. To correct for these errors the differential pulse-height spectrum for positrons was measured with a single-channel analyzer for one of the scintillators. Figure 2 shows the spectrum. The counter threshold setting was at 0.14, thus only about 1.3% of the counts occurred below the threshold. The results presented are corrected for this loss.

The variation of the beam intensity over the sample area was determined by irradiating small scintillators (1/4-in. diam by 1/16 in. thick) located at various positions.

To provide a convenient method to measure the time-integrated intensity, a helium-filled ion chamber was placed in the beam, and the current from the ion chamber was integrated and recorded in digital form on a scalar. Once the time-integrated intensity at the dosimeter position was related to the integrated ion-chamber current, comparisons with the thimble dosimeter were accomplished with no further monitoring with the ^{12}C reaction.

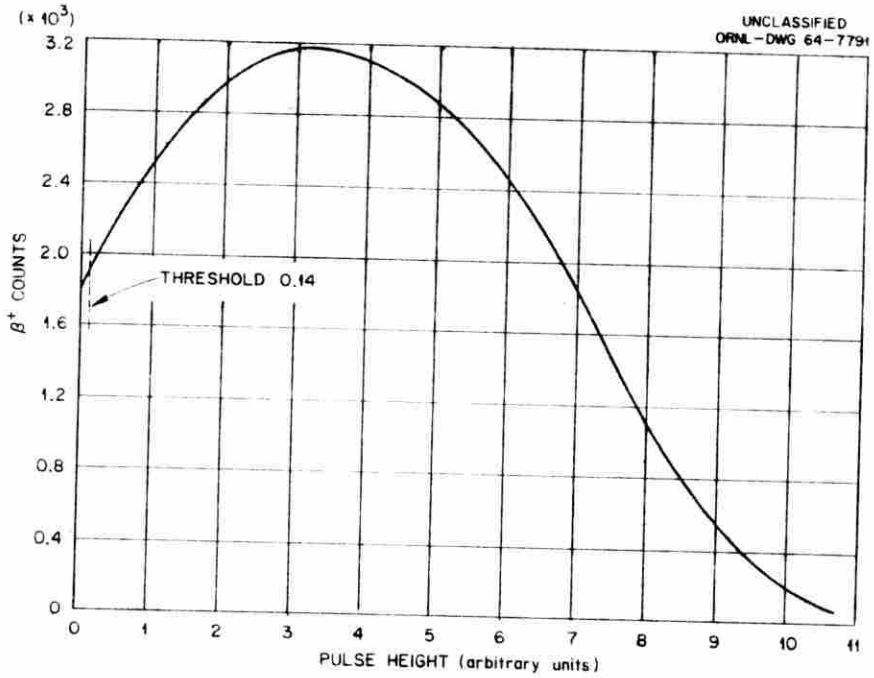

Fig. 2. Carbon-11 Positron Pulse-Height Spectrum in an Organic Scintillator. Threshold was set at 0.14, as indicated by arrow.

c. Comparison with Thimble Dosimeter

The active area of the dosimeter was placed at the center of the beam, and the irradiation was made without any attempt to shield the remainder of the instrument from the beam. However, the beam dropped off rapidly at distances greater than 3/4 in. from the beam center, so that the main body of the dosimeter received little doses. Since the dosimeter is an air equivalent chamber, the dose as calculated from the time-integrated intensity recorded by the helium ion chamber was computed by using the dE/dx for air[8] (taken to be 2.55 Mev cm^2 gm^{-1} at 442 Mev). Table 1 gives the dose calculated from the time-integrated proton intensity measured by the helium ion chamber and the corresponding dose recorded by the Victoreen dosimeter.

Table 1. Comparison of Measured and Calculated Dose

Beam Intensity (Proton/cm^2-sec)	Dose (rads) Victoreen	Calculated[a]
2.8×10^7	132	137 ± 7
3.1×10^7	147	152 ± 8
4.9×10^8	194	204 ± 10

[a] Dose calculated from time-integrated proton intensity measured by helium ion chamber.

The consistency of the results at the three different beam intensities shows that volume recombination in the air within the dosimeter is not important. However, this measurement does not preclude columnar recombination.

[8] M. Rich and R. Madey, Range-Energy Tables, UCRL-2301.

C. BIOLOGICAL METHODS

The biological methods and materials were described in some detail in our previous report.[9] A brief description of pertinent methodology is given in each of the sections of Results of the individual experiments.

D. BIOLOGICAL RESULTS

1. RBE of Fast Charged Particles for Inactivation and Mutation of *Escherichia coli*

G. E. Stapleton, Annie S. Angel, and Alice A. Hardigree

We reported data in the previous Annual Report[10] for inactivation of *Escherichia coli* B/r and inactivation and mutation in *E. coli* (strain AB-531) by 250-kvp x rays and 130 Mev protons. This report contains additional data for 22-, 450-, and 750-Mev protons and for 100-Mev carbon ions. Since last reporting, duplicate irradiations were carried out with all radiations except 750-Mev protons.

The biological materials and methods were presented in some detail in the previous report and will not be repeated here. Aqueous suspensions and thin layers of bacterial cells impinged on Millipore filters were used interchangeably for the various radiations with no detectable differences in cellular response. The latter method was required for irradiations with low energy protons and heavy ions.

Results and Discussion.--Figure 3 shows the inactivation of *E. coli* B/r (ORNL) by x rays and protons of various energies. It is clear that aerobic cells are inactivated with equal efficiency by x rays and protons from 22- to 750-Mev. The RBE or dose required for similar inactivation is constant within 10%.

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[9] G. E. Stapleton and M. A. Bender, ORNL Space Biology Annual Report, June 30, 1963, ORNL-TM-720, pp. 4-8.

[10] G. E. Stapleton, ORNL Space Biology Program Annual Report, June 30, 1963, ORNL-TM-720, pp. 4-9, 15-17.

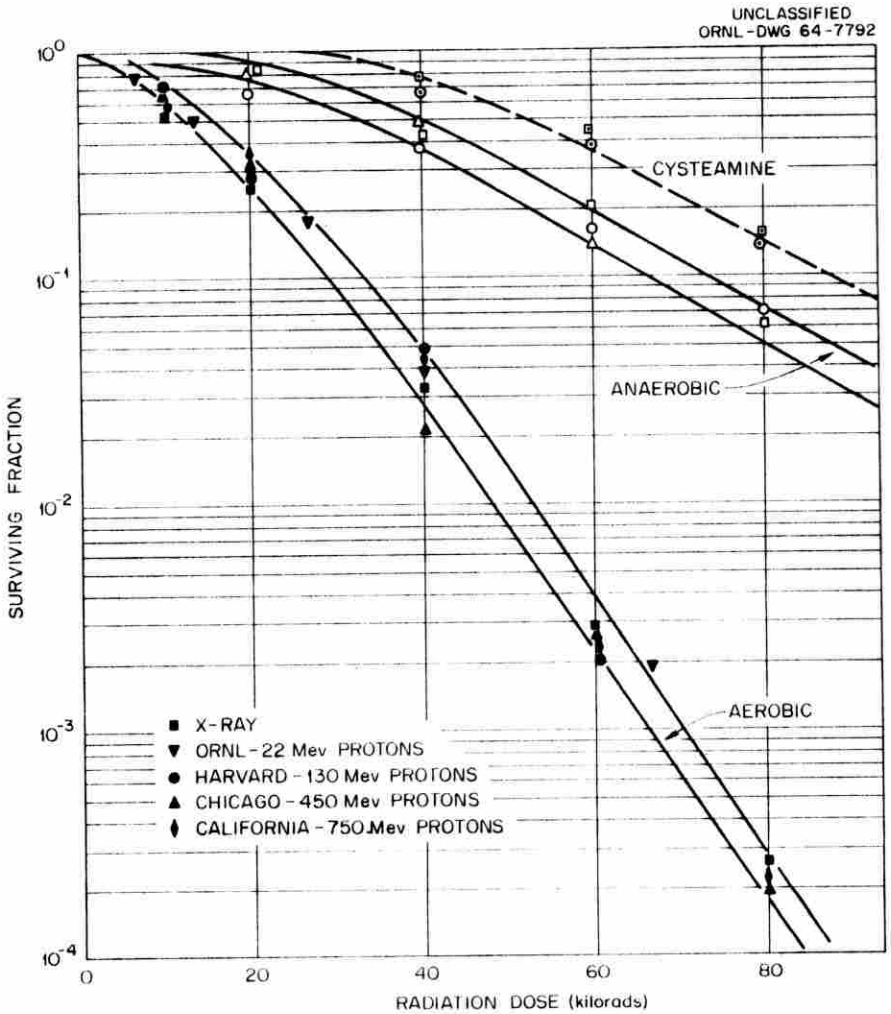

Fig. 3. Surviving Fraction of Escherichia coli B/r as a Function of Dose (Kilorads) for Protons of Various Energies. Survival curves are for cells irradiated aerobically, anaerobically, and in the presence of cysteamine (0.12 M).

The effect of anaerobiosis was investigated in detail with x rays, 130-, and 450-Mev protons. The survival data shown in Figure 3 indicate a constant reduction in inactivation per unit dose for these radiations. All suspensions used for determination of mutation frequencies were anaerobic. Complete mutation-vs-dose relationships are available only for x rays. Only two doses were used for the other radiations, and on this basis the data shown in Table 2 were compiled.

The chemical protection afforded by 0.12 M β-mercaptoethylamine was investigated only for x rays and 130-Mev protons. The data show similar protection capacity from these two radiations.

Table 2 is a compilation of all of the data obtained to date for bacterial inactivation and induction of mutations. The mean LET's for all the radiations and particles other than carbon ions are well below those determined by Brustad[11] to be important for inactivation of another bacterium *Shigella sonnei*. Brustad's data indicate a decreasing RBE as a function of LET for bacteria and phage above 300 Mev cm^2 gm^{-1} when irradiated under aerobic conditions. However, the more common relationship (i.e., increasing RBE versus LET), was found for anaerobic conditions in the range of LET from ~30 to 300 Mev cm^2 gm^{-1}. Anaerobic bacteria show a maximum RBE of ~2.5 in the LET range of ~200 to 500 Mev cm^2 gm^{-1}; decreasing again to 1 in the region of 3000 Mev cm^2 gm^{-1}. Our data are completely consistent with those of Brustad, in that they show increasing RBE versus LET for anaerobic cells and a decrease in RBE for aerobic cells at high LET.

The data on mutation induction are far less reliable than those for inactivation, chiefly because the numbers are small. The frequencies are lower for the K-12 strains than for many strains of *E. coli*, and the doses used are small in the range of 5 to 10 kilorads, where only a small correction is applied for inactivation.

Although the bacterial experiments are of limited usefulness in predicting space hazards for mammals and man, they have served an extremely practical purpose in this survey as a trustworthy biological dosimeter.

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[11] Tor Brustad, "Molecular and Cellular Effects of Fast Charged Particles," Rad. Res. 15, 139-158 (1961).

Table 2. Bacterial Inactivation and Mutation

Radiation	Approximate LET Mev cm^2 gm^{-1}	Inactivation Coefficient[a]		Ratio Aer./Anaer.	Mutation Coefficient Revertants per Survivor per Kilorad	
		Aerobic	Anaerobic		Proline	Galactose
750-Mev protons	2.2	0.14	0.043	3.0	10×10^{-10}	20×10^{-10}
430-Mev protons[b]	2.5	0.15	0.050	3.0	10×10^{-10}	30×10^{-10}
250-kvp x rays	5.0	0.125	0.040	3.1	12×10^{-10}	20×10^{-10}
130-Mev protons	7.0	0.13	0.041	3.1	10×10^{-10}	20×10^{-10}
22-Mev protons	25.0	0.13	0.062	2.1	--	--
100-Mev carbon ions	1850.0	0.11	0.078	1.4	--	--

[a] The inactivation coefficient is the reciprocal of the e^{-1} dose (LD-37) determined from the exponential slope of the survival curves.

[b] The data obtained with the 430-Mev proton beam are somewhat less reliable than the others because the uniform beam area was smaller and the dosimeter system was not cross-calibrated with the other radiations.

2. RBE of Fast Charged Particles for Nuclear Inactivation and Forward Mutation in *Neurospora crassa*

F. J. de Serres B. B. Webber

The *ad-3* heterokaryotic system has been used in assessing the genetic effects of protons of several energies. The protons used thus far have been 750-Mev protons (from the University of California, Berkeley, 184-in. cyclotron) and 447- and 442-Mev protons (from the University of Chicago 170-in. cyclotron). These results are compared with those from 250-kvp x rays and from carbon and helium ions obtained from the Heavy Ion Linear Accelerator at the University of California at Berkeley.

The heterokaryon used is indicated in Table 3. Conidia were irradiated at approximately 0 to 4°C. They were either moist on the surface of millipore filters or stirred while suspended in water or 0.86% sodium chloride solution. The suspensions or filters were kept at approximately 4°C during harvesting and transportation to and from the irradiation site. After irradiation, sufficient conidia to produce 0.5 to 2.0 X 10^6 (but usually 0.8 to 1.0 X 10^6) surviving heterokaryotic colonies per flask were incubated for 6 days at 30°C in aerated 12-liter flasks, each containing 10 liters of a sorbose-sucrose medium supplemented with arginine, adenine, and nicotinamide. The remainder of the general procedure is discussed in some detail in our earlier Annual Report.[12]

a. Results and Discussion

Interpretation of Dose-Inactivation Curves.--The data for survival of heterokaryotic conidia are presented in Table 4. Heterokaryotic inactivation has been shown to be nuclear.[13] The data are best fitted to an equation of the type $y = e^{-\alpha x}$

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[12] F. J. de Serres and B. B. Webber, ORNL Biology Program Annual Report, June 30, 1963, ORNL-TM-420, pp. 6-7, 17.

[13] K. C. Atwood and Frank Mukai, "Survival and Mutation in Neurospora Exposed to Nuclear Detonations," Amer. Nat. 88: 295-314 (1954).

Table 3. Mutant Loci Present in Each of the Components of the Heterokaryon Used in Forward Mutation Studies

Linkage Group	Component 1 (74-OR60-29A)	Component 2 (74-OR31-16A)
I R	hist-2 ad-3A ad-3B nic-2	al-2
III	ad-2	--
IV	--	cot
V	inos	--
VI	--	pan-2

Phenotype of mutants bearing above numbers:

hist-2 -- histidine-requiring;

ad-3 -- purple, adenine-requiring;

nic-2 -- niacin-requiring;

al-2 -- albino or white conidia;

ad-2 -- non-purple, adenine-requiring;

cot -- temperature-sensitive morphological;

inos -- inositol-requiring;

pan-2 -- pantothenic-acid requiring.

Table 4. Dose Survival Data: Proportion of Heterokaryotic Conidia Surviving

Dose (Kilorads)	250 Kv[a,b] X rays	750 Mev Protons	447 Mev Protons	442 Mev Protons	39 Mev[a] Helium Ions	101 Mev[a] Carbon Ions
0	1.00	1.000	1.000	1.000	1.000	1.000
0.9				1.077		
1.0	0.93	0.786	0.828			0.534
1.7				0.830		
2.0	0.86	0.823	0.872		0.674	0.345
4.3				0.813		
5.0	0.59	0.599	0.754		0.474	0.101
8.5				0.654		
10.0	0.53	0.380	0.552		0.344	0.019
17.0				0.340		
20.0	0.29	0.095	0.217			
34.1				0.056		
40.0	0.07	0.010	0.069			

[a]These data are included for purposes of comparison with the proton data.

[b]Survival data for x rays based on mean percentage of survival from four experiments. Dose was measured in roentgens, and absorbed dose may be off by 10-15% because of irradiation procedure.

or $\ln y = \alpha x$, where y = proportion of heterokaryotic conidia surviving the treatment and x = dose in kilorads. The curves best fitting the original data are plotted in Figure 4, where α = the activation constant. Since the RBE of a given radiation is defined as the dose of x rays giving a particular quantitative biological effect divided by the dose of the other radiation that produces the same effect, then the ratio of values of α equals the RBE.[14] The values of α that best fit the inactivation data as well as the computed RBE's for inactivation are given in Table 5.

Interpretation of Dose-mutation Curves.--The data for mutation frequencies are presented in Table 6 and Figure 5. These data can be fitted to an equation of the type $y = ax^b$ or $\log y = \log a + b \log x$, where y = mutant frequency per survivor

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14

$$\ln y = \alpha x$$

$$x = \frac{\ln y}{\alpha}$$

$$\text{RBE} = \frac{x_{x\text{-ray}}}{x_{\text{other}}}, \quad \text{at which } y_{x\text{-ray}} = y_{\text{other}} = y.$$

$$\text{RBE} = \frac{\ln y/\alpha_{x\text{-ray}}}{\ln y/\alpha_{\text{other}}} = \frac{\alpha_{\text{other}}}{\alpha_{x\text{-ray}}}.$$

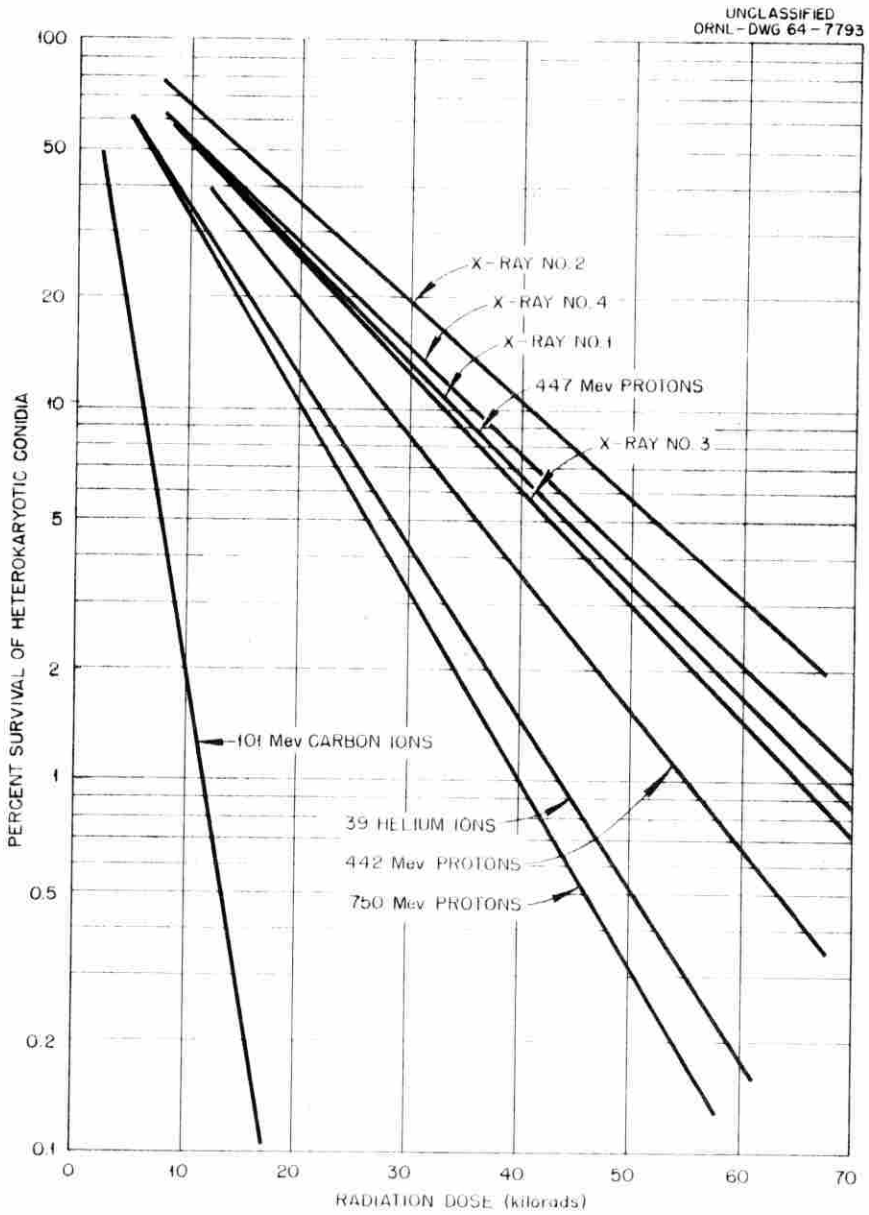

Fig. 4. Survival of Heterokaryotic Conidia (Neurospora crassa) as a Function of Radiation Dose (Kilorads). Data are shown from four separate x-ray experiments and from experiments with 442-, 447-, and 750-Mev protons, 39-Mev helium ions, and 101-Mev carbon ions.

Table 6. Dose-Mutation Data: Ad-3 Mutations per 10^6 Surviving

Dose (Kilorads)	250 Kv[a] X rays	750 Mev Protons	447 Mev Protons	442 Mev Protons	39 Mev[a] Helium Ions	101 Mev[a] Carbon Ions
0[b]	(0.3)	(0.3)	(0.3)	(0.3)	(0.3)	(0.3)
0.9				5.2		
1.0	4.3	6.5	9.8			43.9
1.7				10.8		
2.0	11.4	17.5	35.5		29.8	136.5
4.3				43.9		
5.0	37.3	71.5	37.3		99.8	493.7
8.5				102.3		
10.0	95.7	201.5	87.3		300.1	
17.1				343.8		
20.0	248.9	445.6	256.3			

[a]These data are included for purposes of comparison with the proton data.

[b]A mutation frequency of 0.3×10^{-6} is based on a series of large experiments designed to obtain spontaneous mutants. The zero-dose figures from individual experiments based on small numbers are not used because of their large variability.

Table 5. Parameters and RBE's from DATA for Cellular Inactivation

Mutagen	Approximate LET ($Mev\ cm^2\ gm^{-1}$)	Cellular Inactivation		Mutation					
		a	RBE	a	b	c	RBE for $ad\text{-}3^R$ Mutation	d	RBE for $ad\text{-}3^{IR}$ Mutation
750-Mev protons	2.2	0.1145	1.77	6.50×10^{-6}	1.453	4.85×10^{-6}	1.36	1.35×10^{-6}	1.47
447-Mev protons	2.5	0.0675	1.02	4.03×10^{-6}	1.367	3.11×10^{-6}	0.87	0.62×10^{-6}	1.00
442-Mev protons	2.6	0.0839	1.30	5.79×10^{-6}	1.385	4.43×10^{-6}	1.24	1.06×10^{-6}	1.30
250-kvp x ray	5.0	0.0648*	1.00	4.49×10^{-6}	1.340	3.57×10^{-6}	1.00	0.62×10^{-6}	1.00
39-Mev helium ions	182		1.62	10.82×10^{-6}	1.428	8.47×10^{-6}	2.37	2.05×10^{-6}	1.81
101-Mev carbon ions	1850		6.1	44.77×10^{-6}	1.533	32.5×10^{-6}	9.10	12.0×10^{-6}	4.38

*Average of inactivation constants from four experiments is used for 250-kvp x-ray inactivation constant.

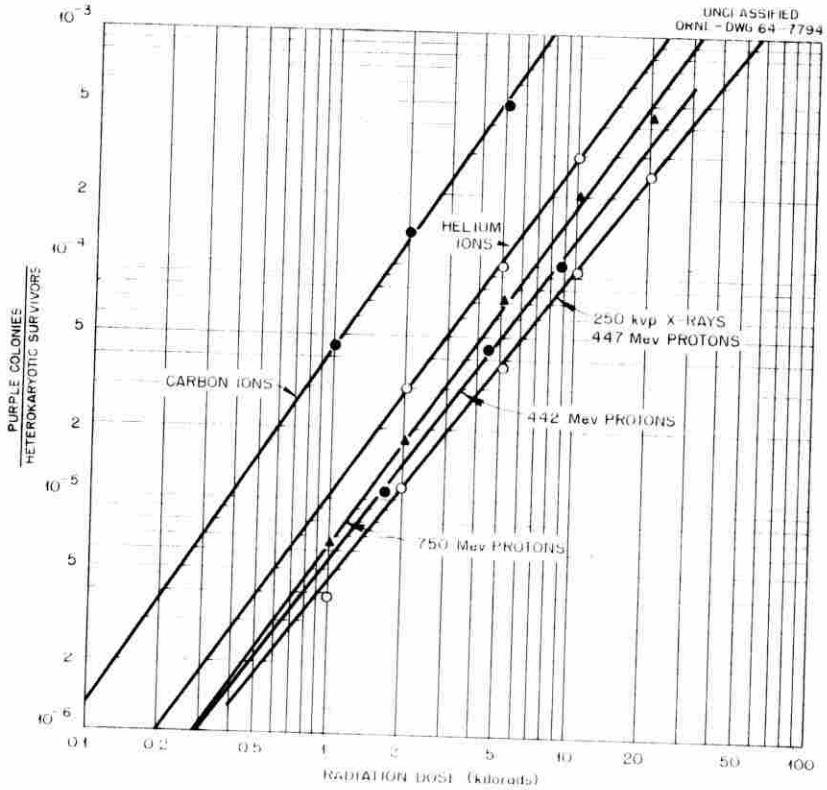

Fig. 5. Number of Mutations at the ad-3 Region in Neurospora crassa per Surviving Heterokaryotic Conidia as a Function of Radiation Dose (Kilorads) for Various Radiations.

and x = dose in kilorads. An expression for RBE can be obtained from this equation.[15] If the slopes, b, of the curves are different (which is especially obvious from a comparison of carbon-ion and x-ray data), then the RBE is dependent upon yield and upon dose. The RBE is independent of dose and equal to $(a_{other}/a_{x\ ray})^{1/b}$ only if the slope of the x-ray curve can be equated with that for the other treatment.

One way of interpreting the data is suggested by a recent study (Webber and de Serres, unpublished) in which x-ray induced ad-3 mutants were shown to include two separate classes of mutants whose induction-kinetics were different. Mutants of $ad\text{-}3^{R}$ (reparable) were shown to increase in proportion to dose, while $ad\text{-}3^{IR}$ mutants (interpreted as deletions) increased as the square of the dose. One might predict a one-hit component and a two-hit component for each of the other radiations on the basis of the composite mutation curves, obtaining constants for such components by fitting data to equations of the type $y = k + cx + dx^2$. (Here y = total mutant

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$$y = ax^b .$$

$$x = \left(\frac{y}{a}\right)^{1/b}, \quad \ln x = \frac{\ln y - \ln a}{b} .$$

$$RBE = \frac{x_{x\text{-}ray}}{x_{other}}, \text{ at which } y_{x\text{-}ray} = y_{other} = y.$$

$$RBE = \frac{(y/a_{x\text{-}ray})^{1/b_{x\text{-}ray}}}{(y/a_{other})^{1/b_{other}}} = \frac{(a_{other})^{1/b_{other}}}{(a_{x\text{-}ray})^{1/b_{x\text{-}ray}}} \cdot \frac{(y)^{1/b_{x\text{-}ray}}}{(y)^{1/b_{other}}} .$$

yield; k = spontaneous mutant yield, approximately 0.3×10^{-6}; and cx and dx^2 equal the estimate yield of ad-3^R and ad-3^{IR} mutants, respectively). Then separate RBE's for one-hit mutants and for two-hit mutants could be estimated.[16] Note that these must be estimates only, but such predictions can be tested by experimental evidence concerning reparability. When this was done for x-ray-induced mutants, there was good agreement between experimental observations and expectation.

Relative Biological Effectiveness of Protons of Different Energies.--The RBE's for killing and the calculated RBE's for ad-3^R and for ad-3^{IR} mutation for protons of different energies are listed in Table 6. Also included in this table are data for 250-kvp x rays, which are used as a basis for the RBE computations, and for alpha particles and carbon ions. The radiations are arranged in order of increasing ionization density. There appears to be little difference among the protons with respect to RBE. (The RBE for inactivation by 750 Mev protons seems a bit high and may reflect differences in the dosimetric procedures or exposure techniques at the University of California and University of Chicago facilities. No such differences were found in the data of the other experimenters. Further survival experiments utilizing protons of different energies from the same source with the same dosimetric instruments are planned, now that they are available at Harvard University.)

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If $b_{x\text{-}ray} = b_{other} = b$, then:

$$RBE = (a_{other}/a_{x\text{-}ray})^{1/b},$$

and RBE's for predicted ad-3^R and ad-3^{IR} mutants will be:

$$RBE_{ad\text{-}3^R} = c_{other}/c_{x\text{-}ray}, \text{ and}$$

$$RBE_{ad\text{-}3^{IR}} = (d_{other}/d_{x\text{-}ray})^{1/2}.$$

Tobias and Manney[17] have summarized work comparing RBE's for particles with different linear energy transfer values. They show that in bacteria, yeast, and human tissue-culture systems there is a peak of relative biological effectiveness at an LET range of 10^2 to 2×10^3 Mev $cm^2 gm^{-1}$ (120-Mev carbon ions), but that there is little variation below ~100 Mev $cm^2 gm^{-1}$ (40-Mev helium ions). This is predicted for the present proton exposures, and the data from the University of Chicago experiments are compatible with this prediction. The relationships in data from the University of California experiments (750-Mev protons, 39-Mev helium ions, 101-Mev carbon ions) are consistent with those found in bacterial or yeast systems, but data from both the 750-Mev protons and 39-Mev helium ions give higher RBE values than might have been expected. It seems more likely that this is due to a difference in the conditions of exposure than a difference in the responsiveness of our assay system. It is clear, nevertheless, that the RBE's for cellular inactivation and mutation induction from the carbon ions are, as predicted, significantly higher than those found with all the other radiations tested.

b. Summary

Experiments utilizing 750-, 447-, and 442-Mev protons, as well as 250-kvp x rays, 39-Mev helium ions, and 101-Mev carbon ions to study cellular inactivation of a balanced dikaryon and to induce forward mutants in the *ad-3* region of *Neurospora crassa* are described. Relative biological effectiveness for cellular inactivation and for mutation induction was computed for the charged particles. The values range from 1.0 to 2.5 for the protons and helium ions, and from 4.0 to 10.0 for carbon ions.

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[17] C. A. Tobias and Thomas Manney, "Some Molecular and Cellular Effects of Heavily Ionizing Radiations," Ann. N. Y. Acad. Sci. 114 Art 1: 16-31 (1964).

3. RBE of Protons and Neutrons as Measured by Survival of Mouse Spermatogonia and Oocytes

E. F. Oakberg E. Clark

Three morphologically distinct types of spermatogonia can be identified in the mouse.[18] Type A, which are the stem cells of the spermatogenic series, maintain a constant population by the process of stem-cell renewal and give rise to an infinite number of spermatogonia of the second type, which has been designated as intermediate (In). Intermediate spermatogonia divide once to form type-B spermatogonia, which, in turn, divide once to form primary spermatocytes. The ability to classify accurately spermatogonia and to time the stages of cell development makes them a good system for quantitation of radiation effects.[19]

Previous experiments indicate that most of the spermatogonia loss following irradiation occurs by the process of cell degeneration within 24 hr after exposure. A period of 72 hr has been found to be an optimum time to score survival after doses of 200 r or less. This time allows degeneration to occur, but it is not long enough for increase in cell numbers by division to exceed the rate of cell loss. The number of apparently normal cells was counted for each mouse in 100-tubule cross sections distributed on the basis of frequency distribution of tubule stages in controls. Type-A, -In, and -B spermatogonia and preleptotene spermatocytes were counted. The type-A cells were both irradiated and scored as type-A spermatogonia; late type-A cells at time of irradiation were scored as In and B spermatogonia; and cells irradiated as the latest type-A and -In stages were scored as preleptotene spermatocytes. Data are presented as experimental/control ratios.

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[18] E. F. Oakberg, "A Description of Spermiogenesis in the Mouse and its Use in Analysis of the Cycle of the Seminiferous Epithelium and Germ Cell Renewal." Am. J. Anat. 99: pp. 391-414 (1956).

[19] _____, "Gamma-ray Sensitivity of Spermatogonia of the Mouse." J. Exptl. Zool. 134: pp. 343-356 (1957).

For the experiments to be reported, animals were exposed to 250- or 280-kvp x rays, 130- and 750-Mev protons, and 14.1-Mev neutrons. A total of 86 F_1 hybrid 101 x C3H male mice approximately 12 weeks old were used in these experiments. Three animals were assigned at random to each radiation treatment. Six control animals were used in the 280-kvp x-ray and 130-Mev proton comparisons, and eight controls were used for the 250-kvp x-ray and 730-Mev proton comparisons. All animals were killed by cervical dislocation, testes were fixed in Zenker-formol and embedded in paraffin, and 5-μ sections were stained by the periodic acid-Schiff technique.

Oocytes in early "arrested" follicular stages in the mouse ovary are extremely sensitive to irradiation, and survival of these cells also may be used to estimate radiation damage.[20] The definitive number of oocytes is determined before birth in the mouse, and estimates of the number of surviving cells are not complicated by cell division as in the male. Since most oocytes degenerate immediately after irradiation, 62 hr is an optimum time to score cell survival in the mouse ovary.

Fifty-four 101 x C3H hybrid female mice, 3-4 months old, were used. Three females were assigned at random to each irradiation group. Four controls were used for the 280-kvp x-ray and 130-Mev proton comparison, and eight controls for the 250-kvp x-ray and 730-Mev proton experiments. Tissues were fixed in Bouins and embedded in paraffin, and 7-μ serial paraffin sections were stained in Heidenhain's hematoxylin and eosin. Oocytes were counted in every tenth section. Data are given as experimental/control ratios.

Results

Data are given in Tables 7-10. It is clear from examination of surviving fractions that 280-kvp x rays, 250-kvp x rays, and 730-Mev protons have the same

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[20] E. F. Oakberg and E. Clark, "Effect of Dose and Dose Rate on Radiation Damage to Mouse Spermatogonia and Oocytes as Measured by Cell Survival." J. Cell. Comp. Physiol. 58 (Suppl. 1): 173-182 (1961).

Table 7. Experimental/Control Ratios for Survival of Type-A Spermatogonia 72 Hours After Irradiation

X Rays				Protons			
280 kvp		250 kvp		130 Mev		730 Mev	
Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control
4.9	0.959	4.7	0.924	5.4	1.01	4.9	0.870
9.6	0.863	9.5	0.845	10.7	1.07	10	0.853
23.2	0.603	23.7	0.556	25.5	0.754	24.9	0.603
47.3	0.404	47.5	0.331	50	0.517	49.7	0.394
96.1	0.235	95.0	0.255	100	0.337	100	0.226
188.2	0.155	190.0	0.166	200	0.163	200	0.164

Table 8. Survival of Late Type-A Spermatogonia as Estimated by Number of In and B Spermatogonia 72 Hours After Irradiation

X Rays				Protons			
280 kvp		250 kvp		130 Mev		730 Mev	
Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control
4.9	0.989	4.7	1.043	5.4	0.962	4.9	0.904
9.6	0.849	9.5	0.799	10.7	0.938	10	0.851
23.2	0.649	23.7	0.539	25.5	0.735	24.9	0.626
47.3	0.151	47.5	0.226	50	0.443	49.7	0.235
96.1	0.025	95.0	0.035	100	0.068	100	0.065
188.2	0.004	190	0.000	200	0.007	200	0.004

Table 9. Survival of Late Type-A and In Spermatogonia as Estimated by Number of Preleptotene Spermatocytes 72 Hours After Irradiation

X Rays				Protons			
280 kvp		250 kvp		130 Mev		730 Mev	
Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control
4.9	1.016	4.7	1.009	5.4	1.029	4.9	0.886
9.6	0.912	9.5	0.801	10.7	0.993	10.0	0.893
23.2	0.655	23.7	0.526	25.5	0.747	24.9	0.512
47.3	0.169	47.5	0.118	50	0.542	49.7	0.147
96.1	0.000	95	0.000	100	0.003	100	0.000
188.2	0.000	190	0.001	200	0.000	200	0.000

Table 10. Experimental/Control Ratios for Number of Type-1 and -2 Oocytes 72 Hours After Irradiation

X Rays				Protons			
280 kvp		250 kvp		130 Mev		730 Mev	
Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control	Dose (rads)	Exptl/Control
4.9	0.717	4.7	0.934	5.4	1.04	4.9	0.973
9.6	0.608	9.5	0.645	10.7	0.809	10.0	0.744
23.2	0.143	23.7	0.213	25.5	0.447	24.9	0.169
		47.5	0.015			49.7	0.004

effect, and that the RBE for these radiations is 1. This is verified in Table 11, where estimated RBE's are given.

The 130-Mev protons, however, appeared to be less effective, with an RBE of about 0.5 or 0.6, except for late type-A spermatogonia, which gave a point estimate of 0.68, which is not significantly different from 1. This was not expected, since the LET of protons of this energy should be comparable to that for x rays. In view of the similar low RBE observed by Stapleton (previous progress report) for bacteria irradiated at the same time, and his observation of an RBE of 1 at a subsequent experiment with 130-Mev protons (this report), we believe that the lower RBE for 130-Mev protons resulted either from exposure techniques or from an error in dose measurement. Caution must be used in evaluating Table 11 owing to difficulties in computing RBE's from survival curves represented by the date of Tables 7-10. While the protons appear to be less effective, most of the upper confidence limits are above 1, and there is no justification at present in concluding that the proton-x-ray RBE differs from 1.

Data from a previous experiment on the effect of 14.1-Mev neutrons from a Cockcroft-Walton linear accelerator are also given in Table 11. The RBE, as compared to ^{60}Co γ rays, was approximately 2, indicating greater effect of particles with a higher LET. Current experiments indicate that the RBE for fission neutrons may be as high as 5.

4. RBE of Protons and Neutrons for Production of Chromosome Aberrations in Human Leukocytes

M. A Bender P. C. Gooch

The protons used in this study were generated by three different accelerators: the Harvard University 160-in. cyclotron, the University of Chicago 170-in. cyclotron, and the University of California 184-in. cyclotron. Use of the Oak Ridge National Laboratory 86-in. cyclotron was also originally contemplated, but difficulties have led to the abandonment of this plan. Proton energies of 50-, 100-,

Table 11. RBE of Protons to X Rays and 14.1-Mev Neutrons to ^{60}Co γ Rays for Spermatogonial and Oocyte Killing

Radiation	Cell Type	RBE		
		Lower 95% Confidence Limit	Point Estimate	Upper 95% Confidence Limit
	Spermatogonia:			
14.1-Mev Neutrons*	A	1.41	1.76	2.76
	Late A	2.19	2.52	2.89
	Late A + In	2.11	2.38	2.69
	Spermatogonia:			
130-Mev protons	A	0.28	0.47	0.70
	Late A	0.41	0.64	0.95
	Late A + In	0.27	0.68	1.40
	Oocytes	0.00	0.28	0.73
	Spermatononia:			
750-Mev protons	A	0.64	0.84	1.10
	Late A	0.52	0.77	1.11
	Late A + In	0.69	0.96	1.34
	Oocytes	0.20	0.66	1.53

*From Oakberg and Clark, 1961

130- (Harvard), 450- (Chicago), and 750-Mev (California) were used in this study. Twenty-two Mev irradiations will be performed with the Harvard machine in August 1964. These irradiations will complete the study of RBE values for proton induction of chromosomal aberrations in human cells. Protons of more than 750-Mev are not available, and the thickness of the necessary tissue specimen makes energies of the order of 20 Mev the practical lower limit because of the short range of low energy protons. Current studies of RBE values for induction of human chromosome aberrations by fast neutrons provide additional information about the effects of lower energy protons. "Knock on" protons of low energy are produced at various depths in the tissue sample, thus circumventing the tissue penetration problem. Experiments have been completed with the 14- and 2.5-Mev neutrons generated by the deuteron-tritium and the deuteron-deuterium reactions in a Cockroft-Walton accelerator. Experiments with fission spectrum neutrons generated by the ORNL Health Physics Research Reactor are in progress.

The chromosomal aberrations studied are induced by irradiation of freshly drawn whole human blood. The investigators are the blood donors in all of the proton experiments and in some of the neutron experiments. Five-ml samples are irradiated in nylon chambers with thin beam-entry windows. After irradiation the leukocytes are separated from the blood by centrifugation and placed in short-term phytohaemagglutinin-stimulated culture. The cells are fixed at 72 hr after pretreatment with colchicine and hypotonic saline solutions. Chromosome preparations are made and scored by standard cytological techniques. Previous studies have shown that in this system the cells examined are in their first *in vitro* mitosis, and that they are in the pre-DNA-synthesis stage of the cell cycle at the time the blood is drawn and irradiated. Virtually all of the chromatid-type aberrations seen in such cultures are thus of spontaneous origin; the induced aberrations are chromosome types, primarily chromosome deletions, dicentrics, and rings. The chromosome deletions show linear dose-effect kinetics. The dicentrics and rings approximate two-hit dose-square kinetics except for irradiations of very high LET (above $\sim$50 Mev-cm^{2}gm^{-1}). The coefficient of production for these two classes of aberrations may be used for

purposes of comparison and calculation of RBE values. Separate studies have established the coefficients of aberration production for 250-kvp x rays (hvl = 2 mm Cu). The value determined for a dose rate of 10 rad/min is used to determine, by definition, the "RBE = 1" values against which the proton and neutron values are compared.

Results and Discussion

A summary of the results of our cytogenetic analysis of the material exposed to proton and neutron irradiation, as well as the material from the 250-kvp x ray exposure, is presented in Table 12. In general, there does not appear to be any consistent increase in the frequency of cells with chromosome numbers, other than the normal human diploid number of 2n = 46, when the cells are irradiated with neutrons or protons. The variation in the frequency of these aneuploid cells is the same as has been seen in similar cultures made for other purposes. The frequencies of chromatid-type aberrations are similar to those seen in unirradiated material. There is no increase in their frequency with increasing radiation exposure, confirming that virtually all of the cells scored were in the pre-DNA-synthesis, or G_1, stage of the cell cycle at the time of irradiation. The frequencies of chromosome-type aberrations in the unirradiated samples are under one percent, as has been found in other experiments, and the frequencies in the irradiated samples increase as expected with increasing dose. All of the expected aberration types were seen.

Only the data for 250-kvp x rays and for 2.5- and 14-Mev neutrons have as yet been subjected to careful statistical analysis. For these irradiations, the coefficients of aberration production were calculated by means of weighted least-squares regression analysis, and their standard errors were determined. Table 13 presents the results of these analyses, together with rough calculations of these values from the other data. RBE values were calculated from them by dividing the coefficient of deletion production for the irradiation in question by the coefficient of deletion production for the 250-kvp x-ray exposures, which, by definition, have an RBE of one. Values for the coefficient of ring and dicentric production by neutrons are not given. They are not comparable with those for other lower-LET irradiations, because of the

Table 12. Chromosomal Aberrations Induced in Human Leukocytes by Protons

Radiation	Dose (rads)	Cells Scored	2n ≠ 46	Chromatid Aberrations	Chromosome-type Aberrations		
					Deletions	Rings	Dicentrics
750-Mev protons	0	200	20	1	2	0	0
	50	200	20	6	9	0	3
	100	200	21	7	14	2	19
	150	200	28	3	19	3	14
	200	200	22	5	19	3	27
450-Mev protons	0	100	2	1	3	0	0
	25	100	4	2	4	0	0
	100	100	5	3	13	0	8
	150	100	15	2	26	0	13
	200	100	12	1	21	1	31
250-kvp x rays	0	411	13	3	1	0	0
	25	300	19	5	11	0	1
	50	300	22	2	17	0	5
	100	500	46	9	32	0	29
	200	400	55	10	75	3	95
130-Mev protons	0	100	6	0	1	0	0
	50	100	11	0	4	0	3
	100	100	16	1	11	0	3
	150	100	8	0	18	0	6
	200	100	14	0	21	2	23

Radiation	Dose (rads)	Cells Scored	2n ≠ 46	Chromatid Aberrations	Chromosome-type Aberrations		
					Deletions	Rings	Dicentrics
100-Mev protons	0	100	10	0	1	0	0
	50	100	2	0	6	0	2
	100	100	5	9	5	1	2
	200	68	1	0	11	4	9
50-Mev protons	0	200	13	6	4	0	0
	50	191	8	5	4	0	3
	100	200	24	5	16	3	12
	150	190	12	5	16	1	13
	200	100	11	7	15	0	26
14-Mev neutrons	0	300	30	7	0	0	0
	50	287	22	5	32	3	12
	100	300	19	3	48	5	25
	150	290	29	8	89	9	52
	200	200	37	3	97	4	62
2.5-Mev neutrons	0	500	54	15	8	0	1
	15	500	38	10	23	3	9
	30	550	42	3	56	5	31
	45	500	60	6	66	8	76
	60	446	46	5	85	9	42
1-Mev neutrons	0	300	14	7	4	0	0
	13	300	12	11	24	5	18
	25	300	20	3	38	6	35
	38	300	35	5	30	5	48
	50	300	48	13	92	6	82

Table 13. Coefficients of Chromosomal Aberration Production for Proton Irradiation of Human Leukocytes

Radiation	Approximate LET ($Mev\text{-}cm^2\ gm^{-1}$)	Coefficient of Aberration Production: Deletions[a] (10^3 X)	Rings and Dicentrics[b] (10^6 X)	RBE[c]
750-Mev protons	2.2	0.6	6.0	0.7
450-Mev protons	2.5	0.9	5.5	1.0
250-kvp x rays	5.0	0.9 ± 0.03	6.0 ± 0.5	1.0[d]
130-Mev protons	7.0	0.9	6.0	1.0
100-Mev protons	8.0	0.7	5.3	0.8
50-Mev protons	13.0	0.4	5.8	0.4
14-Mev neutrons	60.0	2.3 ± 0.2	--[e]	2.6
2.5-Mev neutrons	130.0	2.8 ± 0.2	--[e]	3.1
1-Mev neutron	500.0	50	--[e]	5.6

[a] From $Y = a + bD$; the coefficient is b, expressed in aberrations/cell/rad.

[b] From $Y = cD^2$; the coefficient is c, expressed in aberrations/cell/rad^2.

[c] Calculated from deletion coefficient only.

[d] By definition.

[e] For purposes of comparison, these coefficients would be meaningless because the kinetics of two-hit aberration production change in this LET range, becoming approximately linear for 2.5 Mev neutrons.

change from dose-square to linear kinetics with irradiations with a LET greater than about 50 Mev $cm^2 gm^{-1}$.

With the possible exception of the 50-Mev proton data, the data accumulated so far are entirely consistent with the hypothesis that there is no change in RBE over the range of LET from 2.5 to 13 Mev $cm^2 gm^{-1}$. The 50-Mev proton data are not complete, and in any case the reasonably high coefficient of ring and dicentric production for this energy suggests that the low value of the coefficient of deletion production is an artifact. When the data from the neutron irradiations are included, however, the data are also consistent with the hypothesis that there is an exponential relationship between RBE and LET over the entire range of LET studied. Figure 6 shows the natural logarithm of the LET for all of the radiations used. The regression line is drawn to pass within the standard errors of the values that have been analyzed statistically. A satisfactory test of the hypothesis and calculation of the slope of the best-fit regression line must wait for completion of the analysis of the rest of the data, and in particular for the completion of the 22-Mev exposures.

5. Mammalian Program--Delayed Somatic Effects of Fast Charged Particles in Mammals

A. C. Upton E. B. Darden, Jr.

The purpose of this program is to determine the RBE of high-energy particulate radiation for long-term somatic effects in mammals. The Biology Division has a backlog of such investigations with mice, rats, guinea pigs, and rabbits to determine the influence of the LET of various radiations on late somatic effects such as life shortening, induction of leukemia and other neoplasms, induction of lens opacities, and various other degenerative diseases associated with aging.

The past experiments were carried out with gamma rays, x rays, and neutrons of various energies, the latter radiations resulting in production of high-energy recoil protons produced at various depths in the irradiated animal. The results and methods are set forth in the accompanying reference list.[21-33]

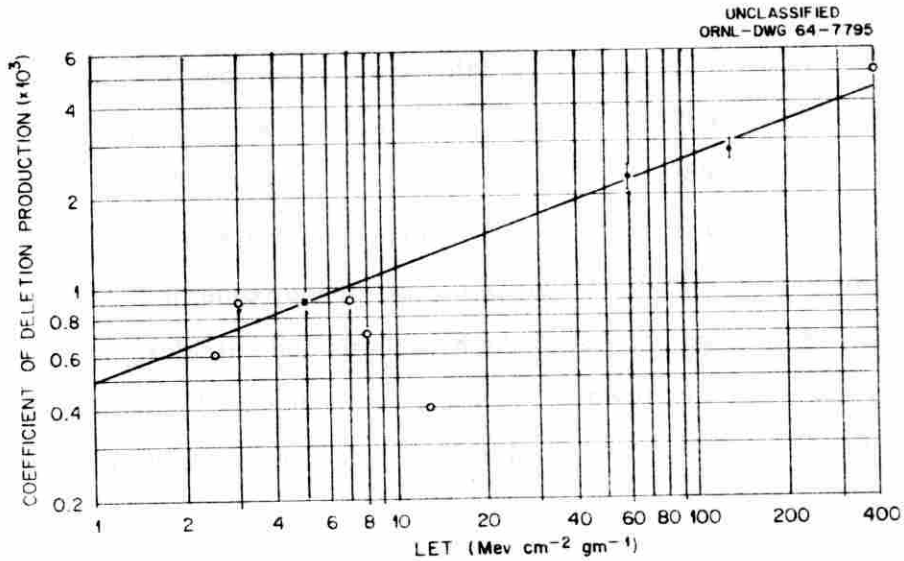

Fig. 6. Natural Logarithm of the Coefficients of Deletion Production for Human Leukocytes Irradiated with Protons and Neutrons of Various Energies, Plotted Against the Natural Logarithm of the Mean LET. The regression line is fitted to pass through the standard errors of the three values for which this calculation was made.

[21] K. W. Christenberry and J. Furth, "Induction of Cataracts in Mice by Slow Neutrons and X Rays," Proc. Soc. Exptl. Biol. and Med. 77: 559-560 (1951).

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The proposed experiments will be carried out with primary beams of protons and other accelerated particles and will be coordinated with allied investigations at ORNL and other AEC- and NASA-sponsored research laboratories.

An air-conditioned mobile trailer will be used to transport animals to near and remote accelerators. The trailer was designed as a field laboratory and animal housing facility to eliminate the stress on animals usually imposed by transportation, and to by-pass quarantine procedures imposed by our own and other research laboratories.

Results and Discussion

The results from previous investigations can be summarized as follows:

(a) Gamma rays were less effective at low than at high dose rates for most, if not all, late somatic effects;

(b) Fast neutrons were generally more effective per unit dose than gamma rays for late somatic effects; and

(c) The effectiveness of fast neutrons for late somatic effects seemed to vary inversely with neutron energy and to be relatively independent of dose rate.

Although the investigations to date do not provide a precise quantitative measurement of RBE with dose, dose rate, and radiation quality, they do indicate broad limits within which each of these variables is effective. More refined experiments, including those proposed herein, should yield definitive information pertinent to assessment of the radiobiological hazards of space flight on higher forms of life.

6. Discussion of Results of RBE Studies

The original concept of this program was to satisfy the need for data related to the relative biological effectiveness (RBE) of these radiations encounterable on space flight, predominantly protons, the energies of which range from a few to hundreds of Mev. Although the lower energy ($<$ 100 Mev) protons predominate in the inner Van Allen belt, higher energy protons are also of interest. We have been able to investigate the lethal, mutagenic, and cytogenetic effects of protons of

energies of 22, 50, 100, 130, 442, and 750 Mev for production of these various biological end points in living cells ranging from microorganisms to human blood cells. Because of the limited range of the lower energy protons, all of the materials could not be subjected to primary beams of these particles. These biological materials, however, have been irradiated with neutrons of energies ranging from 1 to 14.1 Mev, which generate protons in the same energy range as secondary radiations. In addition, the materials that could be prepared in very thin layers were subjected to very high LET primary beams of heavy ions.

The survey is not as complete for any or all materials as would be desired; nevertheless the fragmentary investigation establishes that all of the biological materials show a definite increase in RBE as a function of LET in the range of 100 Mev cm^2 gm^{-1}. Neurospora shows a significant increase in this range for lethality and for both types of mutation. For bacterial killing under anaerobic conditions, the same is true and the same has been reported by Brustad.[34] The saturation RBE as a function of LET has not been established by this study. However, since the RBE of carbon ions ($\sim$2000 Mev cm^2 gm^{-1}) for bacterial inactivation of aerobic suspensions is less than 1, we must conclude from our work, as well as that of Brustad, that the maximum is below this LET. It may well be that all of the effects studied saturate or are maximal in this range.

There are certain inconsistencies in the various data; for example (a) the first experiments (1963) with 130-Mev protons yield RBE values of less than 1.0 for both bacterial inactivation and lethality of spermatogonia and oocytes. A later experiment, which involved duplicate bacterial samples at all doses, yielded an RBE of 1.0 and is described in this report. It is tempting to invoke discrepancies in dosimetry between the two runs, separated in time, but no such discrepancy was found between the two runs for RBE determinations for chromosome aberrations in

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[34]Tor Brustad, "Molecular and Cellular Effects of Fast Charged Particles," Rad. Res. 15: 139-158 (1961).

human leukocytes. This discrepancy has not been solved. Also (b) the data for inactivation of Neurospora conidia by 750-Mev protons are inconsistent with the remainder of the data on RBE versus LET. Although again the first thought is dosimetry error, simultaneous experiments with human leukocytes, mouse spermatogonia and oocytes, and bacteria give little justification for this explanation.

It is obvious from the data presented that much more data would be required to establish the critical RBE versus LET relationship for all of the biological systems used. Nevertheless it has been shown that in the region below 10 Mev protons produce an RBE of greater than 1.0, as was suggested from numerous earlier neutron investigations. The magnitude of the RBE for different materials is obviously different and for different end points in the same living cells.

III. IN-FLIGHT EXPERIMENTS

A. BIOSATELLITE-A EXPERIMENT

The Office of Space Sciences and Applications of NASA has described this program as a series of flights of 3- to 30-days duration in a modified, scaled-up Discoverer satellite to determine the biological effect on plants and animals of weightlessness, radiation, and absence of diurnal cycle. The first flight is scheduled for late in 1965.

The satellite is designed to carry its own radioactive source to supply known doses of radiation and will permit this previous variable to be controlled. Natural radiation will be controlled to less than 2 r/day by the orbital altitude selected.

The temperature, excluding reentry temperature, will be controlled to $24 \pm 2°C$. The experiments will be hermetically sealed in individual containers. These very conditions require rather elaborate ground-based experiments in connection with the viability of the biological experiment. In some cases it is

required to increase the vigor of stocks of organisms to insure good viability under the potential rigors of the experiment.

Short summaries of work in progress for the Biosatellite experiments are included in this report.

B. GEMINI-III EXPERIMENT

At the request of NASA, the Biology Division has undertaken the design and development of an experiment to test for possible unpredicted effects of the radiation encountered in space flight and for possible interactions between radiation and other space flight parameters, such as weightlessness. A number of observations of biological systems subjected to space flights have suggested the possibility of such unpredicted effects.

The Biology Division experiment is to be carried on the Gemini-III manned space flight scheduled for the last quarter of 1964. The experiment will measure the frequencies of various types of chromosomal aberrations in samples of whole heparinized human blood exposed to a known quantity and quality of radiation during the "weightless" phase of the space flight, in unirradiated in-flight controls, and in similar control samples exposed simultaneously on the ground. Both pre- and postflight analyses will also be made of material from the astronauts selected for the Gemini-III mission.

The detailed description of the "package" to Gemini-III and the results of ground-based engineering and biological testing are being prepared in a separate report.

C. PREPARATORY EXPERIMENTS, BIOSATELLITE

1. Preparation of the Habrobracon Stocks for Biosatellite Experiment

R. C. von Borstel Anna Rachel Whiting

In the wasp Habrobracon, females are diploid and heterozygous for sex alleles; normal males are haploid, developing from unfertilized eggs and containing one sex allele. Within an inbred stock, one half of the fertilized eggs have the same sex alleles. The majority of these are inviable, and the few survivors are sterile diploid males. For irradiation experiments, stocks with different sex alleles are used in the crosses, so that viability of controls will be high and experimental data need not be corrected for control inviability. A constant and easily identifiable character difference is also helpful in such experiments.

In order to increase the vigor of one of the sex allele stocks to be used in the biosatellite experiment, males from stock $17o^i$, an ivory eye color, were outcrossed to females from the No. 33 stock, eye color wild-type black. Daughters from these matings were backcrossed to $17o^i$ males other than their own parents. Daughters from these crosses were selectively mated to their own male parent, and each of the lines was then maintained by brother-sister matings.

It was necessary to test these lines against the No. 33 stock to find at least one which had the same sex alleles as the original No. $17o^i$ and different from those in the No. 33 stock. Hatchability of fertilized eggs from such crosses is always high, and diploid males are never produced. Females from a number of the outcrossed, backcrossed, and inbred ivory-eyed lines were crossed with the No. 33 wild-type males and the hatchability scored. One such line was found to give high hatchability and a high female to male ratio. No diploid males were found in 20 separate crosses.

These two stocks (the rederived $17o^i$ and the No. 33) are in excellent condition for the genetic analyses for dominant and recessive lethal and visible

mutations. Analyses of sex alleles in two other wild-type stocks are under way to aid in the tests for the chromosomal translocations that are anticipated in the radiation experiment.

2. Preliminary Data on Survival of Conidia Stored under Satellite Conditions of Time, Temperature, and Atmosphere

F. J. de Serres B. B. Webber

Since the conditions aboard the proposed Biosatellite are somewhat different from those routinely involved in laboratory experiments, it was necessary to determine the optimal conditions for survival of conidia under the restrictions imposed by the vehicle with regard to temperature and atmosphere for the proposed 3- to 30-day orbit.

A series of experiments involving measurement of loss of viability of wet conidia as a function of atmosphere, time, and temperature give the following results:

(a) Conidia survive longer at 0-4°C under any atmospheric conditions than at higher temperatures;

(b) At temperatures above 4°C, the loss of viability with time is much greater under anaerobiosis than under aerobic conditions; and

(c) In air, conidia survive well up to 9 days or more at temperature prescribed for the flight.

To summarize the survival data, satisfactory viability of Neurospora conidia can be obtained under flight conditions, if the package permits a supply of air. Experiments are planned to determine the amount of air required for best viability, and these considerations are being applied to the engineering of the flight package.

Other conditions that will increase viability are also anticipated. These, of course, will have to be tested for their possible influence on radiation sensitivity as well.

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