Title

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Publication Date

1981-06-01

LBL-12848
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

To be submitted for publication

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June 1981

Donner

Biology & Medicine Division

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

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LBL 12848

A Morphological Study on the Cataractogenic Effects of Heavy Charged Particles[1]

by

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[1] This work was supported by the Office of Health and Environmental Research, Divison of the U.S. Department of Energy under contract No. W-7405-ENG-48 and the National Aeronautics and Space Agency under contract No. T35516G.

ACKNOWLEDGEMENT

We sincerely thank Drs. J. Jose and M. Barker for their valuable suggestions and important contributions to training the technical staff. We also wish to thank Mr. J. Lieber and Mrs. Joan Lawson for their excellent technical assistance. The support and encouragement of Dr. E. L. Alpen and Ms. Margaret White are also gratefully acknowledged.

This work was supported by the Office of Health and Environmental Research, Division of the U.S. Department of Energy under Contract No. W-7405-ENG-48 and the National Aeronautics and Space Agency under Contract No. T35516G.

ABSTRACT

The morphological changes of the mouse lens at 15 months after heavy charged particles and x-rays irradiations are described. Radiation treatment, administered to the heads or upper bodies of 4 month old mice, includes 0.05, 0.3, and 0.9 Gy of plateau ^{40}Ar and ^{20}Ne particles, 0.1, 3.2 Gy of stopping ^{12}C particles, and 0.5, 1.5, and 3.0 of 220 KVp x-rays. The changes in other additional groups of mice were evaluated at 4 months after exposed to 0.05, 0.3, and 0.9 Gy of plateau ^{20}Ne particles and at 30 months after exposed to 0.1 and 1.0 Gy of ^{40}Ar stopping particles. A morphological comparison is made between the effect of the similar doses of nonstopping (plateau) and stopping ^{40}Ar particles at 24 months after irradiation. Morphologic differences between cataracts in irradiated and age-matched controls are also described.

At the age that corresponded with 4 months after irradiation, control mice showed little evidence of lens fiber degeneration. At 15 months, lens fibers showed a swollen cytoplasm only in the superficial layers of both the anterior and the posterior regons. At 30 months, degenerative changes in lens fibers appeared at the deep region of the cortex. In the irradiated animals, fragmentation of epithelial nuclei in the bow region was seen at 4 months after plateau ^{20}Ne ions exposure and persisted to 15 months. The lens fibers degeneration was found distinctly at 15 months after exposed to both charged particles and x-rays. These changes included permanent disruption of the lens bow. The cortex was abnormal, as evidence by swollen cells and degenerated nuclei and lysosomal-like structures. These degenerative

changes became progressively more severe at later times (24-30 months) after irradiation. At 24 months after exposure to ^{40}Ar particles, mice exposed in the plateau portion of the Bragg curve (LET $\approx$ 100 Kev/μm) showed more severe lens fiber damage than mice exposed to stopping particles (LET $\approx$ 500-700 KeV/μm). In general, the results indicate that: 1) the degree of degenerative changes in lens fibers increase with increasing radiation dose and sampling time; 2) there were no qualitative differences in damage produced by heavy charged particles and x-rays at 15 months after irradiation; however, the heavy charged particles were considerably more effective than x-rays for inducing morphological changes; 3) radiation-induced injury in the lens fibers showed an LET-dependence when the ionizing density was less than 100 KeV/μm; 4) cataracts produced by heavy charged particles differed in certain morphological features from spontaneous cataracts.

INTRODUCTION

The prospect of manned space mission of extended duration poses a number of problems (1). One is the hazard that might occur due to high energy heavy ion particles (HZE) to which personnel in space will be exposed in a complex radiation environment. The effects of space radiation exposure on the lens epithelial cells of the eye may be of particular concern since this tissue appears to sustain a greater degree of irreparable radiation damage than any other superficial tissues. Although there is a rather extensive body of information on cataractogenesis following low linear energy transfer (LET) radiation (2-8) and high-LET neutron radiation (9-15), only limited data are available on such effects on the lens after exposure to HZE particles (16). No previous morphologic comparison has involved x-rays and HZE particles characterized by different (dose averaged) LETs where lens changes were the endpoint.

The BEVALAC, a heavy ion linear accelerator at the Lawrence Berkeley Laboratory (LBL), is a unique facility that can provide the "man made" cosmic rays. A study at LBL has been designed to explore the LET-dependence of the cataractogenic produced by HZE particles from the BEVALAC (17). This report emphasizes the morphological changes in the crystalline lens of the CB_6F_1 mouse after HZE particles irradiation. The purpose of this study is to determine if the radiation damage produced by HZE particles and x-rays is similar, and to assess any morphological differences between radiation-induced and spontaneous (age associated) cataracts.

MATERIALS AND METHODS

Female CB_6F_1 mice, 4 months old, were given head or upper body exposures to heavy charged particles or x-radiation according to standard procedures used in connection with slit-lamp studies of cataractogenesis (17). Some animals which were available for this study came from experiments on Harderian gland carcinogenesis after exposure to heavy charged particles irradiation (18). Doses, radiation parameters and sampling times are shown in Table 1. Before the animals were sacrificed for the morphological study, the slit-lamp observations for assessment of cataracts were performed and the results have been reported elsewhere (17).

Animals from each dose group and each age-matched (unirradiated) control group were sacrificed at each sampling time. The animals were anesthetized with 0.1 ml nembutal (sodium pentobarbitol). For each animal, the thorax was opened and perfused with the fixative containing 1 percent glutaraldehyde, 3 percent paraformaldehyde and 0.05 percent 1,5-difluro, 2,4-dinitrobenzene through the left ventricle. The eyes were then carefully dissected and placed in 2 percetnt glutaraldehyde in 0.075 M phosphate buffer at 37°C. After 10-15 minutes, the posterior globe was cut off and the eyes were held in the fixative for another 15 minutes at 37°C. The lens and cornea were then separated from the eye and placed in the fixative overnight at 4°C. They were then washed in buffer and placed in cold 2 percent OsO_4 for 1 hour, dehydrated and embedded in the araldite 502. Half micron sections were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss 10A electron microscope.

RESULTS AND DISCUSSIONS

A control mouse lens at 4 months into the experiment showed an intact bow region and well-arranged anterior fibers (Fig. 1A and 1B). After ^{20}Ne exposure, the only abnormality found in the lens was the presence of micro-nuclei (fragmentation of nucleus) in the epithelial cells at the bow region. The production of micro-nuclei in the epithelial cells in the lens has been seen previously as a response to low LET radiation (7). It has been suggested that damage affecting the cell nuclei in bow epithelial cells appeared to initiate the series of pathological events which finally lead to cataractous lesions. Apparently, the lens epithelium is the most sensitive site to charged particles as well as to the other types of radiations. At 4 months after 0.3 or 0.9 Gy ^{20}Ne radiation, the lens fibers at the posterior region seemed to be relatively normal in comparison to the fibers at the anterior region. Some anterior fibers showed a typically swollen cytoplasm containing amphorous materials. Unfortunately, no x-irradiated mice were available so that a direct comparison of anterior-posterior lens changes could be made. Worgul (19) reported that transparency changes were observed during the first few days in the anterior region of the lens after 3.5 Gy of (plateau) ^{40}Ar particles. Soon thereafter the posterior region became involved and lens fiber changes progressed at a greater rate. At doses lower than 0.5 Gy, however, Worgul found the onset of anteior and posterior opacities was approximately the same, and cataracts progressed until 1 year post-irradiation. These results are in contrast to low LET

radiation where cataracts appear first in the posterior subcapsular area. The reason for this difference is not understood currently and should be investigated because both Worgul's results and our findings at 4 months are consistent with the hypothesis that early damage manifestations may not be the same for photons and some high LET heavy charged particles.

At 15 months after exposure to 0.05 or 0.3 Gy ^{20}Ne, the morphology of lens fibers was quite similar to the control group. After 0.9 Gy ^{20}Ne, the nuclei of the bow showed striking abnormalities. At this dose, both the anterior and the posterior lens fibers were irregular in shape and size. Degenerative changes were even more severe in the animals exposed to ^{40}Ar particles. The lens fibers at the bow region showed slight disruption after only 0.05 Gy (Fig. 2). Merrium et al. (20), also reported that initial changes in the lens fibers at the low doses (0.01-0.07 Gy) of ^{40}Ar particles did not differ significantly from those in the control animals. However, degenerative changes progressed significantly unitl one year after irradiation. We also found that after a 0.3 Gy ^{40}Ar exposure, the fibers in the bow and anterior superficial regions contained areas of abnormal swelling and vacuolation. Degenerative changes in the anterior fibers that received 0.9 Gy of ^{40}Ar appeared deeper in the mid-cortical regions than those of the ^{20}Ne irradiation mice. The lens fibers degeneration showed distinctly at both anterior and posterior regions in the x-rays treated groups after 1.5 Gy exposure and the degeneration became more severe after exposed to 3.0 Gy. After

both 0.9 Gy of ^{40}Ar particles and 3.0 Gy of x-rays, irradiation, swellings of epithelium at the anterior pole were developed (Fig. 3A,B). Abnormal nuclei were also seen in the posterior fibers after both radiations. We also found that a much higher x-ray dose (3.0 Gy), in comparison to the low dose of plateau ^{40}Ar particles exposure (0.05-0.3 Gy) was required to induce degenerative changes in the bow region. The breakdown of the bow is thought to be due to the abnormal migration and differentiation of cells which were in the germinative zone at the time of irradiation (21). In general, there was no striking qualitative morphological differences between animals at 15 months after exposed to either plateau (non-stopping) heavy charged particles or x-rays. After 3.2 Gy of stopping ^{12}C particles irradiation, the damage of lens fibers became more severe. These degenerative changes became progressively more prominent at the later stage (24-30 mos.) after irradiation. At 30 months the degenerative changes of lens fibers appeared at the deep region of the cortex in the control animals (Fig. 4). Significant morphological differences were observed in the lenses of irradiated animals and unirradiated animals. Although the average cataract score evaluated by slit-lamp was similar in some animals, the morphological features, however, were quite different. In other words, HZE induced cataracts were different in certain morphological featurs from spontaneous cataracts. HZE particles have the potential for producing thermophysical lesions or "tunnel lesions," which the other low-LET radiations do not produce (22). The role of these lesions in the producton of abnormal fibers by surviving epithelial cells is unknown.

The morphological differences between stopping and nonstopping ^{40}Ar particles were compared at 24 months after irradiations. At lower doses (< 0.5 Gy) there was no apparent difference in the morphological changes for either type of radiation. After higher dose, however, the nonstopping ^{40}Ar particles (LET $\approx$ 100 KeV/μm) were more damaging to the lens fibers structure than were the stopping particles (LET $\approx$ 600 keV/μm) (Fig. 5, 6). From other studies (25), RBE of ^{40}Ar particles for killing intestinal crypt cells was maximum in the plateau portion of the Bragg curve where the LET was ~ 100 KeV/μm and declined with increasing LET in 4-cm spread Bragg peaks.

In summary, four types of changes were involved after heavy charged particles and x-rays: (1) abnormal bow patterns; (b) posterior and anterior migration (or proliferation) of nucleated cells in the cortex; (c) swelling of fibers and development of amorphous areas, especially at the poles; and (d) development of swores of epithelium at the anterior pole, in mice that received either 0.9 Gy of Ar particles or 3.0 Gy of x-rays. It seems that the magnitude of injury is related to dose, and to LET of particles when LET is less than 100 keV/μm. These morphological results are consistent with RBE results based on slit-lamp observations where opacification produced by plateau ^{40}Ar ions was found to be greater than for plateau ^{12}C or ^{20}Ne ions or low-LET x-rays. Although there is considerable morphological similarity in the cataracts produced by low-LET and high-LET heavy charged particles, it is important to examine the histology of lenses of animals early after irradiation in order to determine what the

initial site of radiation damage is for these high and low LET radiations. An early morphological evaluation also provides a means to predict whether cataracts are likely to be induced by a given radiation dose and thus could provide a faster and more accurate means to assess potential radiation risks for personnel in space.

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FIGURE CAPTIONS

Figure 1. Light micrographs of a control lens from 8 month old mouse (4 month into the experiment) showing: (a) intact bow region, X150; (b) well-aligned anterior fibers, X600.

Figure 2. Light micrograph of an anterior region of the lens in an animal at 15 months after 0.05 Gy of plateau ^{40}Ar particles. Swollen fibers in the subcortical region are noted. X 0.

Figure 3. (a) Light micrograph of a swollen epithelium of the anterior pole in a lens at 15 months after 0.9 Gy of plateau ^{40}Ar. Numerous nuclei were accumulated in the epithelial cell. Degenerated areas with pale staining were noted in several places. X350. (b) at ultrastructural level, the degenerated area containing the lipid body (L) and debirs (D), X5000.

Figure 4. Light micrograph of a control lens from an animal at 30 months into the experiment indicating the swollen fibers are in the deeper region of the cortex than those found in the younger animals. X150.

Figure 5. Electron micrograph of degenerated fibers in the anterior region from an animal at 24 months after 0.9 Gy plateau ^{40}Ar particles. The fibers were completely distored and replaced by amphorous debris (D), Lysosomal-like bodies (L) and electron dense globular structures (arrow). Mylein figures (M). X5000.

Figure 6. Electron micrograph of lens fibers in the region just anterior to the bow in an animal at 24 months after 1.0 Gy stopping ^{40}Ar particles irradiation. Partially degenerated fibers with large vacuolus (V) and lysomal like bodies (L) are noted. X3200.

Table I. Radiation dose, parameters and sampling time.

No. of Lenses Examined	Radiation Parameters	Dose (Gy)	Sampling Time (months)
6(3) (a)	570 MeV-plateau ^{40}Ar	0.05	15,24
6(3)		0.3	15,24
6(3)		0.9	15,24
6(3)	570 MeV-SOBP ^{40}Ar (b)	0.1	24
6(3)		0.25	24
6(3)		1.0	24
4(2)	570 MeV-SOBP ^{40}Ar (b)	0.1	30
2(1)		1.0	30
6(3)	470 MeV-plateau ^{20}Ne	0.05	4,15
6(3)		0.3	4,15
6(3)		0.9	4,15
6(3)	400 MeV-SOBP ^{12}C (c)	0.1	15
6(3)		3.2	15
6(3)	220 kVp x-rays	0.5	15
6(3)		1.5	15
6(3)		3.0	15
6(3)	Control	0	4
6(3)			15
6(3)			30

(a) number of animals from each treatment group were examined at each smpling time.

(b) SOBP-animals were irradiated at the distal end of a 4-cm spread Bragg peak (stopping particles).

(c) SOBP-animals were irradiated at the distal end of a 10-cm spread Bragg peak.

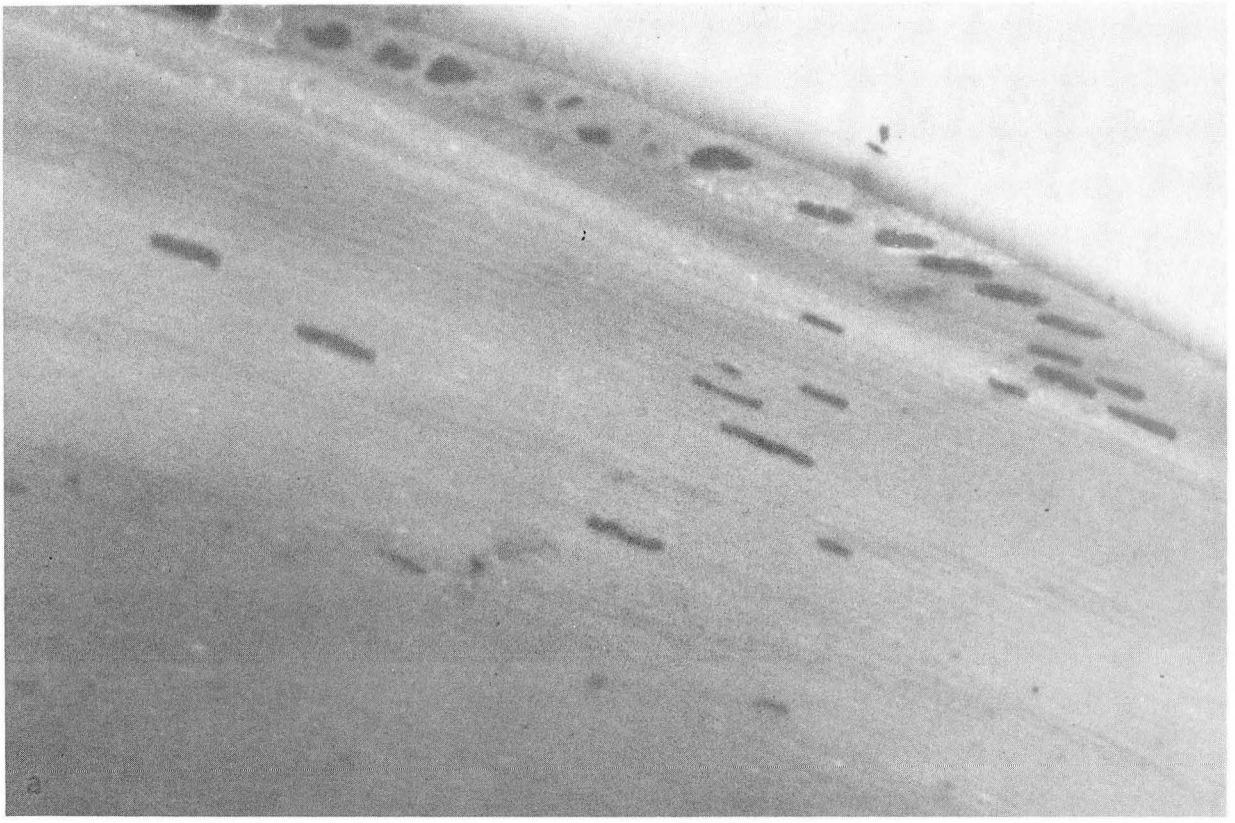

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Figure 1a

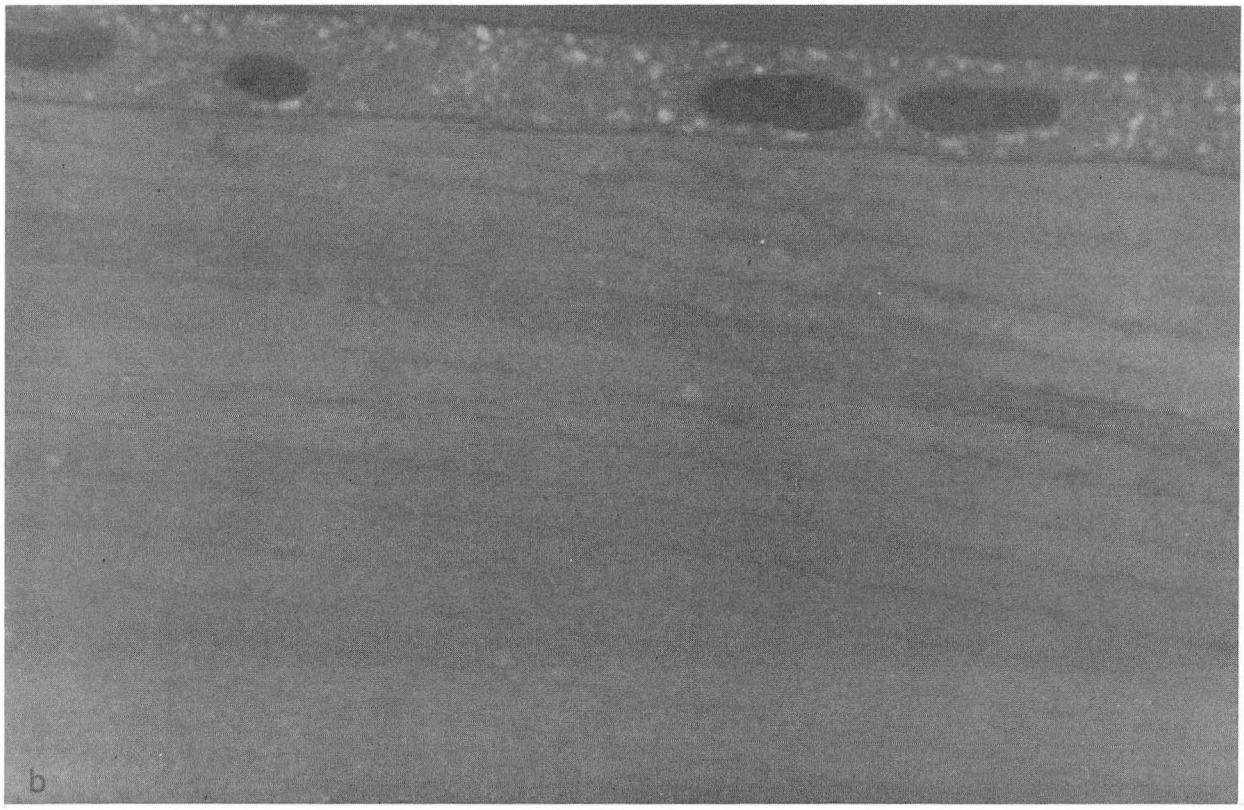

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Figure 1b

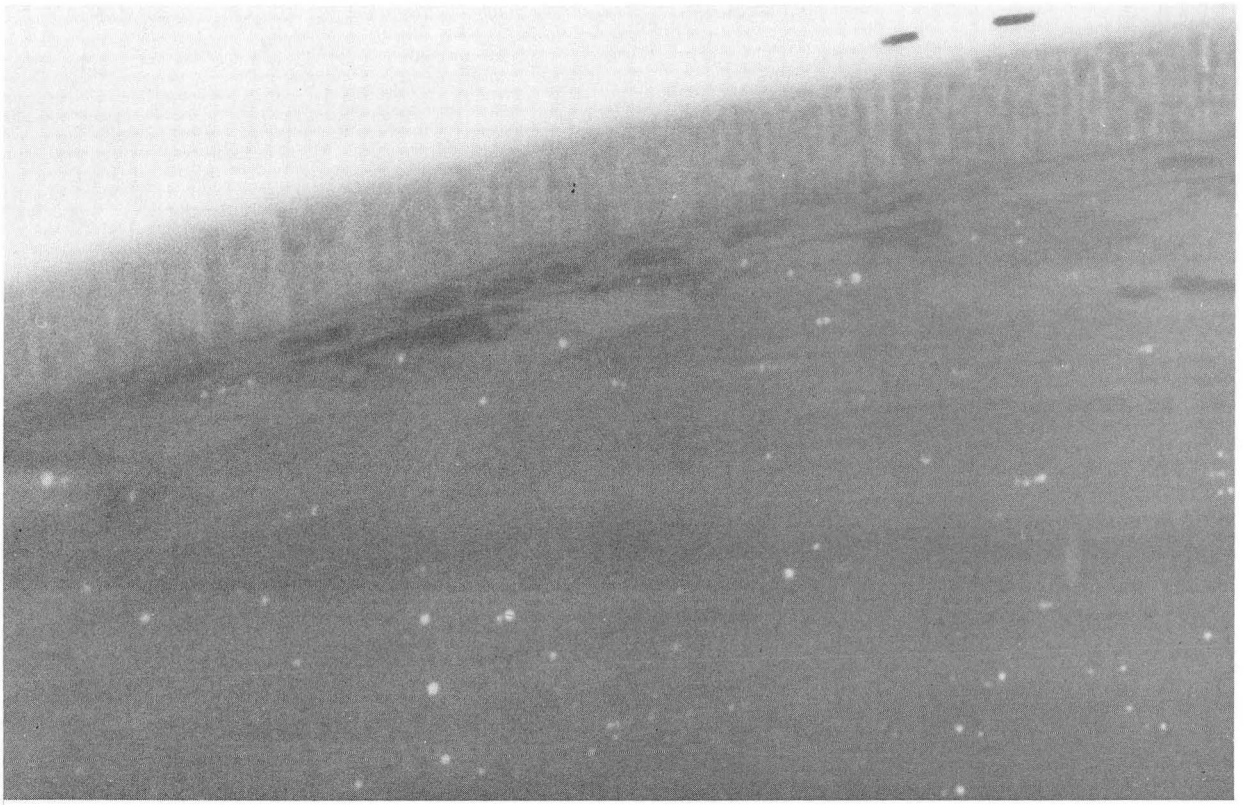

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Figure 2

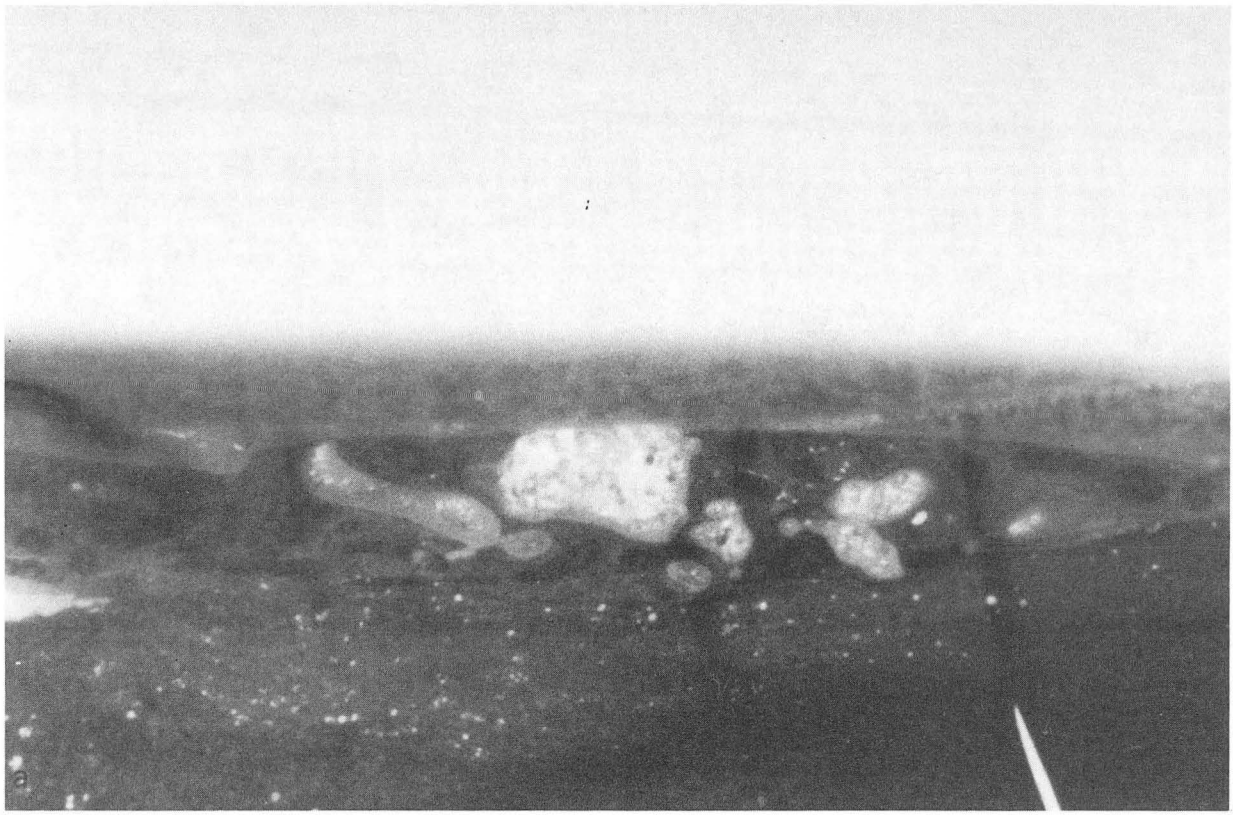

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Figure 3a

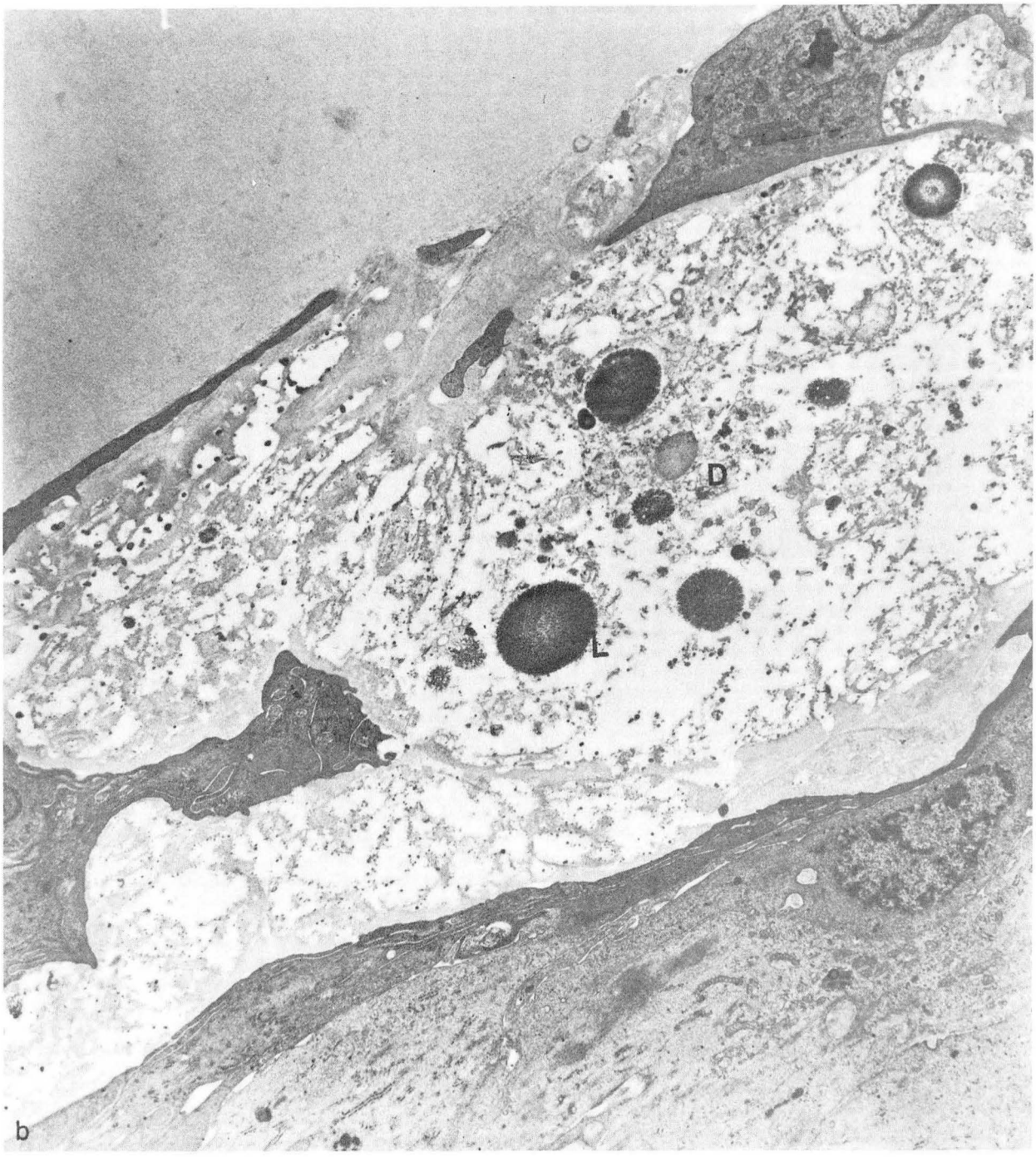

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Figure 3b

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Figure 4

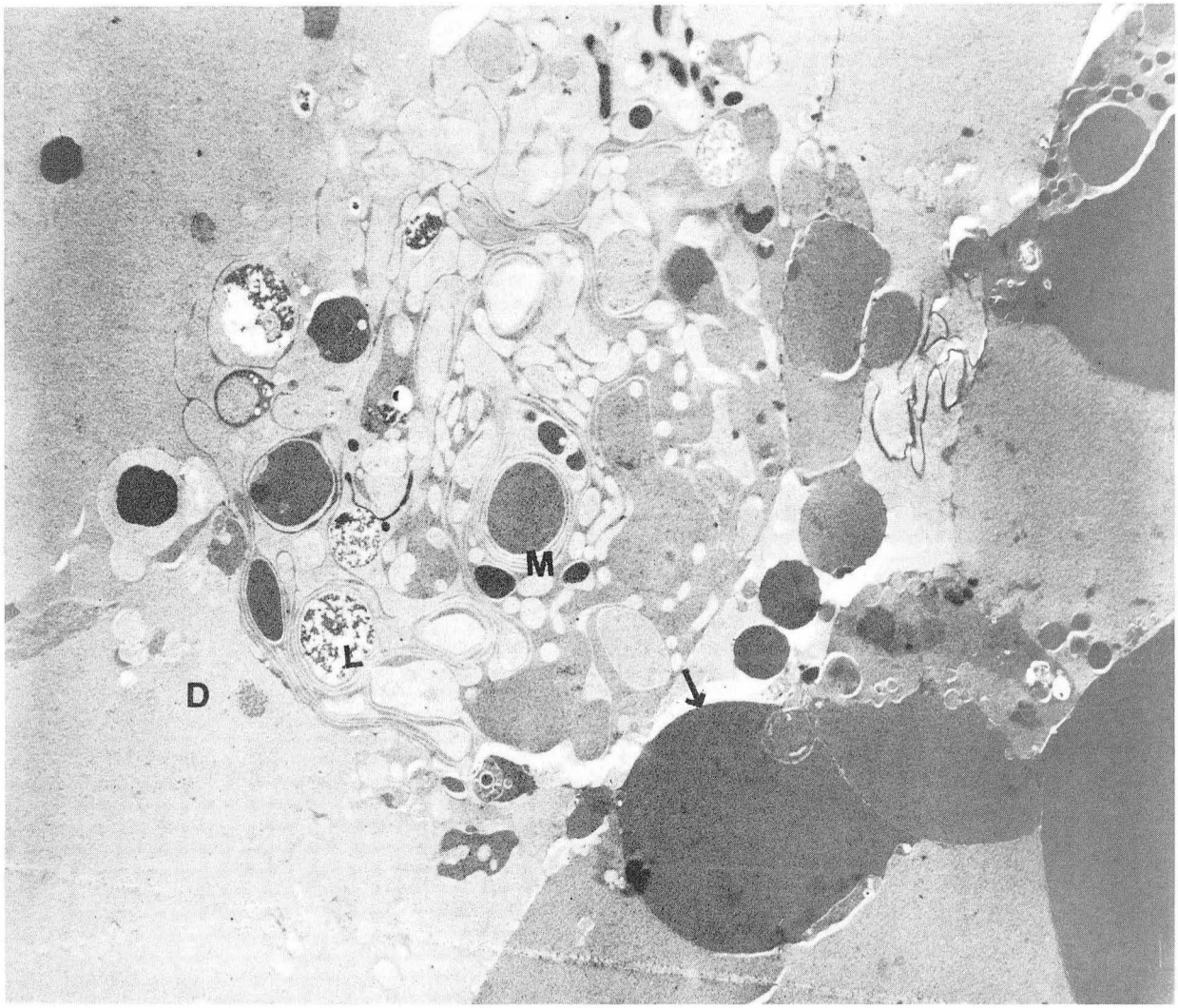

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Figure 5

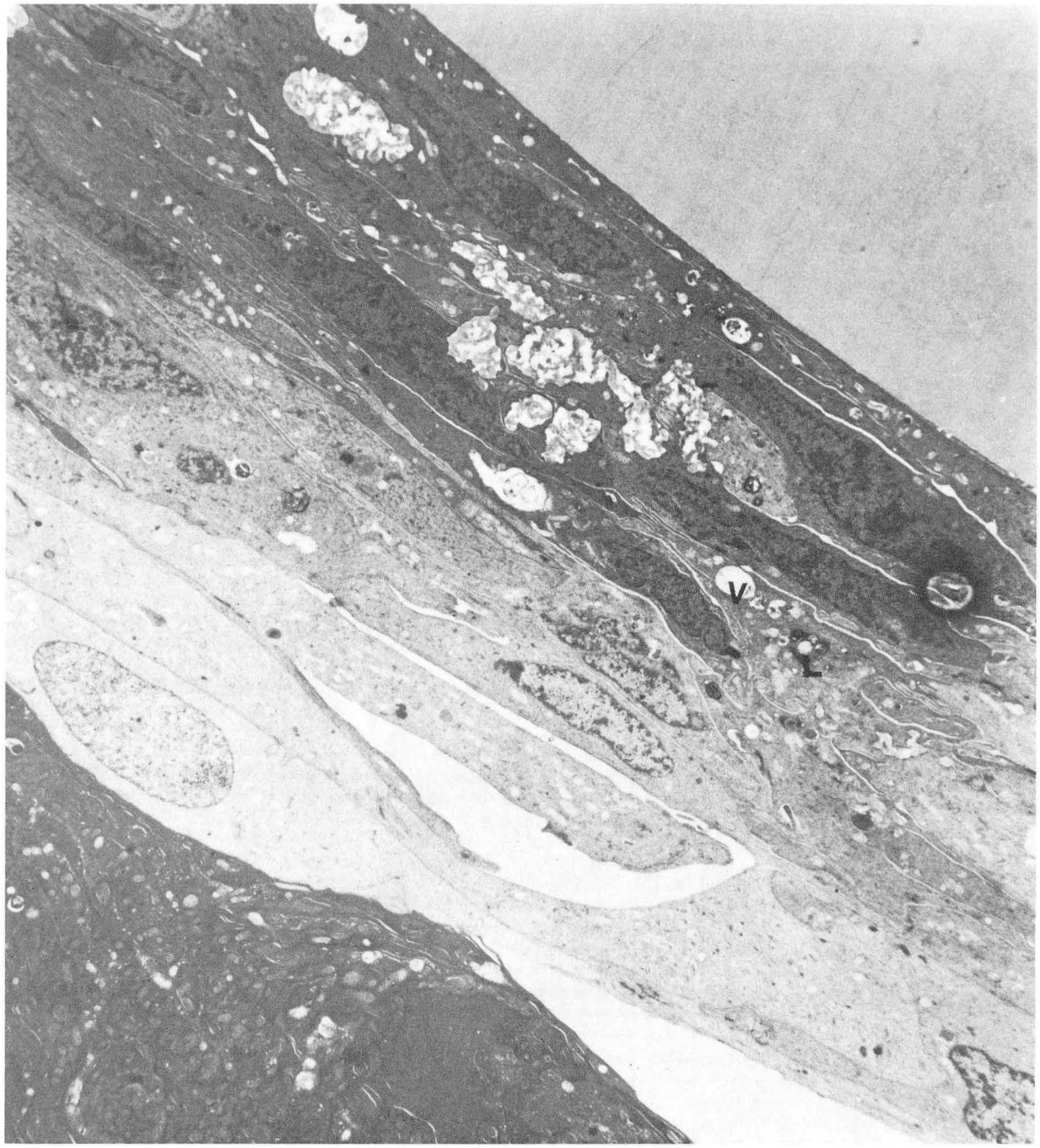

Figure 6

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