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LBL-12654

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

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CATARACT PRODUCTION IN MICE BY HEAVY CHARGED PARTICLES

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March 1981

Biology & Medicine Division

Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

Acknowledgement

This project and preparation of this report benefited from the technical assistance of Dan Judd, Bennett McAllister, Steve Dentone, John Prioleau, Lynn Mahlmann, Andy Shih, Colleen Caudill, Panna Lee, Joan Lawson, and Jon Lieber. The cooperation of Jerry Howard, Tom Criswell, and the Bevalac operation's staff is gratefully acknowledged. Encouragement and early financial support were provided by Dr. E. L. Alpen. Support for the greater part of this research was provided by the Director, Office of Energy Research, Office of Basic Energy Science, Satellite Power System Project, Division of the U.S. Department of Energy under Contract No. W-7405-ENG-48. Partial support for animal care and some of the animals evaluated were from experiments supported by the National Aeronautics and Space Administration.

Abstract

The cataractogenic effects of heavy charged particles have been evaluated in mice in relation to dose and ionization density (LET_∞). The study was undertaken due to the high potential for eye exposures to HZE particles among SPS personnel working in outer space. This has made it imperative that the relative biological effectiveness (RBE) in relation to LET_∞ for various particles be defined so that appropriate quality factors (Q) could be assigned for estimation of risk. Although mice and men differ in susceptiblity to radiation-induced cataracts, the results from this project should assist in defining appropriate quality factors in relation to LET_∞, particle mass, charge, or velocity. Evaluation of results indicated that: 1) low single doses (5-20 rad) of iron (^{56}Fe) or argon (^{40}Ar) particles are cataractogenic at 11-18 months after irradiation; 2) onset and density of the opacification are dose related; 3) cataract density (grade) at 9, 11, 13, and 16 months after irradiation shows partial LET_∞-dependence; and 4) the severity of cataracts is reduced significantly when 417 rad of ^{60}Co gamma radiation is given in 24 weekly 17 rad fractions compared to giving this radiation as a single dose, but cataract severity is not reduced by fractionation of ^{12}C doses over 24 weeks.

Introduction/Background

Manned space flights are now a reality and may increase in the future in connection with use of advanced technologies such as satellite power systems (SPS) to generate electricity. However, many of the risks associated with extended habitation in a space environment remain undetermined. Of particular concern at this time are the hazards that might occur from high energy, heavy charged particles (HZE) to which space-workers will be exposed. Less is known about the biological consequences of HZE particles than about other types of radiation encountered in space. Conventional types of shielding used for radiation protection do not shield out HZE particles. Thus, more information is needed about biological effects of HZE in order to assess their potential adverse health hazards.

Of considerable importance are the effects of HZE particles on the crystalline lens of the eye because this tissue has proven susceptible to x- and gamma-rays and particularly susceptible to the action of other forms of high LET_{∞} (linear energy transfer) radiation such as neutrons (1,2). The study of radiation-induced cataracts has a history that practically coincides with man's initial discovery of radiation. A mere two years after Roentgen's discovery, Chulpecky speculated from ophthalmoscopic examinations that x-rays could induce cataract formation. Very little research on the subject was undertaken, though, until the late 1930's. With the arrival of the nuclear age and World War II, governments and scientists were led to investigate the potential harmful effects on man from many kinds of radiation. This was followed in the 1950s by a second surge of research on the therapeutic uses of radiation, such as in the treatment of cancer, and on potential biological effects from nuclear weapons. With the advent of manned space flights, further investigation was stimulated to probe the effects of radiation including effects of (heavy) charged particles, encountered in outer space.

There are five primary factors that affect the potential for radiation-induced lens opacification in any species. These factors are dose, exposure time (or instantaneous dose rate), dose fractionation, quality of radiation, and age of the animal at the time of irradiation (3). High LET_∞ neutrons are nine to one hundred times as effective as x-rays for producing lens opacification in mice (4). Furthermore, at least twice the dose of low LET_∞ radiation (such as x- or gamma-rays) is required to cause a cataract when the dose is given over an extended period of time rather than as a single exposure (1-4). Thus, there must be a mechanism in the lens to repair the low LET_∞ damage, whereas, there seems to be little or no repair of high LET_∞ damage because the same effect is achieved if a neutron dose is administered on a single occasion or given at low dose rates in many fractions over a long period of time. No information is available regarding repair of lens damage after exposure to HZE particles.

The high susceptibility of the crystalline lens of the eye to densely ionizing radiation suggests that this tissue would also be very susceptible to the effects of HZE particles. Because of this and the fact that astronauts have already reported visual experiences thought to be a consequence of these particles during space flights, we undertook a pilot study (Experiment I) of the survivors of the thirty day lethality study. In this study, LAF_1 mice received from 350-1000 rad of stopping argon particles at the LBL Bevalac. These animals developed opacities quite similar to those described for other forms of radiation in that the opacities began in the posterior subcapsular region of the lens two to three months after radiation. The onset and intensity of the opacity appeared to be correlated with radiation dose (5).

Subsequently we undertook a second pilot study (Experiment II) in which LAF_1 mice were exposed (head only) to 10 to 100 rad of stopping argon particles at the center of a 4 cm spread Bragg peak. Posterior opacities developed seven months after irradiation. Again the density and onset were radiation-dose dependent. Results of these pilot studies were included in thesis submitted in partial fulfillment of the Doctor of Optometry degree at the University of California at Berkeley (5,6). Some results from these pilot studies are presented in this report.

The importance of these pilot studies was that a cataract scoring system appropriate for the mouse was developed, and it was clear we could proceed with a comprehensive study to evaluate relative biological effectiveness (RBE) in relation to LET_{∞} for heavy charged particles. Such an effort seemed highly appropriate because information was lacking on the cataractogenic effects of HZE particles.

Groups of experimental Mice Evaluated by Slit-lamp Biomicroscopy

Further details are provided in subsequent sections of this report, but for purposes of clarity, it is useful to identify briefly here the various groups of mice that have been evaluated during the course of this short project. Table I provides a summary of the various groups (designated by Roman numerals) giving mouse strains, numbers of animals, irradiation dates, and radiation parameters.

Experiment IV: This was the core experiment, the objective of which was to evaluate RBE in relation to LET in mice exposed to HZE particles. Because human space exposures will involve primarily non-stopping HZE particles, the mice were exposed to non-stopping particles in the plateau portions of the Bragg curves of ^{12}C, ^{20}Ne, or ^{40}Ar.

When support for the project was initiated on 1 July 1979, the mice of Experiment IV were being evaluated periodically, but a significant frequency of cataracts had not yet developed.

Experiment III: During the summer of 1979, mice irradiated with stopping ^{2}He, ^{12}C, ^{20}Ne, and ^{40}Ar particles and non-stopping ^{56}Fe particles were scheduled for sacrifice in connection with studies of Harderian gland carcinogenesis. When it was clear that these animals could be evaluated without interference with the Harderian gland study, the decision was made to evaluate them, on a one-time basis, with the hope of extending the LET_{∞} range with which we could gain experience. Additionally, our personnel conducting the slit-lamp biomicroscopic examinations would increase their level of experience and competence.

Experiment V: Extending the range of LET_{∞} values studied also was the rationale for continuing to evalute mice in Experiment II (stopping ^{40}Ar particles) and for initiating evaluations on mice in a life span study. In the latter, designated Skyhook, the mice were exposed to stopping ^{12}C particles or ^{60}C gamma radiation (Experiment V). Examination of these Skyhook mice also provided an opportunity to compare the influence of fractionation (over 24 or 25 weeks) on the cataractogenic action of ^{12}C versus ^{60}C gamma radiation.

Morphologic Studies

A further recent aspect of this project has been light and electron microscopic evaluation of the lenses from irradiated animals. Electron microscopic studies of the lens have not been performed on HZE exposed animals and are rare even for low LET_{∞}-treated animals (7-9). IT is hoped that such cytologic studies (which are not yet complete) will lead to elucidation of the mechanisms of opacification of the lens and to the determination of whether the mechanisms of opacification of the lens are the same or different for high and low LET_{∞} radiation. If expanded to interspecies comparisons in the future, such studies could provide a sensitive early means by which cataractogenic potential may be predicted for various animal species (including primates), thus decreasing the need for further future long term biomicroscopic evaluations of lens opacities to determine radiation risks.

Methods and Procedures

Three strains of hybrid mice were used in these studies. The principle commercial supplier was the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, from which LAF_1 (C57/bl6xCBA) and CB_6F_1 (BALB/cxC57/bl6) mice were purchased and delivered to the Lawrence Berkeley Laboratory at 8-10 weeks of age. The third strain was the B_6CF_1 (C57/bl6xBALB/c) produced at Argonne National Laboratory. The B_6CF_1 stain was used in collaborative studies involving personnel at the Lawrence Berkeley Laboratory, the Argonne National Laboratory, and Oak Ridge National Laboratory in studies of radiation-carcinogenesis using Harderian gland tumors as the end point. The B_6CF_1 mice were shipped to Lawrence Berkeley Laboratory at 50-100 days of age, at 60-100 days were transplanted with two syngeneic pituitary glands inserted surgically beneath the spleen capsule, and given upper body irradiation with charged particles 2-5 weeks thereafter. Unirradiated animals subjected to the same surgical procedures served as the appropriate controls for the experimental group. The CB_6F_1 mice were irradiated at 100-140 days of age and were subjected to a single slit-lamp biomicroscopic evaluation 350-400 days following irradiation.

The three radiation sources used in these studies were a 220 kVp x-ray machine, the Bevalac, and the 184 inch cyclotron. Except for whole-body exposures to argon ions for a 30 day lethality experiment, all animals evaluated for cataracts by slit-lamp biomicroscopy were given either head only or upper-body exposures to charged particles or x-radiation. Fully stripped heavy charged particles were obtained from the Lawrence Berkeley Laboratory Bevalac, a National Facility. The Bevalac combines two accelerators: the SUPERhilac, a heavy ion linear accelerator, and the Bevatron, a proton synchrotron.

Particles are first accelerated to approximately 7-9 MeV in the SUPERhilac and are then injected into the Bevatron through a transfer line where maximum energies obtained are in the range of 2 GeV; ^{12}C, ^{20}Ne, ^{40}Ar, ^{56}Fe ions were extracted from the Bevalac at a preselected energy. The particle energies used in the present studies are summarized in Table I. Mice were irradiated on an optical bench in the Bevalac Cave II apprxoimately 3 meters from the thin mylar window where the incident beam enters the exposure room and passes through air to the exposure location. Interposed in the beam line are lead scattering foils (using 12/64-1/64 inches thick), a collimator, upstream and downstream ionization chambers, a multiwire proportional chamber and, when spread Bragg peaks are used, a spiral ridge filter and a water column. The proportional chamber provides information on localization and size of the incident beam. The collimator reduces the beam size to 3x5 cm. The head and chest of vertically positioned mice were exposed to charged particles by "translating" seven mice through a beam spot while the mice were affixed to a 24.2 cm wide lucite holder. Animals were irradiated under anesthesia; 1.24 mg/mouse of pentabarbital sodium (Diabutal) was administered approximately 5 minutes before irradiation. Dose rates were several hundred rad/min. Shortly after irradiation animals were transported 0.25 miles to LBL Animal Facilities, and were subsequently transported an additional 0.25 miles to animal facilities located in the School of Optometry, on the Berkeley Campus.

Helium ions (920 MeV) were obtained at the Lawrence Berkeley Laboratory 184 inch cyclotron. The beam delivery and dosimetry systems at this facility are very similar to those used at the Bevalac, and anesthetized mice were exposed using the same animal holders used at the Bevalac. Mice were positioned at the distal portion of a 10 cm spread Bragg peak, and the dose rate was approximately 150 rad/min.

Eye examinations for Experiments I, II and III were performed by first sedating each animal with Diabutal. Dilation was achieved by the use of one drop of 1 percent Tropicamide. Subsequently, in Experiments IV and V we did not find it necessary to sedate animals for examination. The lenses were then observed with a slit-lamp biomicroscope. In most instances, the observer did not know the dose or type of radiation that the animal had received.

In Experiment II, with stopping argon ions, 80 animals were divided into 15 groups, 12 of which were irradiated. The remaining three groups were not exposed to irradiation and were designated controls (22 animals). There were four doses of radiation used: 10 rad (14 animals), 25 rad (12 animals), 50 rad (17 animals), and 100 rad (15 animals).

Animals in Experiment II, were examined both just prior to and the day following irradiation. At these times, no opacification was observed in any animal. Approximately 50 percent of the animals in each group were examined at monthly intervals, with each animal being observed approximately every two months. Once an opacity was observed, all mice in the group were examined in each subsequent month.

These observers used the following criteria for grading opacifications: Grade I signified an opacified area of approximately 10 percent of the posterior subcapsular area, grade II was approximately 25 percent, grade III was approximately 40 percent, and grade IV 50 percent or greater. Each grade could be further modified by a plus or minus sign, with plus indicating approximately 5 percent more opacification and minus signifying 5 percent less. The data presented in Figs. 2 and 3 were obtained in this manner. More detailed results are presented elsewhere (6).

After this study, a slightly modified system of cataract grading was developed and is summarized in Table II and Fig. 1. It was hoped that this system would be more reliable. The posterior lens opacities were observed primarily as three types which we have called diffuse, amorphous, and stellate. In the "diffuse" type the subcapsular region may best be described as having a uniform, fairly homogeneous distribution of vacuoles, most of which were similar in size and shape, and sprinkled across the posterior lens in a "salt and pepper" fashion. Lenses in the "amorphous" category had lesions that were discrete, irregular in shape, and located anywhere in the posterior lens area. Size, shape and number varied, but the lesions were nearly always opaque. The "stellate" type was often very like the amorphous type. Generally, however, these were more central in location and had radiating opaque thread-like processes resembling the arms of a star distributed radially around the central opacity.

To prepare the lenses for electron microscopy, the mice were killed by cervical dislocation and the whole eye was placed in 2 percent glutaraldehyde in 0.075 M phosphate buffer at 37°C. After 15 minutes a cut was made in the posterior globe. The eyes were held at 37°C in fixative for 15 more minutes, and then the lens were removed from the eye and placed in buffer at 37°C. The lenses were allowed to equilibrate to room temperature and refrigerated overnight. The next morning they were washed in buffer and then placed in cold 2 percent osmium and refrigerated one hour. At that time, the lens could easily be cut into pieces with a razor. After dehydration in a series of alcohols and propylene oxide, the lenses were embedded in araldite and sectioned on a Sorvall MT-2B ultramicrotome. They were then stained with uranylacetate and Reynolds lead citrate and examined on a Zeiss 10 electron microscope.

Estimates of RBE are provided in this report. They should be interpreted cautiously because shapes of dose response curves for the radiation qualities studied are not yet defined accurately or evaluated statistically. RBE at any level of dose, or at any time when evaluations were made, is dependent on accuracy of dose-response relationships. Moreover, the extent to which the scoring system used provides for a "linear" dose-response is not defined accurately at this time. Therefore, we wish explicitly to qualify our interpretations of RBE estimates on our comments made regarding the LET_∞ dependence of the responses reported here.

Results and Discussions

Experiment I: In our earliest study, we followed cataract development in the lenses of mice that were survivors of the $LD_{50/30}$ study in which the animals received stopping argon ion doses ranging from 350 to 1000 rad. All animals developed significant opacification of the posterior lens within 4 to 6 months. No data are included because of the high doses used and because a semi-quantitative scoring system was only then being developed; however, details of the results have been presented elsewhere (5).

Experiment II: We next undertook an experiment in which animals were irradiated with 10-100 rad of spread Bragg peak stopping argon ions. The results of these experiments are seen in Figs. 2 and 3. It should be noted that the data in these two figures were collected by one group of observers; all subsequent data were gathered by another group, which used a different grading system as indicated in the methods section. Three conclusions are suggested from this experiment:

1. The severity of opacification is dose-dependent. At nine months, the most severe opacifications were observed among mice that received the highest dose, 100 rad (Fig. 2).

2. Latency for lens opacification is also dose-dependent. Opacifications were observed in several animals at seven months after 100 rad, whereas only one animal showed opacification after 50 rad, and no animals showed opacification after 10 or 25 rad at that time (Fig. 3).
3. "Low" doses of 25 or 10 rad produced some opacification of the posterior lens by nine months after irradiation (Fig. 2,3).

Each of these conclusions is consistent with previously published results concerning other forms of ionizing radiations (3,10).

Subsequent evaluations were performed on mice in Experiment II because at low doses, latency could be quite long, and the severity of opacifications could also increase.

At nine months after irradiation, only one animal in the 10 rad group showed lens opacification. We therefore evaluated the animals again at 18 months (Table IIIa). At that time, all mice given 10 rad showed lens opacities. The average opacification score was similar (2.6-2.9) at that time, in mice that received 10, 25 or 50 rad, and we assumed that the lack of dose-dependence was due to a plateauing of the response by that time. Upon re-evaluation of 23 months (Table IIIb), the data suggested that plateauing might, in fact, not have occurred; however, the differences obtained are not significant and could be within the range of variability of our scoring system.

It is also clear that we have not determined a minimal dose of argon ions below which no lens opacification will develop. It appears that the low doses have a fairly long latency, and further studies are necessary to determine if opacities can be induced by even lower doses after long latencies.

Experiment III: Among the first animals available for study in this project were those involved in an ongoing experiment (see Table IV) that concerned induction of Harderian gland tumors by HZE particles. Animals were sacrificed at 450-500 days of age (340-390 days after irradiation), and it appeared prudent to collect data on cataracts from these animals prior to sacrifice. Although the data obtained yielded information on only a single time point, it was felt that the information could be useful because of the various particles involved in the study. Cornea and lens lesions were scored according to an early version of our scoring system (Table IV). The criteria were different from those used for experients IV and V. In fact, it was the conscientious observations made on these animals that led to the formulation of the scoring system summarized in Table II and Fig. 1.

Although the average scores were computed, we consider the data potentially unreliable and not comparable to data collected from experiments IV and V. Two factors largely accounted for the unreliability. The first is that our experience level was low at that time because these were among the first animals that our team members had evaluated, and the use of Diabutal anesthesia inhibited the normal tearing process, and drying of the cornea actually interferred with the slit-lamp biomicroscopic evaluations. Subsequently, procedures were modified such that the eye was irrigated with saline (0.85 NaCl) during evaluation, thereby alleviating corneal drying. We have considerable reservation regarding even the qualitative results summarized in Table IV where the responses are tallied only as normal vs. abnormal. Our reservation about the qualitative results is based on the fact that the results are not totally coherent. Little in the way of dose-response relationships, which we had expected, was actually observed; also the number of lens lesions scored after 20 rad of ^{56}Fe decreased when the same animals were examined a second time one month later. The absence of clear-cut dose relationships could be attributable to a plateauing of response by 350-390 days after irradiation. Had the mice been evaluated at an earlier time, dose-response relationships might have been evident. Results forthcoming from Experiment IV will identify dose-time-plateau relationships and may aid with reevaluation of results from Experiment III. Because photographs were taken of lens lesions in some of these animals we are confident in stating that posterior lens abnormalities can be produced in at least some animals exposed to 5-20 rad of ^{56}Fe ions. Another important observation made on ^{56}Fe-irradiated mice concerns corneal neovascularization. The original age examination records will have to be studied again before quantitative statements are prudent, but we are prepared to comment here that a small number of mice exposed in the plateau portion of the ^{56}Fe Bragg curve showed extensive neovascularizations. Several thousand eye examinations have been performed during the course of our investigations, and the only corneal neovascularizations observed were among mice exposed to ^{56}Fe. Because neovascularization is most frequently associated with traumatic injury, the possibility exists that very heavy charged particles produce a unique mode of injury. Clearly, sequential eye examinations, supported by light and electron microscopy, should be given high priority for the future.

Experiment V: Two separate evaluations have been completed on mice in the life-span study designated Skyhook I (Experiment V). The principle objective was to determine the extent to which fractionation of a ^{12}C dose influenced the cornea and lens responses. Because a sparing affect of photon dose fractionation is well estabished, only two groups of gamma-irradiated mice that received the same total dose were evaluated. The results summarized in Table V show a large sparing effect when the gamma dose of 417 rad was administered in 24 weekly fractions of 27 rad over approximately six months. In contrast, when ^{12}C doses were administered in 24 fractions of 1.7, 3.3, or 5.0 rad, rather than as single doses of 40, 80, or 120 rad, the average scores were elevated significantly in comparison with the scores from animals that received the same single dose. Thus, it appeared that ^{12}C dose fractionation enhanced or accelerated the cataractogenic process. However, firm conclusions regarding enhancement could not be based on a single set of observations. Consequently, some of these animals were reevaluated approximately two months later. This enhancement phenomenon was not confirmed (Table VI). This table has another important feature in that it demonstrates something about the accuracy of our measurements. In fact, the results, if correct, would have suggested that the cataracts might have decreased in density during the period between observations (compare this to Table V). Neither we nor others studying cataract formation due to radiation have ever reported regression of cataract formation. Therefore, we required our evaluators to examine the same animals on three different days very closely spaced. We found that each observer was consistent, but that there may have been as much as a scoring unit difference between the obs rvers. Thus, the apparent decrease in the opacity between the results given in Table V and VI is most probably due to differences in scoring from observe to observer.

In spite of the fact that the scoring system is subjective and does suffer from problems of observer bias, we feel that the results shown in Tables V and VI demonstrate that there is definitely no sparing induced by fractionation of HZE dose. Further, based on the degree of opacification following 120 rad of ^{12}C ions, at 334-360 days, the RBE is probably not much greater than 3.5

Experiment IV: In this experiment, we studied cataract induction in animals irradiated with x-rays and with argon, neon, and carbon ions in the plateau region of the Bragg curve. At the onset, it was postulated that HZE particles might have an RBE of 10 in comparison with x-radiation at low radiation doses.

Six months after the animals had been irradiated, only minimal lens changes were observed. The dose-response relationship was not clear-cut, and no significant differences emerged among the lenses irradiated with the different ions (Fig. 4).

This situation changed at 9 months after irradiation. As is shown in Fig. 5, the degree of opacification appeared strongly dependent upon the ion used. At the highest dose (90 rad), all types of particles produced unmistakable opacification, the extent of which is correlated with the expected LET_∞ dependence of the response. That is, the degree of opacification increased progressively with increasing estimated values of LET_∞: namely, carbon (~10 keV/μm), neon (~30 keV/μm), and argon (~100 keV/μm).

From these data, one cannot accurately determine the RBE for HZE particles in relation to x-radiation, but there is no question that the RBE is less than 10, at these doses. Whereas the x-ray animals given 900 rad had developed total lens opacification at 9 months, the 90 rad argon-induced cataracts were approximately grade 3, the neon about grade 2 and carbon grade 1. If one considers the data in Fig. 5, one observes that 60 rad of argon induces about as much opacification as 300 rad of x-rays; thus, the RBE may be of the order 5. The RBE for carbon would then be about 159/90, or less than 2.0.

The data at 11 months did not demonstrate as clear-cut a dependence of RBE upon LET_∞(Fig. 6) because of discrepancies for neon at 60 and 90 rad. The opacities in these animals appeared to have decreased by as much as one unit during the 2 month period following the 9 month observation. We consider this not a real change, but due, as described above, to the subjectivity of the measurement. The data from the animals at 13 months appear more consistent with that at 9 months, thus supporting this conclusion (Table VII).

Comparison of average cataract scores at 11 and 13 months indicated very little change (or perhaps only a slight increase) in the degree of opacification for all doses of all ions (Fig. 6). Thus, it appeared that the opacification could be plateauing. However, in argon-irradiated animals that we have been observing over a period of two years (experimental group II, Table I) it appeared that the mean cataract score did not plateau, but rather continued to progress (Table IIIb). Based on the data collected at 13 months from animals in Experiment IV, we felt it was necessary to continue to evaluate those animals to ascertain if there was an apparent slowing or cessation of opacification. The data collected at 16 months after irradiation (Fig. 7) confirmed our previous observations on argon-irradiated animals in which an appreciable increase in average cataract score occurred between 18 and 23 months after irradiation. At all doses of argon ions in Experiment IV, the average cataract scores increased between 13 and 16 months. The statistical significance of these increases has not yet been determined. The largest relative increases occurred at 15, 30, and 60 rad, with the relative increases being comparatively small at 90 rad. This probably means that our scoring system has greater sensitivity at lower levels of lens opacification (1-25 percent) than at higher levels of opacification (see Table II). Consequently, estimates of RBE should probably be made on data at a time when average cataract scores range between 1.5-2.5. This matter is currently under assessment, and the results available from aimals in Experiment IV are being replotted based on the fraction of animals at risk that show a given average cataract score, e.g. 2.0, as a function of time after irradiation. This is the type of data analysis used by Bateman et al. (1) for estimation of RBE. A similar RBE for plateau ^{40}Ar ions, and a somewhat lower estimate for plateau ^{20}Ne ions, has been reported for rabbits (13).

As was true at 9 months, the 16 months data are also consistent with an RBE estimate in the range of 3-5 for argon ions and somewhat lower for neon. Regarding carbon, the average cataract score at 16 months following 90 rad is lower than the average cataract score for 50 rad of x-rays (Fig. 7); thus, the RBE for plateau carbon ions would appear to be less than 1.0.

Because of the higher LET_∞ associated with stopping particles in comparison with non-stopping particles (plateau region), higher average cataract scores would be expected for stopping particles at LET_∞ values below 100-200 keV/μm. At higher LET_∞ values, the effect per rad would be expected to diminish because a significant portion of the ionization energy is wasted, i.e., "overkill." Questions regarding scoring reliability/reproducibility notwithstanding, the present results suggest a greater effect per rad for stopping particles in comparison with non-stopping for single doses of both ^{12}C and ^{40}Ar ions. This result is surprising for ^{40}Ar ions. At about 12 months after 80 rad of stopping ^{12}C particles, the average cataract score was 2.5 (Table V) whereas, a dose of 90 rad, deposited by the plateau portion of the Bragg curve, resulted in an average score of 1.2 at 13 months (Table VII). Also, at 16 months after 15 rad of ^{40}Ar plateau particles, the average score is ~1.5 (Fig. 7), whereas, at 18 months after 10 rad of ^{40}Ar stopping particles, the average score is ~2.7 (Table IIIa). The 2 month difference in observation time somewhat complicates these comparisons between stopping and non-stopping ^{40}Ar particles. Nonetheless, the results strongly indicate that stopping ^{40}Ar particles would show a higher RBE than plateau particles if a definitive study were to be conducted. This RBE/LET_∞ relationship seems highly important to explore because stopping ^{40}Ar particles are probably characterized by a LET_∞ of 400-500 keV/μm and would be expected to be above the peak RBE/LET_∞ range of 100-200 keV/μm. Based on conventional wisdom regarding

RBE/LET_∞ relationships for cell killing, RBE should exhibit a maximum value in the LET_∞ range of 100-200 keV/μm.

As is true with all other forms of radiation, the major lens changes were first observed in the posterior lens. The various forms of opacifications are diagrammed in Fig. 1. It should be noted that at about 18 months, several of the animals began to develop an anterior opacification that was fairly discrete and located in the central pupillary area (Fig. 9). Their discreteness has prompted us to call them "buttons." This opacification will be examined at the light and electron microscopic level in the near future.

The mouse has been criticized as an experimental model for cataract studies due to its high spontaneous rate of opacification. We did not find this to be a problem with these animals. Due perhaps to a difference in the strain selected, we saw little opacification in our unirradiated controls at 18 months. With longer times, the control lenses showed some aging changes (Table IIIb), and the loss of transparency covered a large diffuse area of the posterior cortex. However, this opacity was certainly not like those caused by radiation and, seemed almost like a subtle change in texture. By contrast, the changes in the irradiated animals were quite discrete and dense.

At this time, we have undertaken limited electron microscopic evaluations of lens opacities induced by HZE particles. These have included lenses only from animals irradiated with argon ions two years earlier (Experiment II animals, Table I). Significant changes were observed in these animals. As is typical of other forms of radiation, there is permanent disruption of the lens bow and the cortex is otherwise abnormal, as evidenced by swollen cells and by what appear to be degenerated nuclei and lysosome-like structures. Some of these changes may be observed in the photomicrographs in Figs. 10a-e. Deeper layers of the lens were normal in morphology, thus being consistent with the suggestion that HZE-induced cataracts, like other radiation cataracts, occur as a consequence of early effects on the epithelial cell layer (11).

Conclusions

Results indicate: 1) HZE particles produce posterior lens opacities in mice with considerable facility; and although no definitive RBE can be assigned at this time for the varous charged particles used in this project, it is expected to be greater than 1.0 and could be of the order of 5 for ^{40}Ar or ^{56}Fe particles after a single dose.

2) posterior cataracts were observed in animals given "low" single doses of 5, 10, or 20 rad of ^{56}Fe ions or 15 rad of ^{40}Ar ions;

3) the time of cataract expression and magnitude of injury are related to dose and to LET_∞ of the particle such that response increases with increasing LET_∞;

4) ionization produced by stopping ^{40}Ar particles, characterized by an LET_∞ of 400-500 keV/μm, appears more cataractogenic than ionization produced by non-stopping ^{40}Ar particles. Although the contribution of fragments to the dose is not known accurately, this suggests that the maximum RBE for cataractogenesis occurs at an LET_∞ value greater than 100-200 keV/micron;

5) dose fractionation reduces the cataractogenic effects of ^{60}Co gamma radiation but definitely does not reduce the effects of ^{12}C radiation;

6) no strong evidence exists at this time for the production of unique lens lesions by HZE particles, but the presence of anterior lens "buttons" in some HZE particle-irradiated mice (and their apparent low frequency in x-irradiated mice) requires further study;

7) at this time, no firm conclusion can be drawn as to whether the opacification plateaus as suggested by Bateman et al. in studies of neutron cataract formation (12). Our scoring system differs consididrably from theirs

inasmuch as they attempted to correlate the number of discrete opacities with radiation dose. It is our observation that early discrete opacities merge with time. Thus, there might be an apparent plateauing of the number of opacities, yet the density of the opacity could continue to increase. Our long term studies support this strongly.

Whether anterior opacities will progress or remain stable is unclear at this time. It is interesting that Worgul has determined that in rats, at high dose levels, anterior changes occur prior to the onset of the posterior opacities. At low doses, he found that the onset of anterior and posterior opacities was approximately the same (Worgul, personal communication). In our experience, the posterior lens changes always preceeded the appearance of anterior buttons;

8) light and electron microscopic examinations of the lens cortex, especially the bow region, of animals two years after argon irradiation also suggest considerable similarity in the nature of the cataracts produced by argon ions and x-rays. It is, however, important to examine the histology of lenses of animals early after irradiation to establish the initial site of damage for high and low LET radiations. It is possible that the early effects on the lens could be quite different, but that the lens has a rather limited way in which to respond morphologically as the opacities develop, so that at late stages, the different ions might produce quite similar histologic changes, even though the initial damage may be different. Early histologic examiatnion could thus help to determine whether there might be more than one mechanism contributing to HZE-induced lens opacification.

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Table I: Mice from Various Experimental Groups Evaluated for Corneal and Posterior Lens Damage
Number of Mice Examined
Irradiation Controls

Experimental Group	Mouse Strain	No. Mice Irrad.	Examined Controls	Irradiation Date	Radiation Parameters
I $LD_{50/30}$ Survivors	LAF_1 males	113	8	10/31/77 11/01/77	570 MeV ^{40}Ar – 4 cm SOBP[a]
II Cataract Study	LAF_1 males	80	22	07/11/78	570 MeV ^{40}Ar – 4 cm SOBP
III Harderian Gland Carcinogenesis	B_6CF_1 females	53			600 MeV ^{56}Fe – Plateau[b]
		190			570 MeV ^{40}Ar – 4 cm SOBP
		189		Variable	425 MeV ^{20}Ne –10 cm SOBP
		168			400 MeV ^{12}C – 10cm SOBP
		37			920 MeV ^{2}He – 10 cm SOBP
			12		Transplant Controls
IV Cataract Study	CB_6F_1 Males	90		04/06/79	220 kVp x-rays
		80		03/28/79	570 MeV ^{40}Ar – Plateau
		90		03/03/79	470 MeV ^{20}Ne – Plateau
		90		03/09/79	400 MeV ^{12}C – Plateau
			90		Control
V Life Span Study	CB_6F_1 Males	51		02/17/79	225 MeV ^{12}C – 4 cm SOBP Single Dose
(SKYHOOK)		48		12/08/78	225 MeV ^{12}C – 4 cm SOBP – 24 Fractionated Doses
		14		01/12/79	1.2 MeV ^{60}Co – Single Dose
		21		01/09/79	1.2 MeV ^{60}Co – 24 Fractionated Doses
			30	12/08/78	Controls

* a SOBP = Spread out Bragg peak

b Four mice were exposed simultaneously by positioning them at different positions in the plateau portion of the Bragg curve; the orbits were positioned carefully in a 1 cm beam spot where the dose rate range from ~0.1–1.0 rad/min.

Table II. The area (percent) of the posterior lens affected was estimated and given a numerical grade as follows:

Numberical Grade	Percent of Posterior Lens Affected
0	0
0.5	1-5
1	5-15
2	15-25
3	25-50
4	50+

A visual impression of our approximate grading procedure is conveyed by Fig. 1

Table IIIa: Cataracts in the Posterior Lens of LAF_1 Male Mice at 18 Months After Exposure to Stopping ^{40}Ar Ions (4 cm SOBP)

Dose (rad)	No. Eyes	Average Score ± S.E.
0	31	0.5* ± 0.09
10	21	2.7 ± 0.14
25	20	2.9 ± 0.18
50	22	2.6 ± 0.14
100	13	3.6 ± 0.14

* significantly different ($P < .01$) from all irradiated groups.

Table IIIb: Same Animals Examined at 23 Months After Irradiation

Dose (rad)	No. Eyes	Average Score ± S.E.
0	26	2.2 ± 0.17
10	18	2.6 ± 0.17
25	18	3.1 ± 0.17
50	24	3.0 ± 0.11
100	6	4.0

Table IV: B_6CF_1 Female Mice from Harderian Gland Experiments that were Evaluated by Slit-Lamp Biomicroscopy at 340-390 Days after Irradiation

Group	Date Observed	No. Eyes Examined	Dose (rad)	Cornea Abnormal/ Total	Post. Lens Abnormal/Total
Transplanted Aged Controls	7/11/79	24	0	1/24	1/24
920 MeV ^{2}He a	06/18/79	24	40	2/24	I b
	06/18/79	30	80	0/30	I b
	06/18/79	10	160	02/10	7/10
	06/18/79	8	320	1/8	6/8
470 MeV ^{20}Ne c	06/18/79	28	20	1/28	10/28
	06/18/79	12	40	0/12	7/12
	06/18/79	8	80	2/8	3/8
570 MeV ^{40}Ar c	06/28/79	28	40	7/28	I b
	06/28/79	28	80	12/38	I b
	06/28/79	22	160	1/22	I b
600 MeV ^{56}Fe d	06/18/79	24	10	13/24	12/24
	06/18/79	22	20	3/22	9/22
600 MeV ^{56}Fe d	07/13/79	12	5	1/12	4/12
	07/13/79	24	10	9/24	5/24
	07/13/79	24	20	13/24	8/24

a) distal portion of 10 cm spread out Bragg Peak (SOBP)
b) inconclusive observation due to corneal clouding caused by anesthesia
c) Center of 4 cm spread out Bragg Peak (SOBP)
d) Plateau portion of Bragg curve

Table V: Corneal and Lens Response at 334-360 Days Following Single or Fractionated Doses of 225 MeV Carbon Ions (4 cm SOBP) or ^{60}Co Gamma Radiation

Radiation Dose (rad)	No. Eyes	Single Dose Average Score ± S.E. Cornea	Single Dose Average Score ± S.E. Post Lens	No. Eyes	Fractionated Dose (24) Average Score ± S.E. Cornea	Fractionated Dose (24) Average Score ± S.E. Post. lens
^{12}C 40	40	0	1.2 ± 0.07	32	0.08 ± .06	2.3 ± .13[a]
^{12}C 80	32	0	2.5 ± .09	32	0.04 ± .09	3.5 ± .10[a]
^{12}C 120	30	0	2.7 ± .08	32	0.00 ± .07	3.8 ± .13[a]
^{60}Co 417	28	0.14 ± .08	3.2 ± .08	42	0.07 ± .07	0.70 ± .06[b]
Controls						
0	26	0.4 ± .12	0.6 ± .10			
0	16	0.6 ± .13	0.4 ± .06			
0	18	0.6 ± .15	0.5 ± .08			

a) Average cataract score at 334-360 days after the first of 24 weekly fractions was increased significantly (P=<0.01) in comparison with the same total single dose.
b) Reduced significantly (P=<0.01) in comparison with the single dose.

Table VI: Lens responses at approximately 420-470 days following single or fractionated doses of 225 MeV ^{12}C ions (4 cm SOBP) or ^{60}Co gamma radiation. Animals from the same dose groups were re-examined on different days to estimate scoring reproducibility.

Dose (rad)	No. Eyes	Average Score	S.E.	Days at Risk
		^{12}C Single Dose		
40	40	1.45	.09	418
80	32	2.38	.07	418
120	14	3.14	.10	418
120	16	3.06	.06	419
		^{12}C 24 Fractionated Doses		
Dose (rad)	Eyes	Average Score	S.E.	Days at Risk
40	24	1.23	.15	460
40	8	1.75	.16	462
40	21	1.81	.09	472
80	22	2.09	.13	462
80	8	2.75	.16	472
120	32	3.34	.09	472
0	22	0.36	.06	460-472
		^{60}Co Single Dose Radiation		
417	22	3.27	.10	425
		^{60}Co Fractionated Radiation		
417	40	1.28	.07	428

Table VII: Average cataract score for Group IV mice (see Table I) at 13 months after irradiation.

	Dose (rad)	No. Eyes	Average Score		S.E.
XRAY	50	28	1.21	±	.22
	150	34	1.79	±	.12
	300	34	2.76	±	.07
	600	30	4.00 [a]		-
	900	36	I [b]		
CARBON	5	36	0.48	±	.05
	15	36	0.66	±	.05
	30	36	0.65	±	.04
	60	34	0.90	±	.06
	90	26	1.21	±	.10
NEON	5	34	0.53	±	.05
	15	34	0.78	±	.05
	30	36	0.97	±	.09
	60	30	1.32	±	.09
	90	32	1.38	±	.09
ARGON	5	28	0.79	±	.05
	15	28	0.95	±	.05
	30	34	1.62	±	.10
	60	26	2.69	±	.12
	90	26	3.00	±	.10
CONTROLS	0	40	0.16	±	.04

a) One case of 6 mice showed >50 percent opacification and was assigned a score of 4.0; because of corneal clouding caused by anesthesia, observations on an additional 9 mice were difficult, but all probably had >50 percent opification.

b) Corneal clouding precluded meaningful examination.

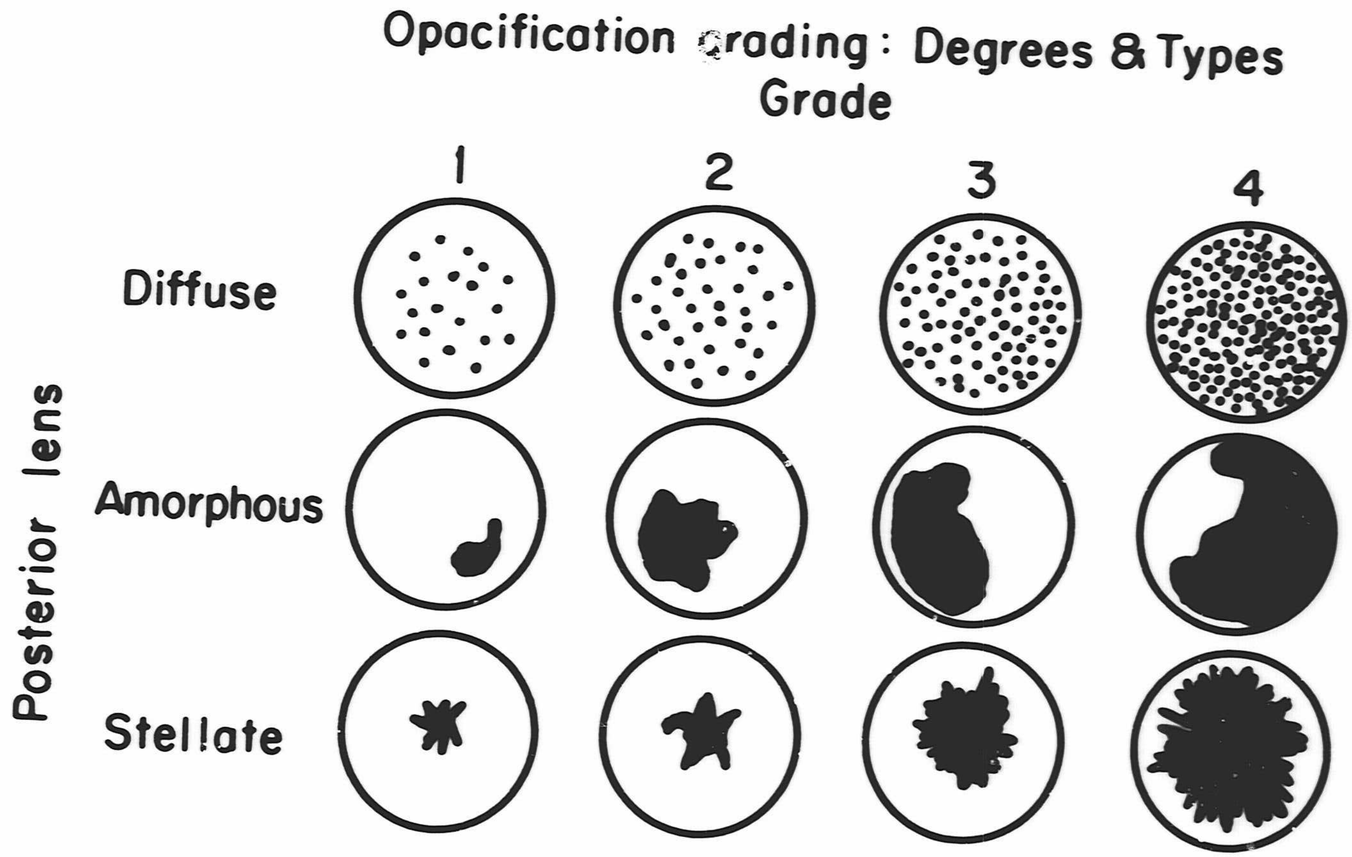


Figure 1. Artistic representation of the types of lens opacities observed and the grading system that has been developed to describe the degree of opacification.

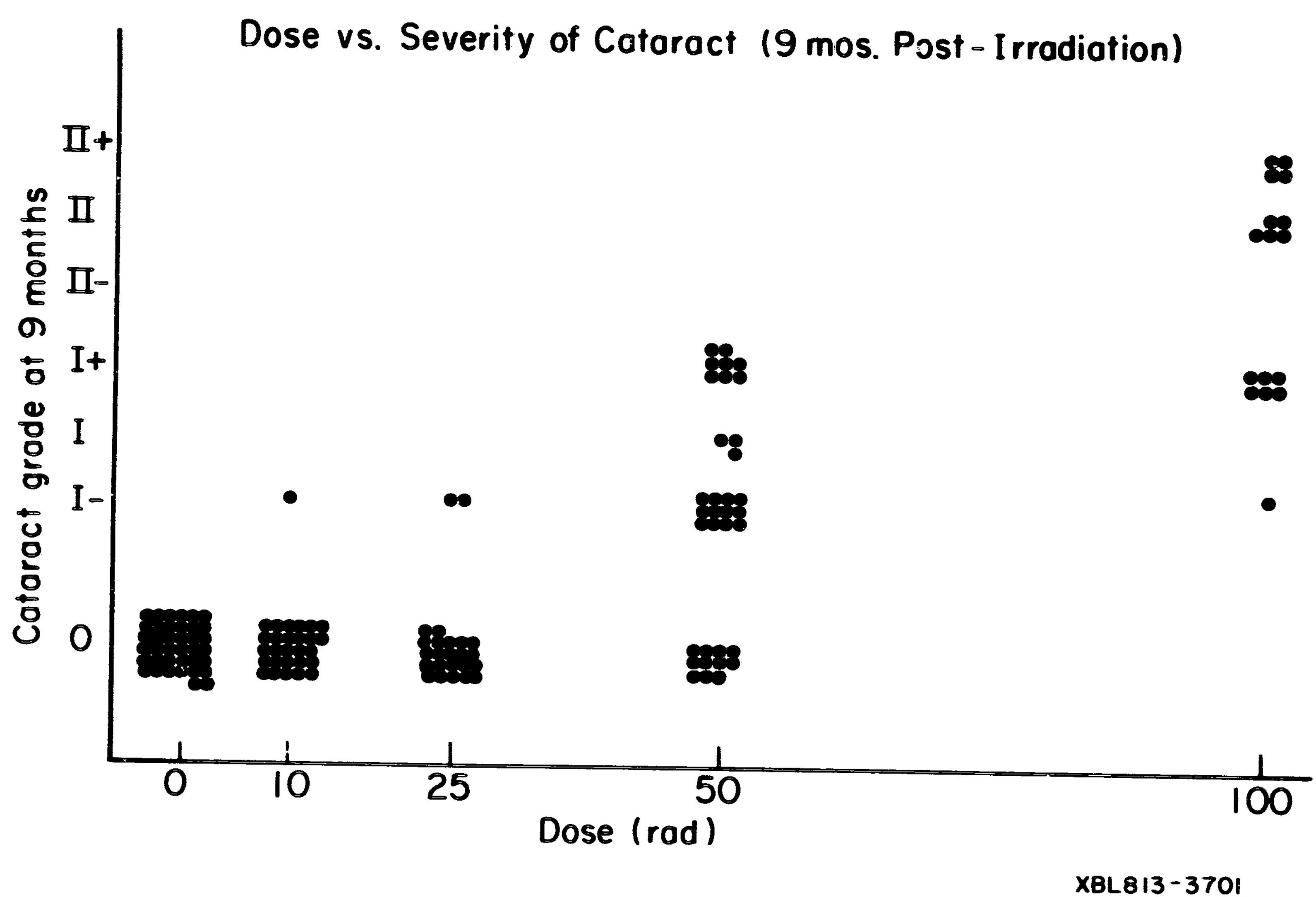


Figure 2. Severity of cataracts at nine months in LAF mice irradiated with spread Bragg peak argon ions (Experiment II). Each dot indicates one animal.

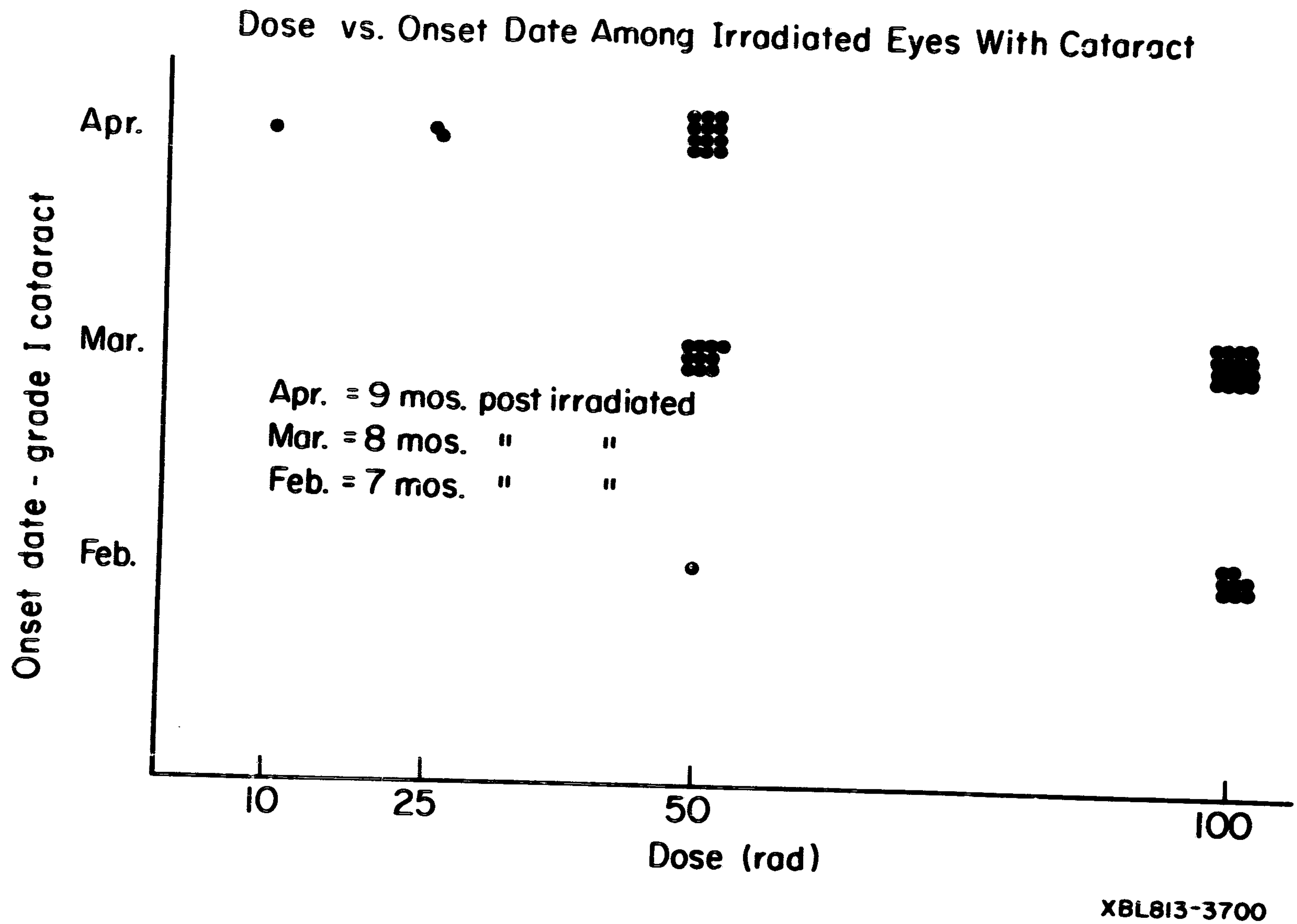


Figure 3. Onset date of a grade 1 opacification in mice irradiated with spread Bragg peak argon ions. Each dot represents one animal in Experiment II.

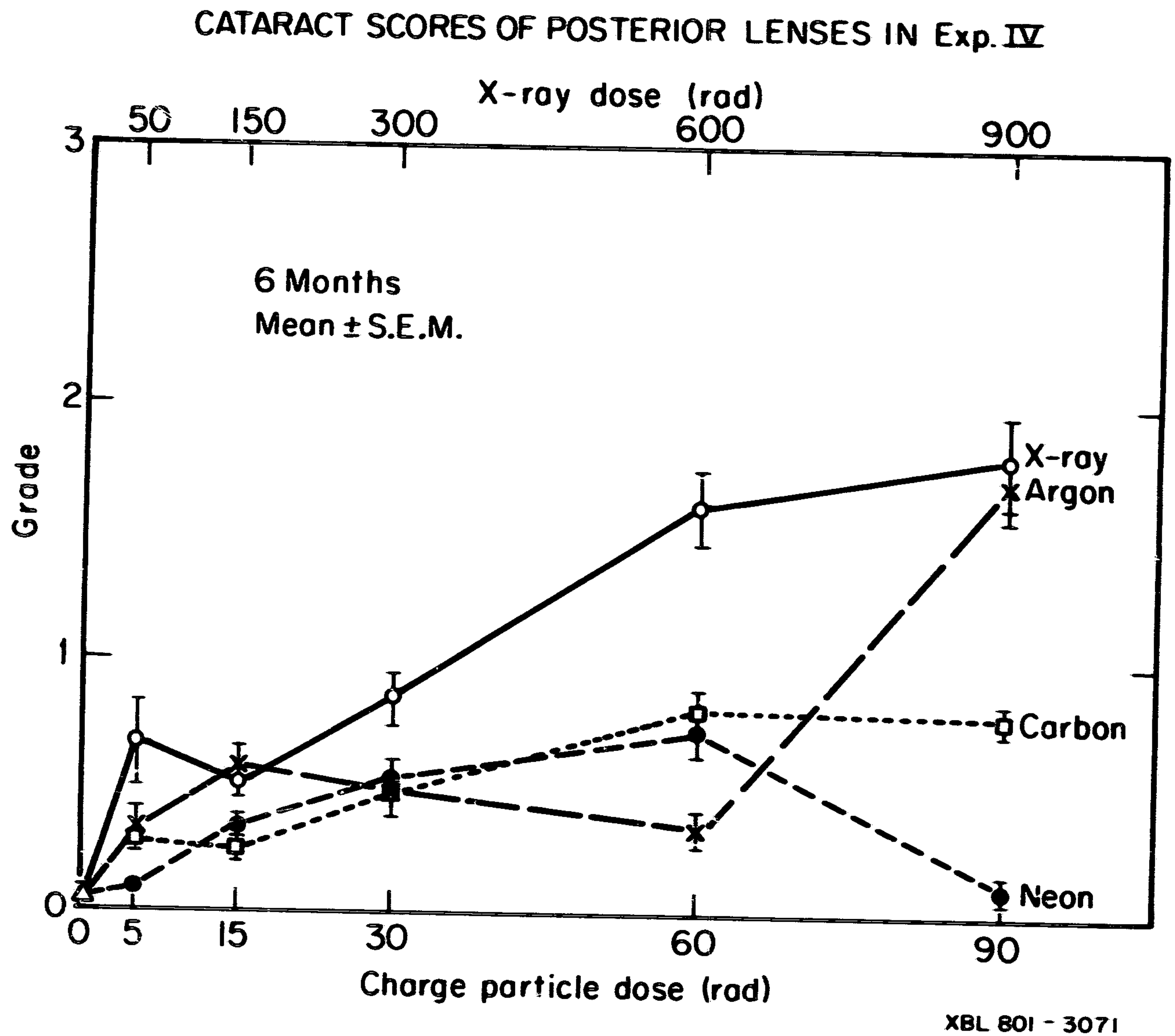


Figure 4. Average severity of posterior lens cataracts in mice six months after irradiation with plateau argon, neon or carbon ions. The bars indicate the standard errors of the mean. The average score among unirradiated controls was nil.

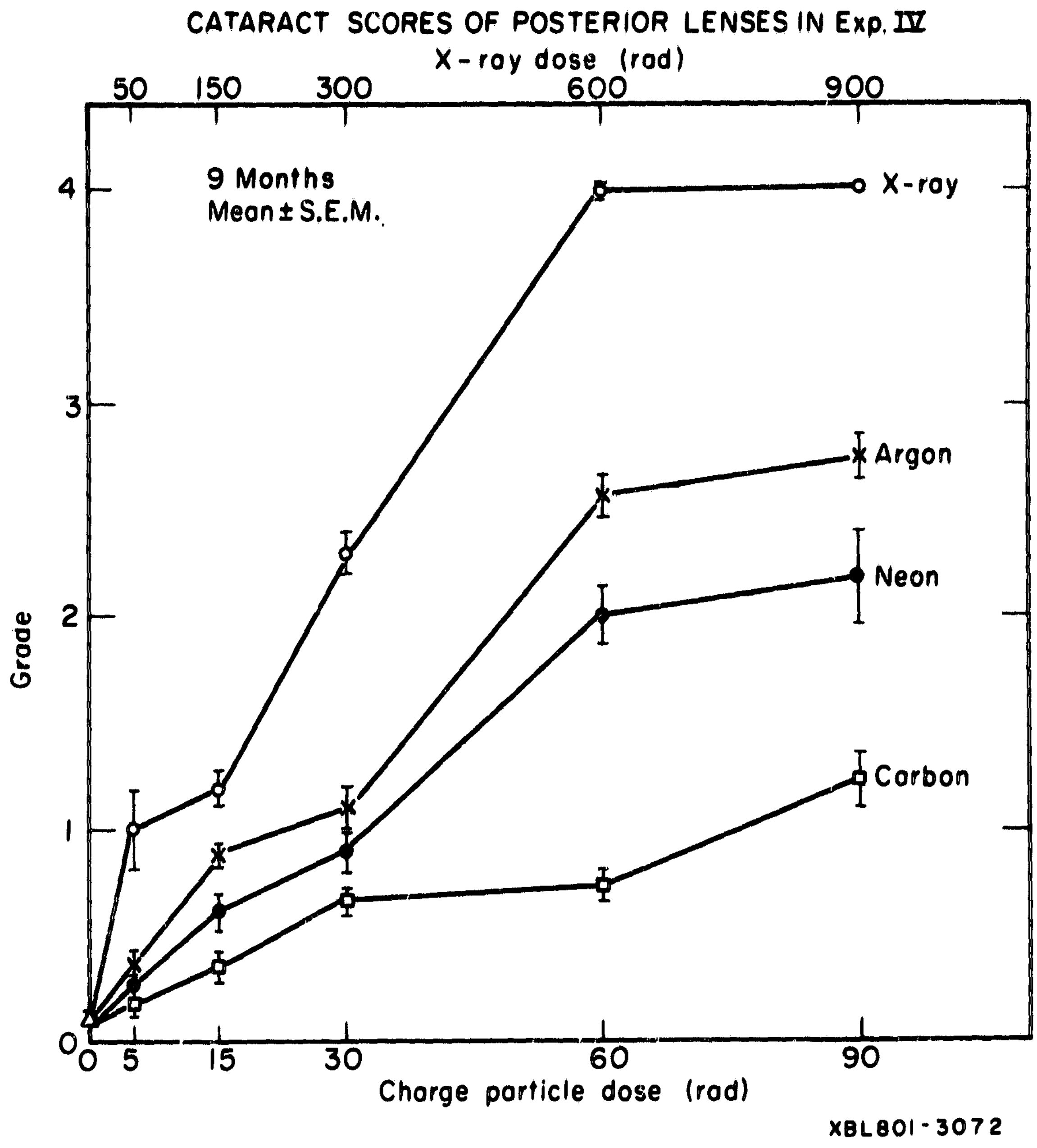


Figure 5. Average severity of posterior lens cataracts in mice nine months after irradiation with plateau argon, neon or carbon ions. The bars indicate the standard errors of the mean. The average score among controls was nil.

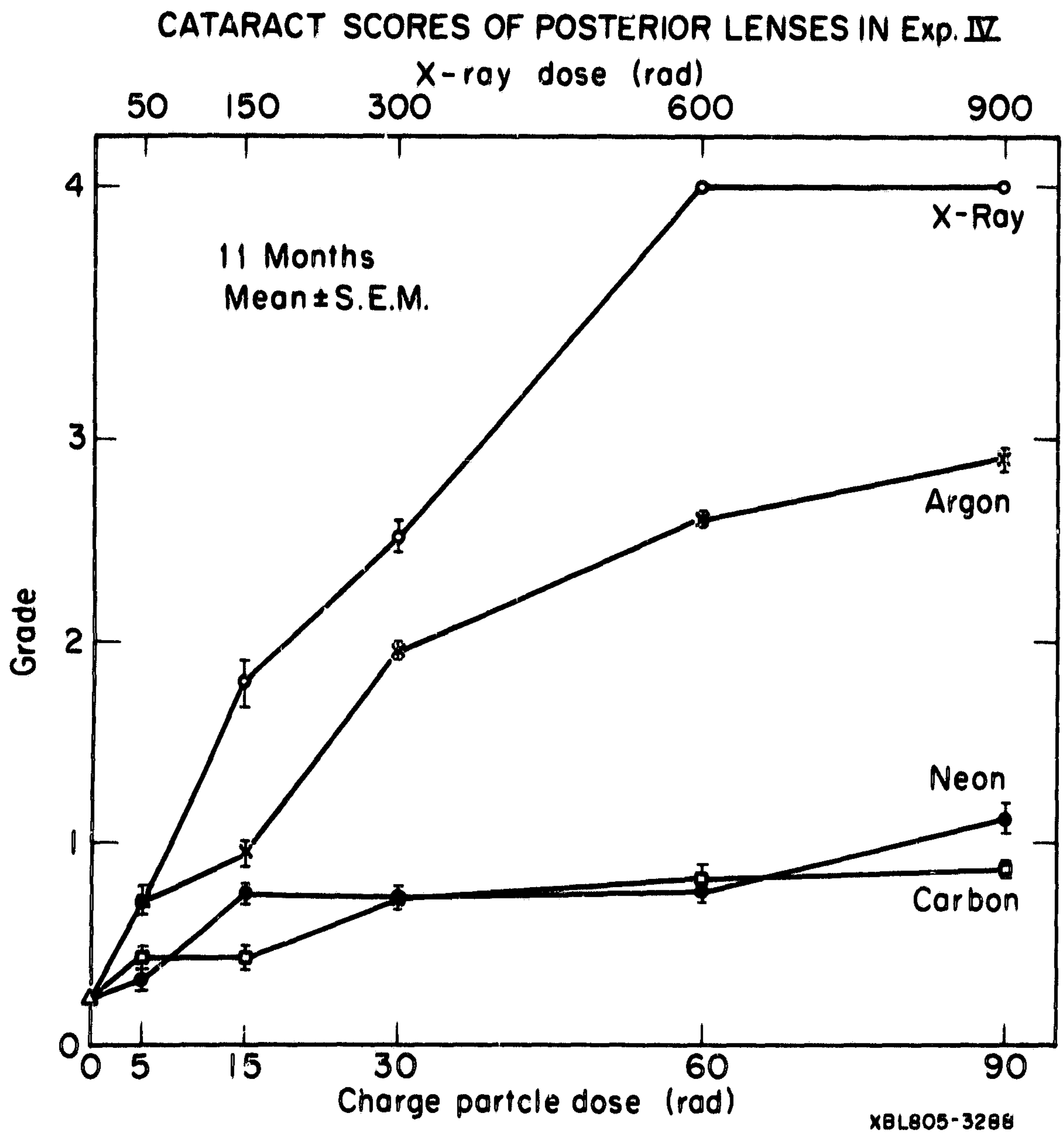


Figure 6. Average severity of posterior lens cataracts induced in mice eleven months after irradiation with plateau argon, neon or carbon ions. The bars indicate the standard errors of the mean. The average score among controls was nil.

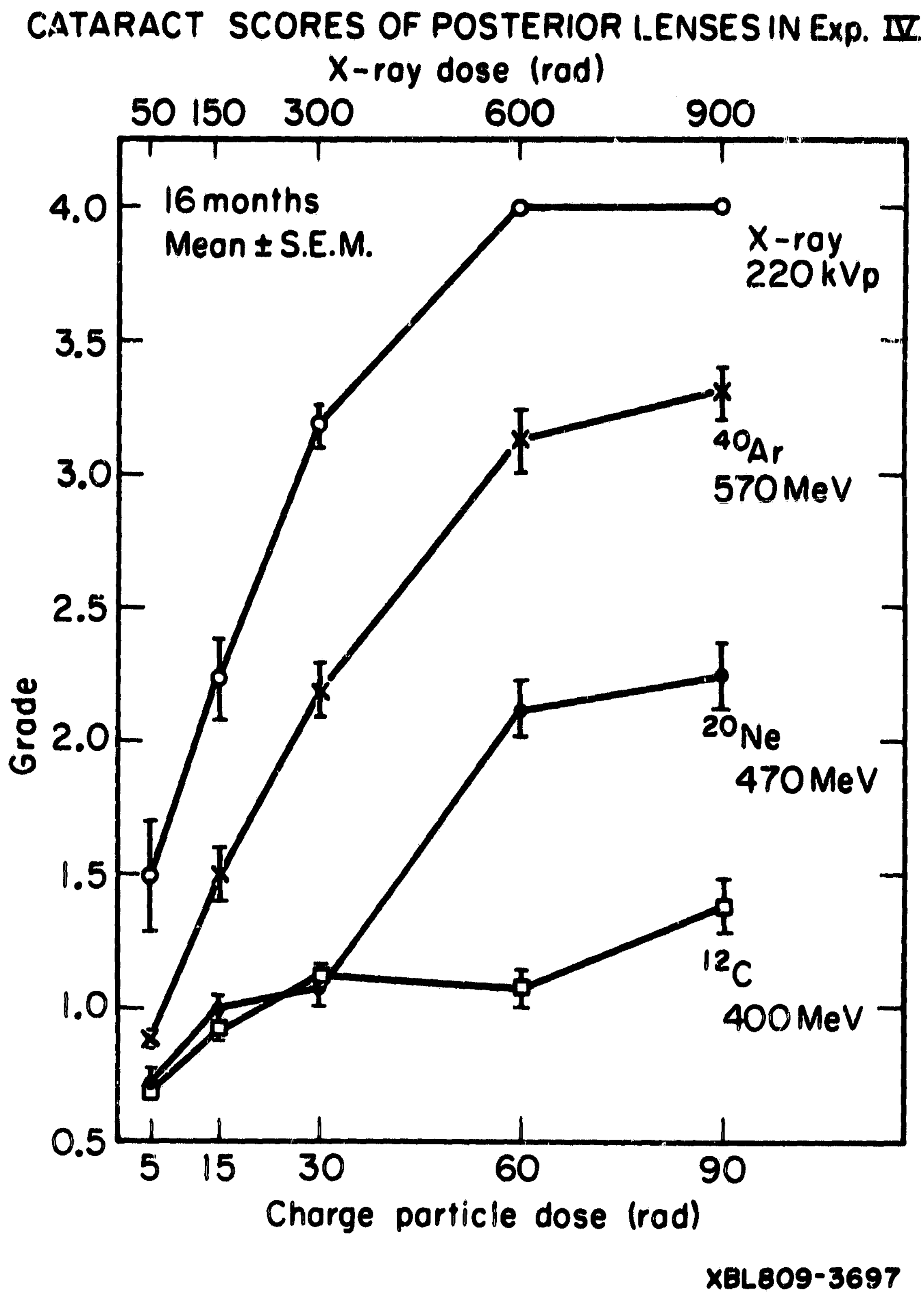
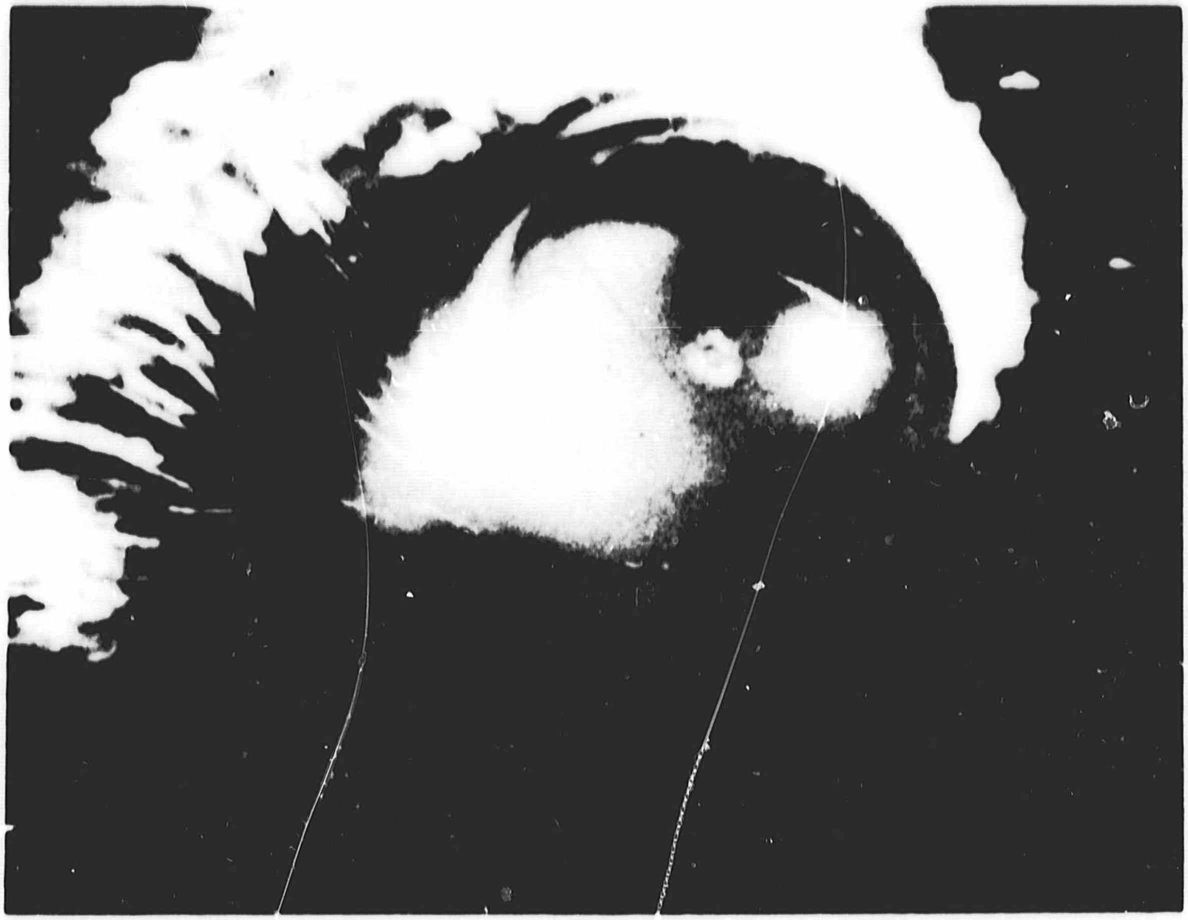


Figure 7. Average severity of posterior lens cataracts induced in mice sixteen months after irradiation with plateau argon, neon or carbon ions. The bars indicate the standard errors of the mean. The average score among unirradiated controls was 0.46 ± 0.07.



XBB 815-4296

Figure 8. Slit-lamp photograph of the lens of a mouse that had been irradiated with 90 rad of 570 MeV ^{40}Ar 12 months earlier. Grade 3 stellate opacity.

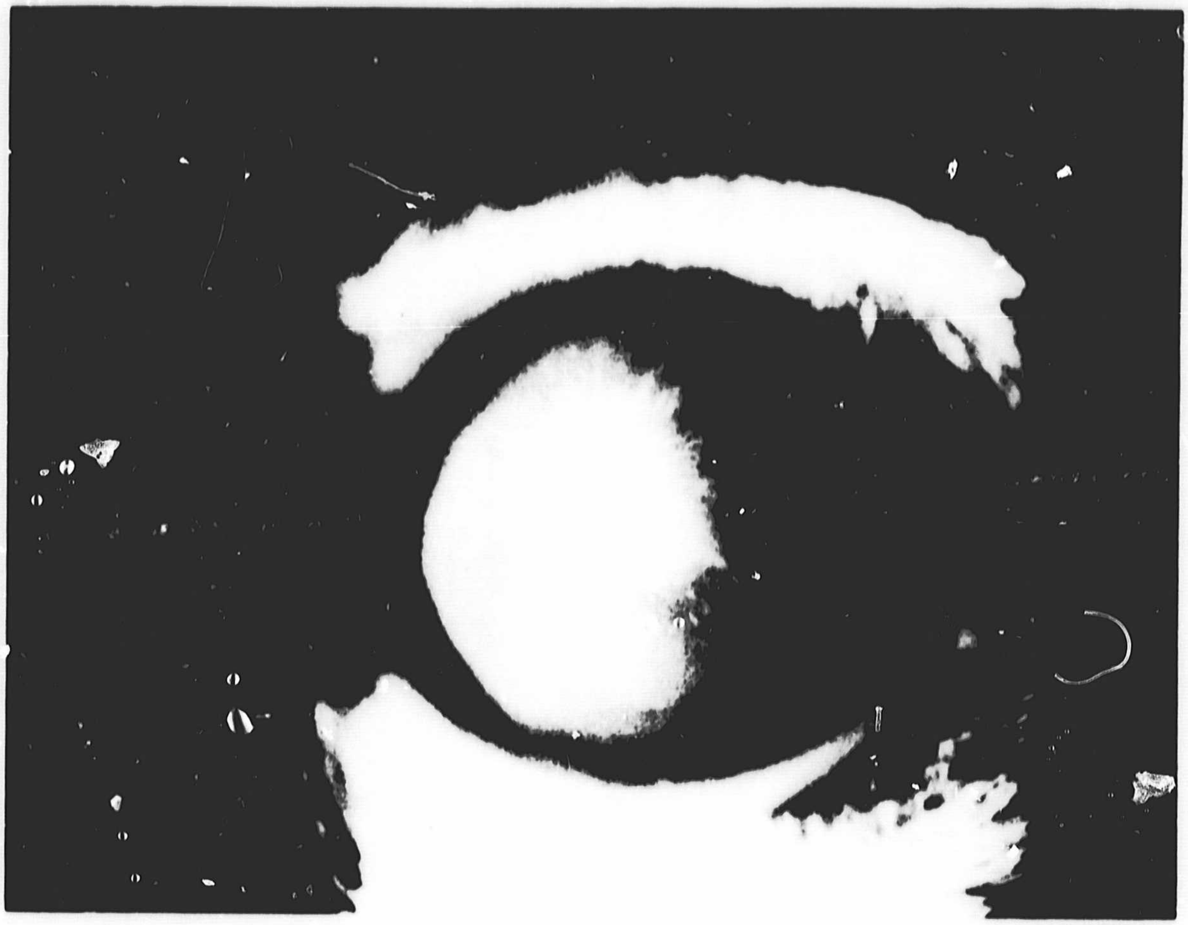


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Figure 9. Slit-lamp photograph of the typical "button" observed in mice irradiated with HZE particles.

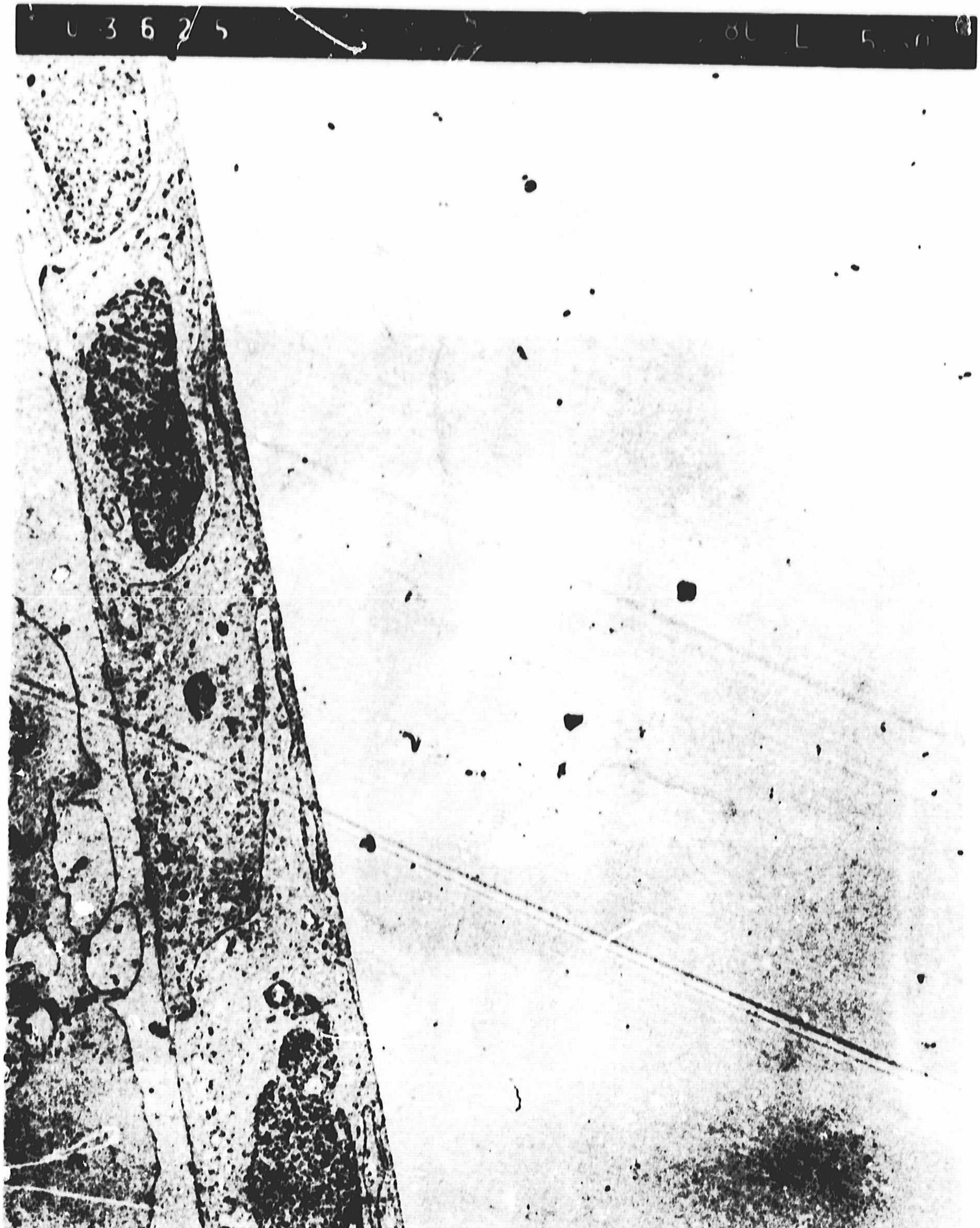

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Figure 10a. Electron micrograph of a region of the lens just anterior to the bow in an animal that had been irradiated two years earlier with 100 rad of 570 MeV ^{40}Ar (5,500x).

XBB 8010-11766

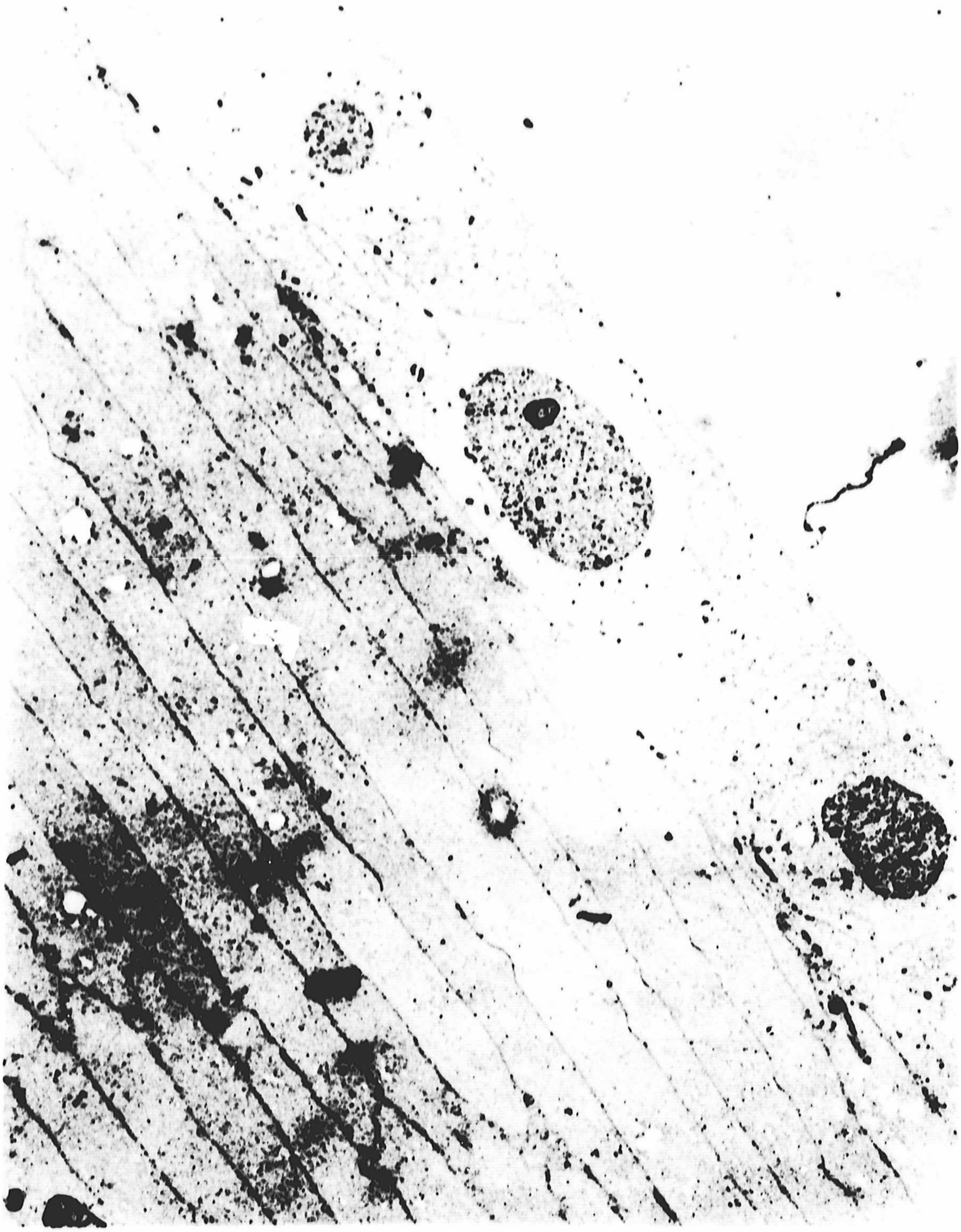
Figure 10b. Same lens as in Fig. 10a in an adjacent region. Note the disruption of the cortical fibers (5,500x).

XBB 815-4295

Figure 10c. Electron micrograph of a region of the lens just anterior to the bow in an animal that had been irradiated two years earlier with 25 rad of 570 MeV ^{40}Ar (4,400x).

XBB 809-10476

Figure 10d. Region of the lens adjacent to that in Fig. 10c (4,400x).



XBB 809-10475

Figure 10e. Electronmicrograph of the lens from a control (unirradiated mouse). Note the regularity of the cortical fibers (4,400x).