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BIOLOGY DIVISION
THE ORNL SPACE BIOLOGY PROGRAM

Annual Report
Period Ending June 30, 1965

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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.

SEPTEMBER 1965

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee
operated by
UNION CARBIDE CORPORATION
for the
U. S. ATOMIC ENERGY COMMISSION

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SUMMARY

This report describes work performed to July 1, 1965 in the AEC-NASA Space Biology Program through an interagency agreement with the U. S. Atomic Energy Commission and the National Aeronautics and Space Administration. At present the general space radiobiology program supports chiefly the high-energy proton studies on several mammalian systems, including cataractogenesis, shortening of life span, and acute lethality. Development of the Oak Ridge Isochronous Cyclotron facility, pertinent dosimetry, and results to date are reported. Results obtained in mock-up experiments in preparation for the Biosatellite A Project are also reported. The preparation and participation by members of this Division in the Gemini IV experiment are described in periodic special reports and are not covered in this report.

I. PROTON-RBE PROJECT — BIOLOGICAL RESULTS

1. RBE OF PROTONS AND OTHER FAST CHARGE PARTICLES ON VARIOUS BIOLOGICAL SYSTEMS

Most of the data obtained on this part of the program, which includes experiments other than those on whole mammals now in progress, were reported previously¹ and are summarized here.

Protons of energies ranging from 22 to 750 Mev are not significantly different from 250-kvp X rays in biological effectiveness. Heavy carbon ions are less efficient per unit dose than are the other radiations for aerobic cells, as was found by Brustad et al.² for a related bacterium. An oxygen effect was shown for all radiations used, and chemical protection by β -mercaptoethylamine was shown to be equally efficient for protons and for X rays.

Neurospora conidia show an increase in RBE as a function of LET (200 to 2000 Mev cm^2/gm), as do all of the systems tested except the bacterial system. The increase in RBE was found for lethality as well as for two classes of mutations. The RBE of 100-Mev carbon ions was found to range from 4 to about 9, depending on the effect studied. An anomaly was found for RBE of 750-Mev protons, which suggests the possibility that high-energy secondary particles might be detected with this biological system.

Experiments on lethality in mouse spermatogonia and oocytes and those on production of chromosome aberrations in human leukocytes show increased RBE in the LET region of 100 to 200 Mev cm^2/gm . The highest RBE found in either system is about 5.

It is clear from the several systems studied that protons of energies from 22 to 750 Mev have similar biological effectiveness at the cell level, the possible exception being the inactivation and mutation efficiency of 750-Mev protons in Neurospora.

¹The ORNL Space Biology Program Annual Report, June 30, 1964, ORNL-TM-924.

²T. Brustad, Advan. Biol. Med. Phys. 8, 161-220 (1962).

The fact that the *Neurospora* system yields a high RBE for high-LET radiations suggests, but certainly does not prove, that this experimental system can detect low-yield, high-LET secondary particles resulting from protons in this energy range.

All of the systems studied, except bacteria, yield increasing RBE as a function of LET in the range of 100 to 2000 Mev cm²/gm, resulting from heavy ions as primary particles or from low-energy protons or other secondaries resulting from neutron irradiation.

The data reported are for the most part consistent with those reported in the literature for cellular systems by Mortimer,³ Barendsen,⁴ and Todd.⁵ The experiments fail to estimate the maximum RBE for any system. Such information would be valuable, especially for *Neurospora*, for lethality of mammalian germ cells, and for chromosome aberrations in human leukocytes.

³R. K. Mortimer, T. Brustad, and D. V. Cormack, Univ. of California Report, UCRL-11387, 1964, pp. 35-53.

⁴G. W. Barendsen, Ann. N.Y. Acad. Sci. 114(1), 96-113 (1964).

⁵C. A. Tobias and P. W. Todd, Univ. of California Report, UCRL-11387, 1964 pp. 25-34.

2. MAMMALIAN STUDIES WITH 35-MEV AND 57-MEV PROTONS IN THE OAK RIDGE ISOCHRONOUS CYCLOTRON

E. B. Darden, Jr. R. S. Bender⁶ A. C. Upton

Introduction. — With the completion of the Biology Facility at the Oak Ridge Isochronous Cyclotron, proton irradiation experiments with mice were initiated there in the fall of 1964, as planned.⁷ Initially, protons with energies above 35 Mev were not produced in the ORIC, so we confined our first studies to effects on the mouse lens (see below) because of the relatively short range of these protons in tissue. Since protons of energies up to 57 Mev have subsequently become available there, studies have been made to explore the use of 57-Mev protons for whole-body irradiation of mice, as well as to investigate their effects on the lens (see below).

Results and Discussion. — Because cyclotron time is at a premium, we began irradiating mice in groups of four or more in a broad defocused proton beam (6 1/2 to 7 in. diam). With these conditions, one needs particularly to know the variation in intensity across the effective area of the beam. In the early experiments, semi-quantitative means such as observation of phosphor screens and densitometric analysis of sheet film placed in the beam were used for estimation of cross-sectional uniformity, supplemented by measurements with small (1 X 6 mm) fluororod dosimeters distributed in the beam.

We soon found that a faster and more sensitive method was required to detect beam inhomogeneities when setting up for irradiation of animals. Accordingly, we have built and tested a remotely operating scanning device consisting of a pair of thimble-type chambers which can be positioned in at least 12 preset locations within the effective area of the beam (Fig. 1). The readouts of these instruments (Radocon dosimeters) give a measurement of both rate and integral output. Experience has

⁶Electronuclear Division

⁷A. C. Upton and E. B. Darden, Jr., The ORNL Space Biology Program Annual Report, June 30, 1964, ORNL-TM-924, p. 39.

shown that the only change in beam properties likely to occur unnoticed during an exposure is an alteration in the field of the positioning magnets, which tends to change the effective area of the beam and thus produces nonuniform exposure. Hence, when beam mapping has been completed, we can move these chambers to opposite positions just inside the beam and effectively monitor it during subsequent exposures.

Protons of 57-Mev energy are sufficiently energetic to traverse the body laterally in young (8-week-old) mice, according to range-energy considerations and dose measurements with small (1 X 6 mm) glass fluororods in phantoms. Although calculations and measurements indicated that radiation to tissue on the exit side of the beam would have LET values 30% or more greater than that to tissue near the entry side, the variation in average LET was reducible to a few percent by rotating the mouse body on its axis; i. e., fluororod measurements at depths of 1 to 12 mm in a 22-mm-diam phantom irradiated in this manner agreed within $\pm 3\%$. Accordingly, for whole-body irradiation, a drive mechanism has been built which permits up to four mice (restrained in thin-walled centrifuge tubes) to be rotated simultaneously in the exposure field (Fig. 2).

As a result of the satisfactory outcome of the preparatory studies described above, whole-body irradiation experiments to study the RBE of 57-Mev protons for life-shortening, leukemogenesis, carcinogenesis, and other delayed somatic effects in mice, as outlined previously,⁷ are scheduled to begin in the immediate future.

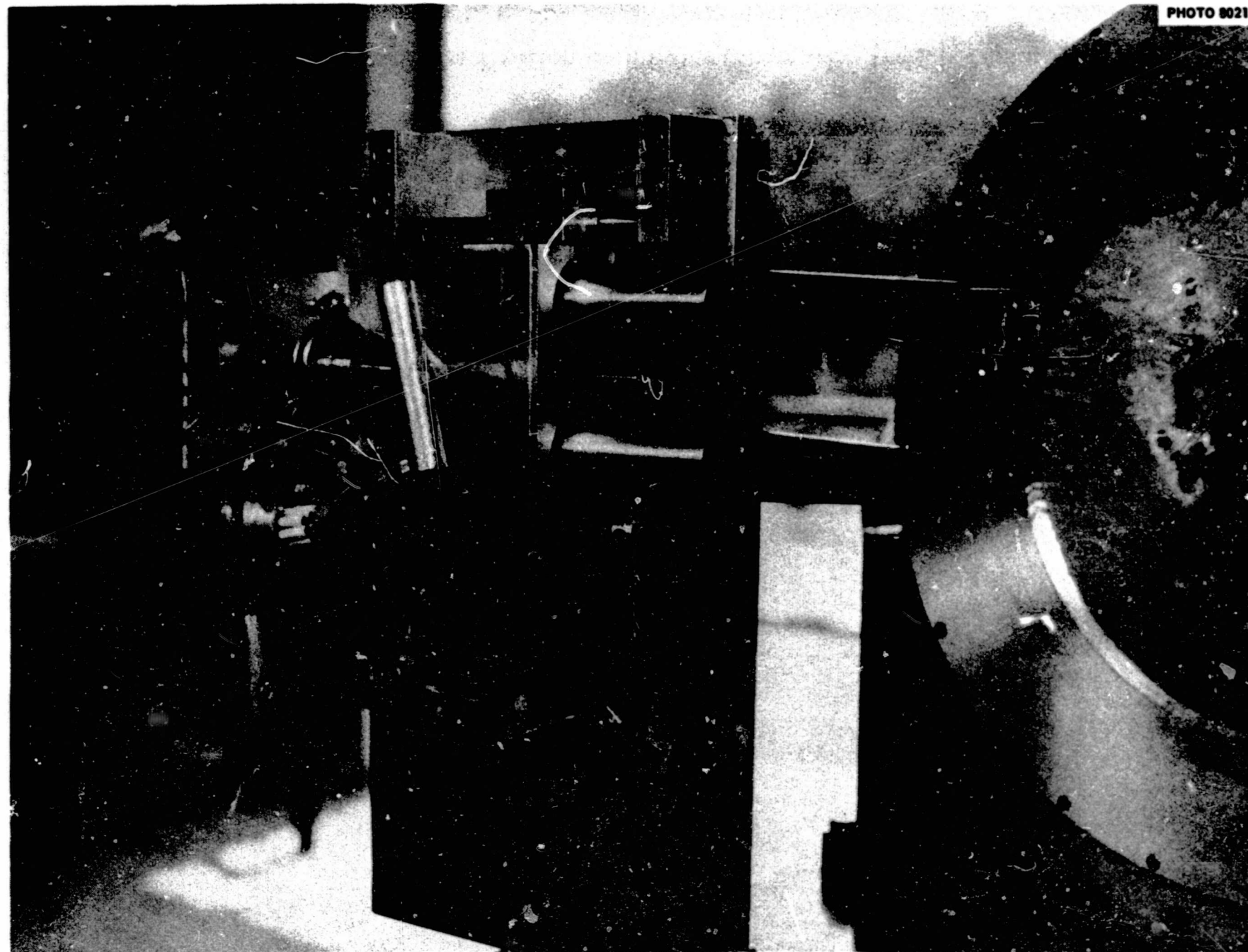


FIG. 1. Scanning Device for Mapping Cross-Sectional Intensity of Proton Beam. Ion chambers are in exposure position (beam from left). Part of front window of beam dump is visible behind chambers on right.

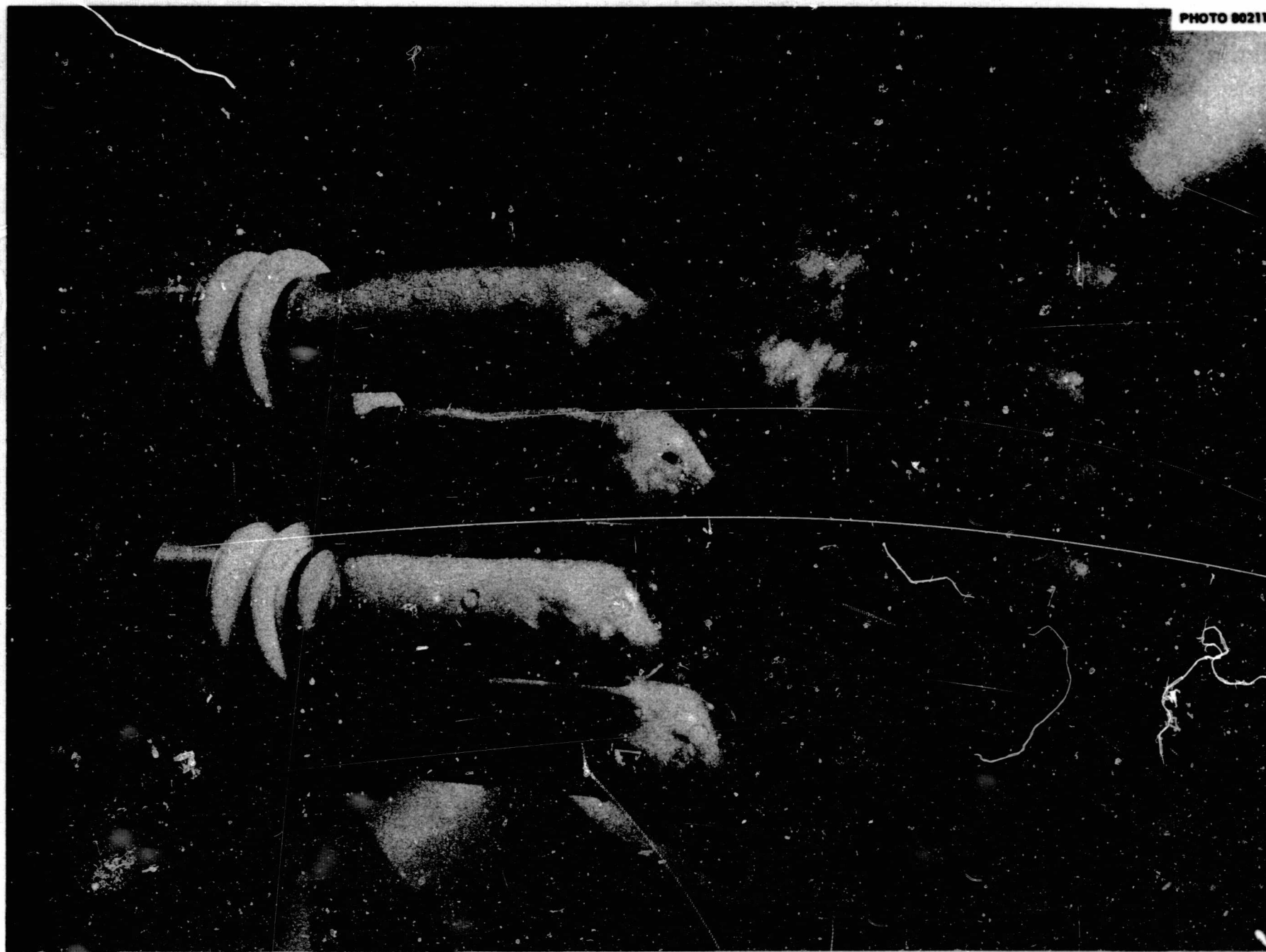


FIG. 2. Mice in Exposure Position on Rotator (beam from left). Mylar window of beam dump is in background.

3. PRELIMINARY STUDIES OF LENS OPACIFICATION IN MICE FOLLOWING EXPOSURE TO 35- AND 57-MEV PROTONS

E. B. Darden, Jr. K. W. Christenberry A. C. Upton J. W. Conklin

Introduction. — To determine the RBE of protons of various energies for induction of cataracts, 10-week-old RF/Un female mice were irradiated in the Oak Ridge Isochronous Cyclotron with 35-Mev protons, and resulting opacities of their lenses were assayed as described previously.⁸ Since then, experiments have been undertaken with 57-Mev protons in which anesthetized mice of similar age were irradiated head-on, so that both eyes received a single absorbed dose of 0 to 2000 rads.

Results and Discussion. — Results with 35-Mev protons (Fig. 3) resemble those with X rays (Fig. 4) in showing a rapid rise in lens opacification with time, which is dose dependent during the first 50 to 200 days. Results to date with 57-Mev protons (observations to 150 days) are similar to those with 35-Mev protons. From these preliminary results, we can tentatively infer that the RBE of 35- to 57-Mev protons for the induction of cataract in the mouse is not greatly different from that of X rays (Fig. 5).

⁸E. B. Darden, Jr. et al., Biol. Div. Semiann. Progr. Rept., Feb. 15, 1965,
ORNL-3768, p. 110.

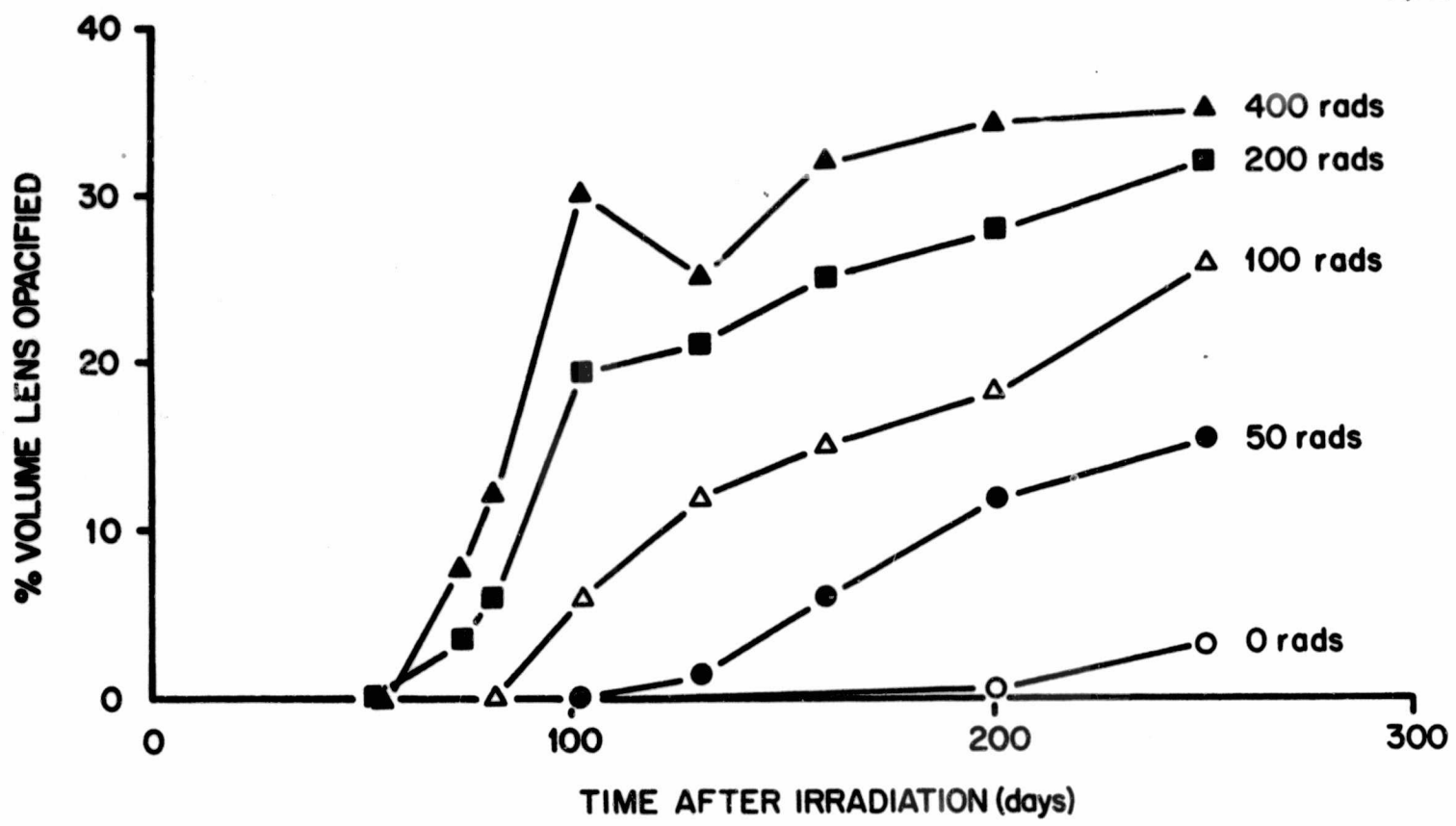


FIG. 3. Lens Opacification Following Irradiation of Eye with 35-Mev Protons.

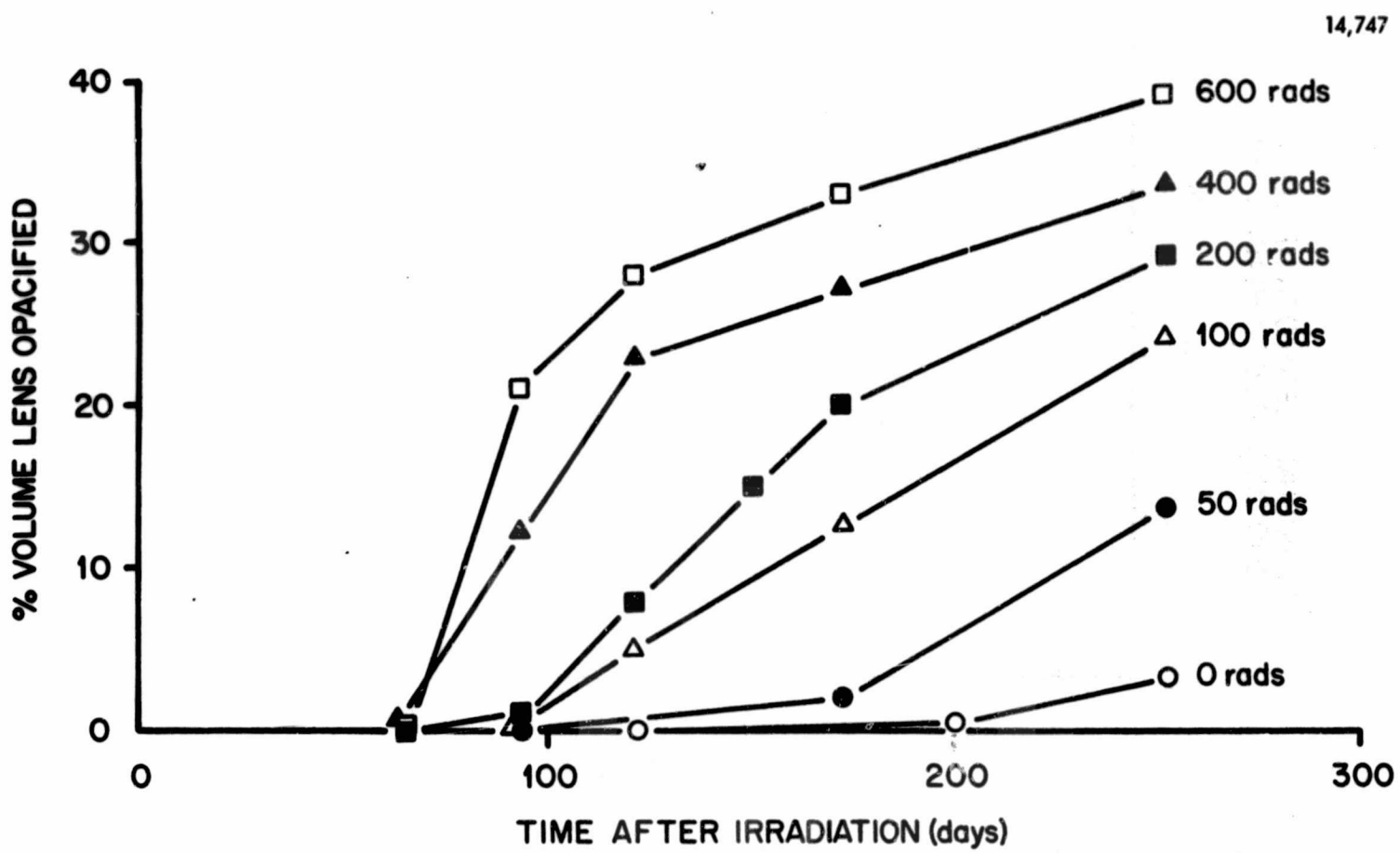


FIG. 4. Lens Opacification Following Irradiation of Head with 300-kev X rays.

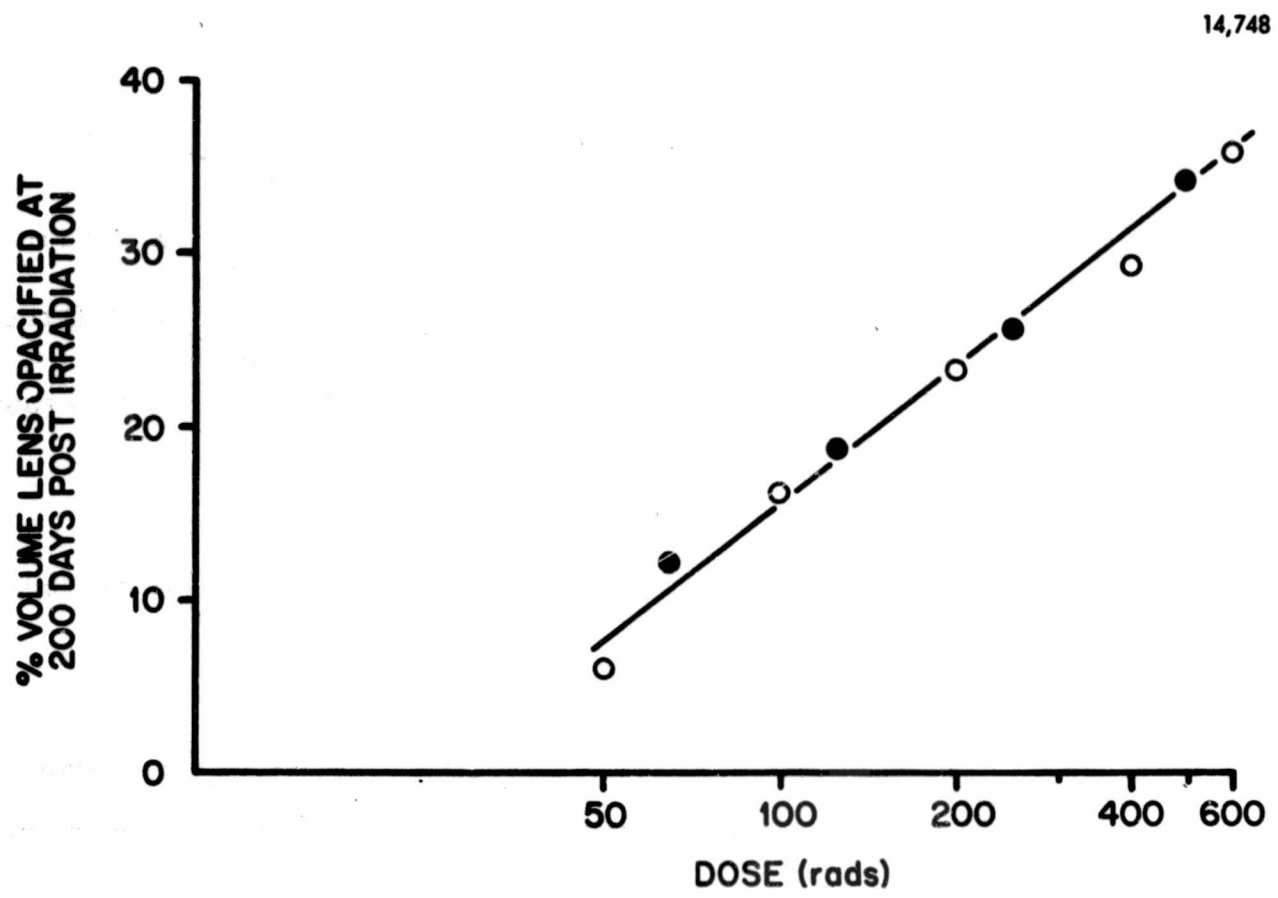


FIG. 5. Severity of Lens Changes at 200 days Post Irradiation.

- 35-Mev protons
- 300-kev X rays

II. BIOSATELLITE PROJECT

1. GENETIC EFFECTS OF A SIMULATED FLIGHT PROFILE ON PROTOTYPE PACKAGES FOR THE HABROBRACON BIOSATELLITE EXPERIMENT

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Diane J. Goins Mary Lou Pardue¹⁰

Introduction. — The Biosatellite experiment is designed to test synergistic effects of weightlessness and radiation, as well as other possible space-flight conditions, in a 3-day flight of a satellite that will contain its own radiation source as one of the controls. A number of organisms, including the parasitic wasp *Habrobracon*, are being prepared for the experiment, expected to be held in June 1966. The purpose of this experiment was to test the radiation subassembly and prototype packages with *Habrobracon* in conjunction with all other radiation experiments under four separate conditions. These conditions were (1) the simulated flight profile (centrifugation and vibration), (2) the γ -irradiation (from a low-level ^{85}Sr - γ source), (3) the simulated flight profile plus the irradiation, and (4) the nonprofile-nonirradiation control. It was assumed that this would provide an adequate test of the flight hardware, as well as provide preliminary ground control data for the eventual orbital flight.

Dosimetry. — Toshiba glass rods were used to measure the doses. Their readings were standardized by glass rods exposed to a National Bureau of Standards ^{60}Co - γ field. Their energy dependence was checked by an ORNL ^{137}Cs - γ field calibrated by a Victoreen chamber which in turn was standardized by NBS. There was no energy dependence. These rods were encased either in nylon, polyethylene tubes, or aluminum. Other glass rods, encased in plastic, lead, or copper, were used to make qualitative estimates of the amount of scattered radiation. Dosimetry was performed only on the packages receiving the gamma radiation, not on the control packages or on the profile-only packages.

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¹⁰Department of Biology, Purdue University, Lafayette, Indiana.

It was found that the radiation subassembly does not follow dose-square relations. From the ratios of the measurements of dosimeters encased in plastic, lead, and copper, it seems that the scattered gamma radiation does not contribute too much to the total dose (<10%). Precise determination of the contribution of scattered radiation of different wavelengths would require extensive further experimentation.

Results. — From the data obtained and by using the equations of von Borstel and Rekemeyer,¹¹ a dose-action curve for sperm survival (1.0 - dominant lethality) was constructed. This is shown in Fig. 6. It is apparent that there is no clear evidence for a synergistic effect for the flight profile and the radiation for induced dominant lethality in sperm.

¹¹R. C. von Borstel and M. L. Rekemeyer, Genetics 44, 1053-1074 (1959).

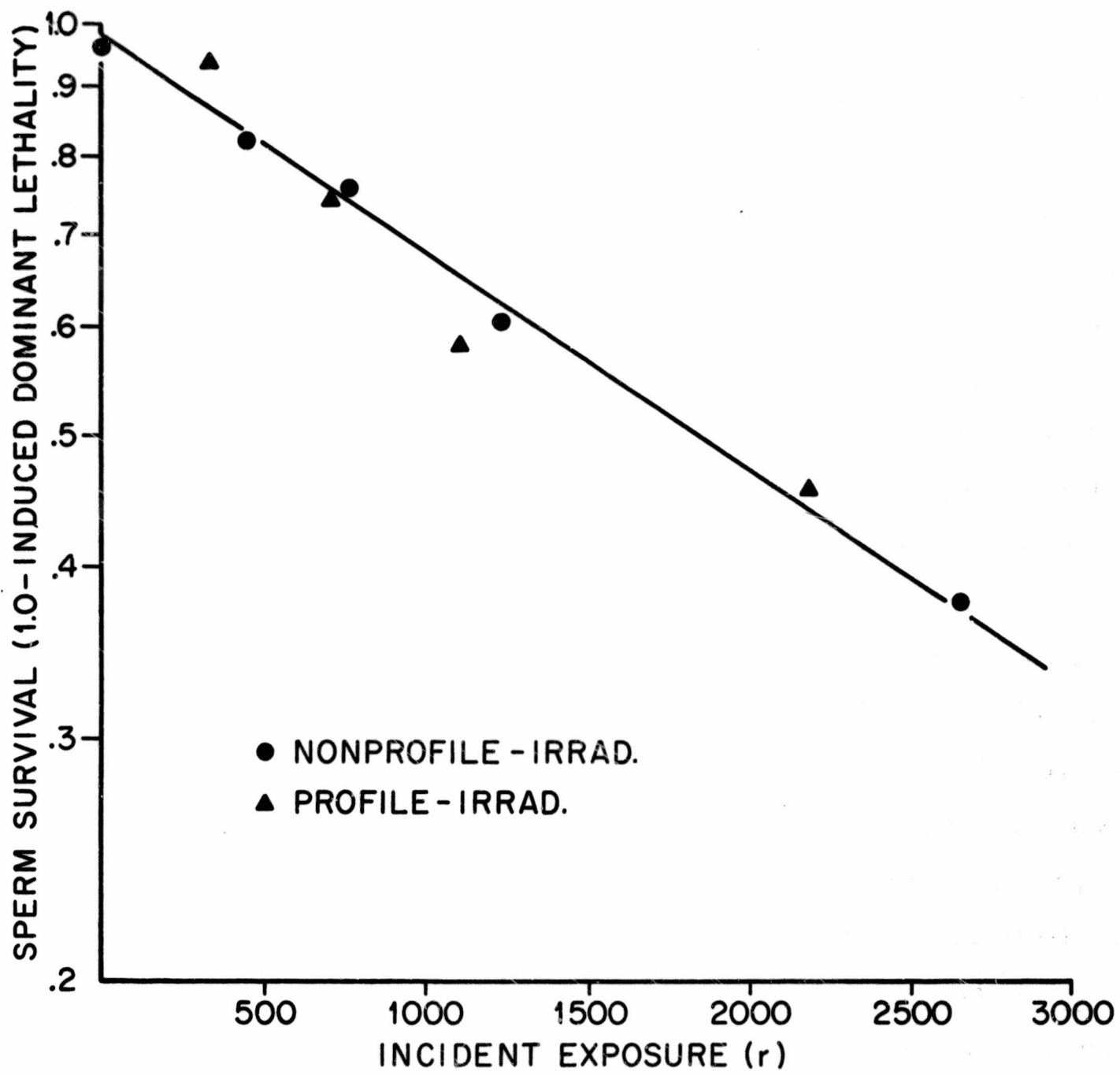


FIG. 6. Dose Response for Embryo Dominant Lethality Induced in Sperm.

2. THE GENETIC EFFECTS OF SIMULATED FLIGHT CONDITIONS ON THE PROTOTYPE PACKAGES FOR THE NEUROSPORA BIOSATELLITE EXPERIMENT

F. J. de Serres B. B. Webber

In preparation for the Biosatellite Experiment of June 1966, ground-based experiments are being performed both to test the design of the Neurospora prototype packages and to provide a preliminary biological assay of the effects of the acceleration, impact, and vibrational parameters, alone and in combination with radiation. In April 1965 a testing program was designed to provide such an assay. The genetic effects on Neurospora conidia in prototype packages subjected to (1) simulated flight profile alone, (2) radiation profile alone, or (3) the simulated flight profile in combination with the radiation profile were compared with (4) untreated controls.

Neurospora conidia were packaged and transported in the prototype packages. Treatments were designed to approximate the intensities of vibration and the gravitational forces during acceleration and impact actually experienced during satellite launching and reentry. Gamma ray doses from an ^{85}Sr source were administered continuously over a period of about 66 hr. These were estimated to be 2900, 1670, and 640 rads for three groups of conidia subjected to radiation alone and 2270, 1400, and 555 rads for three groups of conidia subjected to radiation and the simulated flight profile. The frequency of forward-mutation in the *ad-3* region among the heterokaryotic surviving conidia (as described previously¹²) was used to assay the genetic effects of the four different treatment conditions.

Results and Conclusions. — In Fig. 7 the mutation frequencies are plotted against estimated doses for the irradiated conidia. Unirradiated conidia (with or without vibration and centrifugation) typically exhibited forward-mutation frequencies of about 0.5 mutant per 10^6 survivors. There is no evidence for an

¹²B. B. Webber and F. J. de Serres, Proc. Natl. Acad. Sci. U.S.A. 53: 430-437 (1965).

enhancement of the mutagenic effect of gamma radiation by the vibration and centrifugation profiles used. Included in Fig. 7 are two estimates of mutation rate from a low-intensity 250-kvp X-ray experiment carried out under laboratory conditions, which indicates that the quantitative results from the gamma experiment are consistent with our expectations.

It may be concluded that the Neurospora conidia maintain approximately normal viability and radiosensitivity during storage in the Neurospora prototype packages for the period of time required for the proposed biosatellite experiment. Furthermore, the experimental results permit the tentative prediction that the mutagenic effectiveness of low-intensity gamma irradiation should not be affected by the vibration and forces of acceleration associated with launching and reentry of the biosatellite.

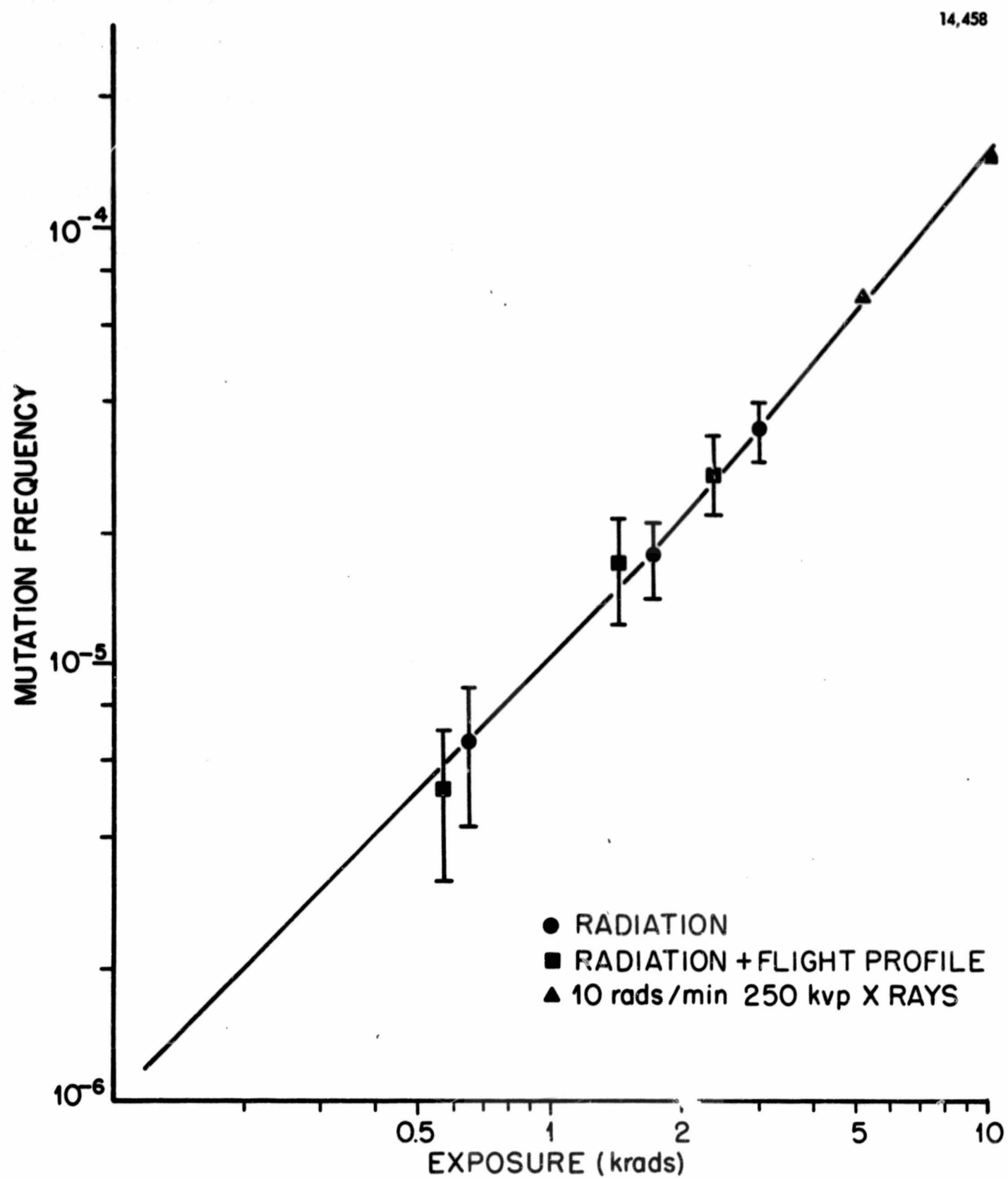


FIG. 7. Forward-Mutation Frequencies Obtained with the Neurospora Test System with Gamma Irradiation Alone or with Radiation in Combination with Simulated Flight Profile as Compared with Forward-Mutation Frequencies from Exposures to 250-kvp X rays (10 r/min).