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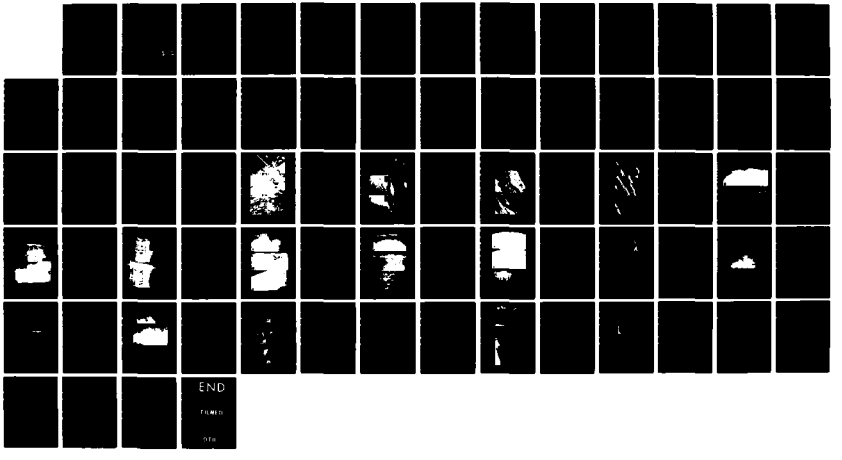

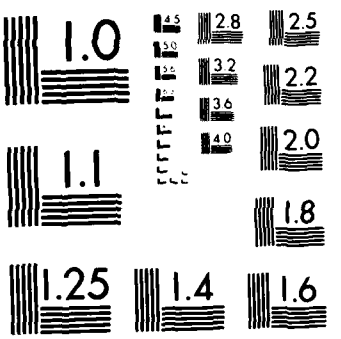

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MICROWAVE-INDUCED CATARACTS OF THE EYE LENS: STRATEGIES FOR MODELLING AND PREVENTION IN VITRO AND IN VIVO

Annual Summary Report
(Sept. 1, 1981-May 30, 1982)

DR. JOHN R. TREVITHICK

June, 1982

Supported by

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Rat eye lens, microwave irradiation, pulsed and continuous waves, tissue culture, medium 199, elevated temperature, globular degeneration, cataract vitamin E prevention.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Rat ocular lenses were studied after fixation and critical point drying of the tissue by scanning electron microscopy (SEM) following exposures to elevated temperatures and/or microwave irradiation in a thermostatically controlled chamber. In this way, the temperature of the lens bathing medium was set independently of the temperature increase normally associated with application of microwave power. Irradiations were done at three final temperatures and five specific absorption rates (SAR) for two durations. These were accomplished at 915 MHz in WR975 waveguide with either pulsed (Pu) or continuous wave (CW)

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radiation of equal average power. The parameters of the (Pu) radiation were selected to maximize the production of thermoacoustic expansion.

In addition to the types of damage noted on previous progress reports: (1) immediately after pulsed microwave irradiation, holes in cell membranes (2) after incubation for 48 hr, globular degeneration including very large globules at high SAR's and 37°C, this work has investigated (1) possible reciprocity between time and SAR; and (2) threshold dosage (for 6 min exposures) for damage to lenses in vitro by pulsed microwaves at low average power ratings but high peak power in the pulses.

SUMMARY

Over the four year period of this grant, the initial aims of this project were to develop techniques for incubating intact rat lenses in vitro in order to study the development of cataracts when lenses are exposed to elevated temperatures and/or microwave irradiation. We planned to (1) establish cataractogenic temperatures and SARs for irradiation in vitro and (2) investigate the mechanisms of cataractogenesis in such lenses.

Initial studies indicated a linear correlation between depth of cataractous globular degeneration and temperature when the lens was exposed to a short period of temperature elevation and postincubated for a period of 48 hr. This unexpected linear relationship was found between 37°C and 50°C; in addition, at 47°C and 50°C some very large globules were formed. Surprisingly, apparently because they had been fixed by the high temperature. D-α-tocopherol acetate when added to lenses before incubation at 41°C, prevented most of the globular degeneration observed at this temperature.

In initial attempts to expose the lenses to microwaves, a system was devised to rapidly circulate thermostatted coolant around the lens while it was being irradiated. This system permitted experimental separation of heating effects in the lens from effects due to electromagnetic radiation, since there was no measurable temperature elevation in the lens with respect to the surrounding medium even at highest microwave exposure levels tested. Irradiation was performed for two exposure times and at three SAR values.

The results of the irradiation indicated that the effect of the electromagnetic radiation itself could be considered to be equivalent to heating, since at the highest dose rate and 37°C, large globules were formed, which would otherwise have been reported at 47°, equivalent to a temperature elevation of 10°C. Pulsed irradiation at high SAR values appeared to result in holes in the surface of cells, a novel observation which could indicate that thermoacoustic expansion is causing mechanical damage to cell membranes. A preliminary estimate of amount of damage experienced as a result of total dose level of microwaves is consistent with the idea that the amount of damage is roughly proportional to the total dose delivered to the lens, and that a reciprocal relationship exists between dose rate and time required to cause a defined amount of globular degeneration. Further work done to explore the possibility of such reciprocity, which has important implications for personnel who are chronically exposed to low levels of microwaves as well as those suffering from the effects of acute exposure, has supported an approximately reciprocal relationship, but has indicated that the peak pulse power has an important effect on the amount of damage observed in lenses fixed immediately after exposure to pulsed microwave irradiation. Because pulsed irradiation appears to cause more damage than continuous wave (CW), these findings may have important implications for setting safety standards.

FOREWORD

A. List of Professional Personnel Employed on This Project

Principal Investigator	-	Dr. John R. Trevithick, Ph.D.
Research Associate	-	Dr. P. Jill Stewart-DeHaan, Ph.D.
Research Associate	-	Dr. Madhu Sanwal, Ph.D.

B. Animal Care

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science - National Research Council, U.S.A.

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INTRODUCTION

Since the collaborative development of an in vitro technique for experimentally distinguishing the cataractogenic effects of microwaves due to (1) elevated temperatures and (2) the electromagnetic field (Stewart-DeHaan et al, 1980) several generally accepted concepts regarding microwave cataractogenesis have been felt to deserve more detailed scrutiny. Such ideas as a temperature threshold for microwave cataractogenesis, which have been developed almost exclusively for CW microwaves, have ignored the possibilities for mechanical damage to the eye and its lens as a result of the thermoacoustic expansion caused by high energy pulsed microwaves (Pu) similar to those commonly in use in military radars.

In the experiments reported here, we used the system we have devised for microwave irradiation of rat lenses in vitro. This permits the temperature of the lens bathing medium to be varied independently of the irradiating field, and examination of the lens by SEM following fixation (1) immediately after irradiation, or (2) following 48 hr incubation in M199. The changes are compared with known changes involved in the course of cataractogenesis induced by several toxic agents such as elevated glucose levels in M199, elevated temperature alone, or toxic drugs such as hygromycin B.

In these experiments we wished to approach in a preliminary way the questions: "Is there a reciprocal relationship between time and dose rate of microwaves (defined as Specific Absorption Rate, S.A.R.) required to induce a certain amount of cataractogenic damage in a lens?" We have explored as well the minimum dose and pulse parameters necessary to cause damage discernable in lenses fixed immediately after irradiation.

In our previous (1981) progress report we presented preliminary data outlining (1) several biochemical analytical techniques we have been developing to permit analysis of ATP, NADPH, NADH, vitamin E, O_2 superoxide radical levels and lens protein alterations during the course of the cataractogenesis and (2) high voltage electron microscopy which we hope to apply to single lens fiber cells from the equatorial area in which the initial changes in microwave cataracts occur.

MATERIALS AND METHODS

a) Microwave Exposure Apparatus

Rats (150-200 g) were sacrificed by decapitation and the lenses removed. Lenses with intact capsules were placed in M199 in culture tubes or in an apparatus in which they could be bathed in circulating phosphate buffered saline (PBS) at controlled temperatures while simultaneously being subjected to microwave radiation. Intact lenses were exposed to elevated temperatures for periods of up to one hour, followed by incubation in M199 at 35.5 °C for two days. Some lenses were pre- and post-exposure incubated in M199 with 2.4 μM D-α-tocopherol (vitamin E) added in order to explore possible prophylatic effects for exposure to elevated temperatures.

Microwave radiation was delivered to the lens holder in two modes, Pu and CW. The lens holder and waveguide structure and the exposure system block

diagram were illustrated in our 1981 progress report and in the attached paper, (Stewart-DeHaan et al, 1982, in press). In the CW case, the signal was generated by a Hewlett Packard (HP) 8690B Sweep Oscillator with an HP 8699B plug-in, filtered by an HP 360B low pass filter to remove harmonics, amplified by an Amplifier Research 4W1000 amplifier and a Varian VZL-6943G1 TWT amplifier and then filtered again to remove harmonics and noise generated by the TWT. The power was adjusted to the desired level by setting the output level from the sweep oscillator. In the Pu case, the signal was generated by an Epsco PH40K and passed through a PAM-TECK UTA1017 isolator to protect the pulsed source from excessive reflected power. In either case, the signal was passed through a coax-to-waveguide adaptor (Dielectric Communications C-42061-501). A reflectometer (Dielectric Communications D-40588-502) was used in conjunction with HP 435A power meters to monitor forward and reflected powers. The impedance match to the lens holder was done using a triple stub tuner designed and built at Walter Reed. Typically, it was possible to adjust the tuner so that the reflected power was 15-18 dB below the forward power with the lens holder in place. The lens holder was located 1/4 guide wavelength from the waveguide shorting plate placing the lens holder in the maximum of the electric field. The vertical position of the lens holder was adjusted so that the lens was approximately at the center of the waveguide.

b) Microwave Exposure Conditions

Average transmitted powers were 60W, 20W, 6W, 2W, 1W, 0.5W and zero W for the shams. The corresponding SARs (as determined by measuring temperature elevation at the lens site with the coolant flow off) were 1.0-1.2 W/g, 300-400 mW/g, 100-200 mW/g, 20 mW/g, 10 mW/g, 5 mW/g and zero respectively. Initially, the bath offset temperature to meet the final temperatures was 1.4-1.6 ^{o}C at 60W of average power (with coolant flow), 0.3-0.4 ^{o}C at 20W and very close to zero at 6W and below. The bathing medium was PBS. Flow rate was 600 ml/minute. More recently modifications to the circulating coolant system have permitted reductions in the offset temperature required to approximately 0.3 ^{o}C at the highest SAR with proportional reductions at lower SARs.

In the pulsed case, average power was set by variation of the pulse repetition rate. The peak power and pulse rise time were constant at about 24 KW and 0.1 to 0.5 µsec, respectively. The pulse width used routinely was 10 µsec optimum for thermoacoustic expansion; in experiments testing peak power it was sometimes varied to 48 KW and pulse width varied from 5 µsec to 20 µsec at a maximum.

Lenses were fixed either immediately after irradiation or after the 2-day incubation in M199. After treatment with Karnovsky's fixative for 48 hours at 4 ^{o}C the lenses were transferred to 0.1 M Na-cacodylate buffer, dehydrated in an alcohol series to acetone and critical point dried from CO_2. Each lens was quartered and attached to an SEM stub with silver daube paint, sputter-coated with gold-paladium and examined in a Hitachi HHS-2R SEM at an accelerating voltage of 20 KV.

c) High Voltage Electron Microscopy of Tissue Culture and Lens Fiber Cells

These techniques were described in our progress report for 1981.

d) Cryological-SEM-EDX Sample Examination

Using funds having been granted for this year matching funds (150,000) from the University's Academic Development Fund, the purchase of the instrument package for this technique is underway. It is expected to be installed and operating early in 1983.

Fresh lenses are frozen on a cryostage, using octanol to attach it to the cryostage, and freeze-fractured. The frozen fractured specimen is transferred on a cold stage cooled with liquid nitrogen to the SEM specimen chamber and examined. A suitable area is chosen for x-ray analysis and the spectrum recorded. Two dimensional analysis of three elements simultaneously can be performed, giving a picture which corresponds to the SEM picture.

e) Histochemical Sample Preparation and Staining

This was described in 1981.

f) Water-Soluble Embedding Method for Light Microscopy

This was described in 1981.

g) Biochemical Techniques

Techniques for the following were described in 1981:

Protein, adenylate nucleotides, NADPH, NADH, superoxide anion, glutathione, vitamin E and Vitamin C.

NEW RESULTS

As described in the attached paper and appendix with figures 1-25 we found mostly equatorial damage, more with pulsed than CW at the same dose, and an approximate reciprocal relationship between time of exposure and dose rate required to produce a particular depth of degeneration (Fig. 1-4 summarize this). The comparable regimens required to produce approximately the same dose are illustrated in Figure 5. The depth of damage (globules or granular cell surfaces) shown is illustrated in Fig. 6, which indicates similar depths of damage for diagonally related boxes in Fig. 5.

Further figures document the morphological features of the damage found by scanning electron microscopy (Figures 7-10).

High resolution light microscopy (Figures 11-20) illustrates typical morphological changes in such lenses by a new version of a conventional technique. Figures 21-23 illustrate scanning electron microscopy of changes observed in lenses fixed immediately after irradiation at low doses of pulsed microwaves, but at elevated peak power; such high peak powers would be found in the vicinity of military airport and ship-borne radar installations.

Figures 24 and 25 illustrate preliminary development of techniques for measuring modulation transfer function of irradiated lenses, using a computerized technique for determining spatial frequency.

Table 1 after Fig. 25 in the appendix, represents a preliminary attempt to explore whether the ATP level changes markedly, in leses incubated under cataractogenic conditions (such as elevated glucose). A corollary of this hypothesis, that one might expect minimal charges under conditions in which no cataracts may be seen. As can be seen, even though standard errors are rather large, the specific activity of ATP in fresh lenses is low in epithelium and nucleus, but high in the cortex. This pattern is reversed between epithelium and cortex in control lenses incubated for 3 or 4 days, although they increase somewhat in the cortex in vitamin E (VE) - treated lenses. In the presence of glucose, cortical ATP appears much higher than controls after 3 and 4 days, while in the presence of glucose and VE, similar levels of ATP (to fresh lenses) are obtained after 3 days. By 4 days this similarity has diminshed somewhat but cortical ATP levels are still devated over controls, however epithelial ATP levels are higher than might be expected.

Extension of these experiments to heated lenses and to microwave-irradiated lenses, with larger numbers to obtain better statistics is planned for the near future.

DISCUSSION

In considering the possible hazards of microwave irradiation for military personnel, it is appropriate to consider not only the effects of acute damage which might develop following inadvertent exposure to high doses under combat conditions, but also possible long-term effects which might ensue following low level exposures over a longer period of time. Although such experiments could be conducted in a living animal, it appeared advisable, in beginning these experiments, to use a more sensitive system in which the cataractogenic response occurs rapidly and large doses which would ensure that a measurable effect was found. Threshold studies will be conducted later once an appropriate level to check is established.

The in vitro system developed for exposing lenses in medium to the stress of chemical compounds, temperature, and irradiation permits the precise manipulation of these factors, whereas, in vivo, only an approximation is possible. In addition, the various types of damage and their degree or extent are more readily and rapidly detectable and assessable; for example, incubation with 55.6 mM glucose in M199 results, within 2 days, in development of both globular degeneration and opacity, while the same process in the intact diabetic animal would require as much as 6-8 weeks to reach a similar degree of degeneration.

Taken with the attached paper, these results indicate damage at high peak power (48 KW) using dose rates of 1 and 2 watts (approximate SAR 10 and 20 mW/g, respectively), but not at the lowest dose rate studied, which corresponds to 0.5W (SAR 5 mW/g approximately). The type of damage seen at such low doses in immediately fixed lens samples, consisted of some foam and globular degeneration and rather large areas of cells with granular cell surfaces. The holes seen at higher dose rates, in Fig. 1-20, appear to be a later stage in a progressive series of events in membrane damage. Such

membrane damage appears to be related to physical damage due to pressure waves occurring as a result of the thermoacoustic expansion caused by pulsed microwaves (Foster and Finch, 1974), since these effects are found to be more pronounced when at the same average power, the microwaves are delivered in pulsed mode rather than as continuous wave. The theoretical basis for such physical damage has been discussed by Lin (1978); he ascribes the sound waves to thermoelastic expansion. Some uncertainty remains regarding the correspondence between his theoretical treatment of thermoelastic stress and the measured differences in experimental damage to the irradiated rat lenses, which we have found. A particularly vexing question is why increasing the pulse peak power or the power per pulse appears to cause significantly more damage even though the average power and total dose are held constant. As Lin admits (page 157) "The sound pressure generated in a surface-stress-free spherical head model is, therefore, smaller than known results." (apparently by a factor of about 6.5). This discrepancy, which he addresses by modifying the model, indicates the need for further development of theoretical treatments.

The development of a technique for measurement of modulation transfer function of fixed rat lenses appears to be proceeding satisfactorily. The modulation transfer function is measured by the percent of total power in the peak for the correct spatial frequency, by comparison to the background or random noise distributed among the various spatial frequencies, which is plotted as a function of spatial frequency (number of bars per mm). As can be seen in the control lens tested, this approximate function (expressed in arbitrary spatial frequency units derived from the various grating frequencies), is a decreasing function of increasing spatial frequency.

Special notice should be taken of the apparent proportional increase in depth of globular degeneration, as a function of dose rate, for pulsed irradiation and the similar extent of damage observed for different SARs at times which resulted in a similar total dose being delivered to the sample. The possibility of reciprocity, generally recognized as a feature of ionizing (but not of nonionizing) irradiation, has not been suggested to date for microwave irradiation; these data support the idea that such a relationship is approximately true for the short times we used. If also true in vivo, such reciprocity could have important implications for long term chronic exposure to low levels of pulsed microwave irradiation. This would imply that long term exposure to a low level of pulsed microwave irradiation, such as might occur in military personnel working in the vicinity of a radar installation, might result in increased risk of cataractogenesis. For examples, in vitro even though little damage can be seen after 2W exposure for 6 min, more extensive damage is noted after exposure to 2W pulsed energy for 20 min (Fig. 13). This makes it seem very likely that if exposures at low dose rates are conducted for a long enough period of time (sever hr).

The mechanism of cataractogenesis in the isolated lenses following exposure to microwave irradiation is still not well-defined. Nevertheless, certain features of this process were suggested by the experiments on high voltage electron microscopy reported in our 1981 progress report. The peculiarly similar appearance of the cytoskeleton in microwave-irradiated fiber cells, and tissue culture cells treated with cytochalasin D, is consistent with the idea we have presented previously, that in cortical cataracts the critical change leading to globular degeneration and opacity is a change in the cell cytoskeleton (Mousa, Creighton and Trevithick, 1979). Further work which needs to be done to confirm this and to define the biochemical and physical changes (i.e. in ATP, Na/K ratios and levels, NADPH and glutathione) etc. which may be responsible for this cytoskeletal change,

is proposed in an accompanying application for continuation of this grant. At present this work is awaiting adequate morphological characterization of the damage so that appropriate conditions for the biochemical studies may be chosen. However, our preliminary ATP determinations in lenses incubated to simulate cataractogenesis are consistent with an involvement of ATP in the cytoskeletal changes.

The biochemical results on cataractogenesis in vitro induced by heat, microwaves and glucose are consistent with several possible mechanisms for globular degeneration:

1. The lowering of cortical ATP levels would be expected to result in supercontraction of the actin microfilaments.
2. The increased level of superoxide radicals with time would be expected to result in oxidative damage to sulfhydryl groups, or oxidation of unsaturated fatty acids, and proteins of membranes, as might the previously reported (by Kinoshita's group) decreased or of reduced pyridine nucleotide or glutathione, reported for radiation cataract recently by Reddy's group (Giblin 1980) level of Vitamin C.
3. A decrease of potassium/sodium ion ratio and increase in calcium, which might result from cell membranes with leaks or holes, would be expected to cause a change in contraction of cytoplasmic microfilaments, since potassium is required for the process of microfilament contraction.
4. The possibility of some prostaglandin, or thromboxane, or prostacyclin metabolite generated to a larger extent acting on a cell membrane or cytoskeletal receptor; this of course would be reduced by biological antioxidants such as BHT, glutathione and vitamin E.

Experimental design in the future is expected to be directed towards analyses which will enable us to determine whether these mechanisms (singly or in combination) actually function, once approrpriate conditions of irradiation are selected.

ACKNOWLEDGEMENTS

We wish to acknowledge the excellent technical assistance of Mr. Leon S. Butler and Miss Margo Miller for performing the surgical procedures to remove the lenses. We thank Mr. Warren Foster for his contributions in designing and building the glass lens holders. Grants from the U.S. Army medical Research and Development Command (DAMD-78-G-9449), the MRC of Canada, National Eye Institute (USA) (EY1927) and the E.A. Baker Foundation of the CNIB are acknowledged with thanks.

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TABLE 1

PRELIMINARY EXPERIMENTS EXPLORING LEVELS OF
ATP IN EPITHELIUM, CORTEX AND NUCLEUS OF RAT LENSES INCUBATED IN M199
UNDER CATARACTOGENIC CONDITIONS

			Lens Area ATP levels (ng/ g protein ± Standard error)		
Days Incubated	Condition (number lenses)	Vitamin E	Epithelium	Cortex	Nucleus
3	Fresh lens (3)	-	0.230 ± 21	3.04 ± 0.50	0.166 ± 0.006
3	Control (2)	-	1.90 ± 1.09	0.0121 ± 0.0002	0.0055 ± 0.0017
3	Control (2)	+	0.829 ± .793	0.0392 ± 0.046	0.0045 ± 0.002
3	56 mM Glucose (2)	-	1.50 ± 1.67	0.526 ± 0.49	0.0213 ± 0.012
3	56 mM Glucose (2)	+	0.664 ± 0.077	2.68 ± 3.40	0.0738 ± 0.098
4	Control (2)	-	2.22 ± 2.52	0.109 ± 0.149	0.0059 ± 0.001
4	Control (2)	+	22.9 ± 31.1	0.935 ± 0.25	0.0158 ± 0.006
4	56 mM Glucose (2)	-	2.39 ± 0.78	1.19 ± 1.12	0.045 ± 0.027
4	56 mM Glucose (2)	+	2.21 ± 0.38	1.34 ± 1.10	0.143 ± 0.005

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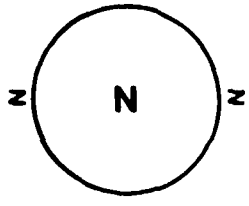
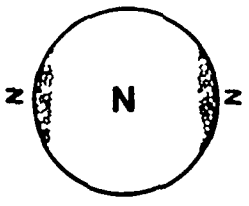
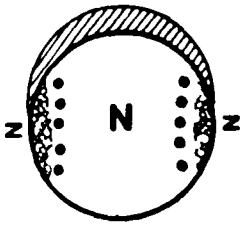
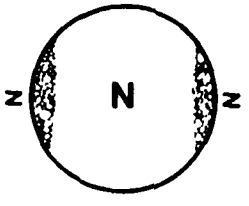
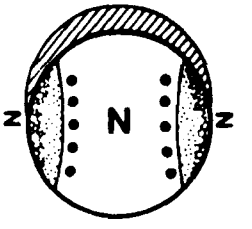
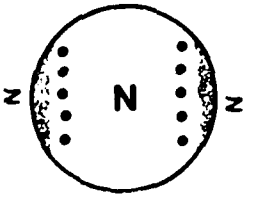
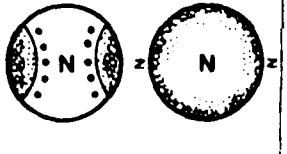
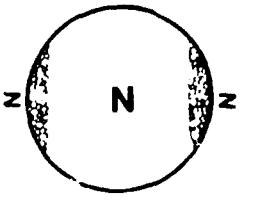
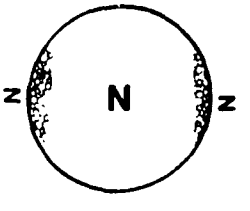
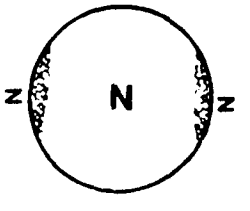
APPENDIX

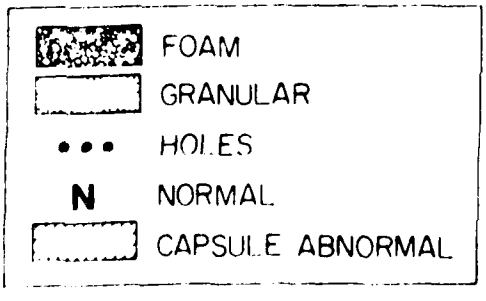
The attached appendix (Figures 1-25, Tables 1 and 2 and letters) illustrates some of the pathological microwave bioeffects found to date in experiments using 915 MHz irradiation, changes observed in immediately fixed lenses after low doses of irradiation, preliminary experiments aimed at determining modulation transfer function of lenses, some biochemical data, and some details of techniques which may be useful in analysis of protein gels.

Figures 1 & 2. Comparison of Damage to Rat Lens In Vitro After Exposure to Pulsed and Continuous Wave Microwave Irradiation.
All lenses were fixed in Karnovsky's fixative after irradiation with microwaves at power levels for times indicated. Irradiation at 915 MHz was performed in phosphate - buffered saline (PBS); at the end of the irradiation the lens was immediately removed from the PBS and placed in Karnovsky's fixative at 4 C. After 48 hr, the lens was transferred to 0.1M cacodylate buffer at 4 C for a minimum of 48 hr; then it was dehydrated through an alcohol series to acetone and critical-point dried from CO_2, prior to examination by scanning electron microscopy with an Hitachi HHS-2R microscope. All samples were examined in triplicate.

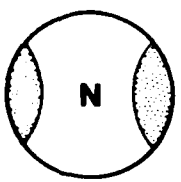
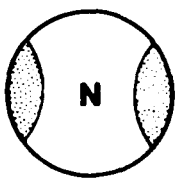
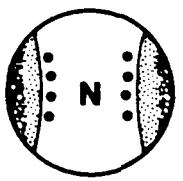
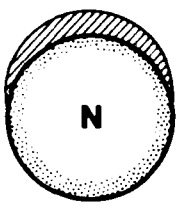
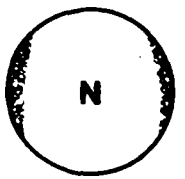
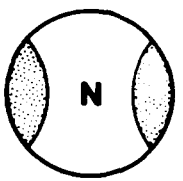
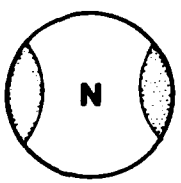
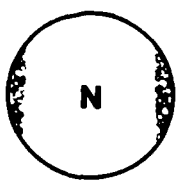
Damage found is illustrated as shown in the figure by symbols. Most damage occurring was found at the equator of the lens. Pulse conditions were: pulse width 10 μ sec, peak power 24kW; interpulse intervals were varied to obtain correct average powers. Pulse variations were studied at 2 W at 65 W average power (SAR 40 mW/g and 1.2 W/g, respectively). At fixed energy per pulse and fixed total energy, increased peak power increased damage evaluated by SEM. Still greater increase in damage (at constant total energy delivered) was observed at twice the energy per pulse (240 mJ and 480 mM, respectively). Indeed, the "threshold" for cortical fiber cell fenestration at 37 C was reduced from 400 mW/g to 40mW/g.

CONSTANT FACTORS 37°, 6 MINS, QUICK FIX

PULSE	2W	6W	20W	65W
24 KW 10 MICROSEC	 120 MILLISEC	 40 MILLISEC	ND 12 MILLISEC	 3.7 MILLISEC
48 KW 5 MICROSEC	 120 MILLISEC	ND 40 MILLISEC	ND 12 MILLISEC	 3.7 MILLISEC
48 KW 10 MICROSEC	 240 MILLISEC	ND 80 MILLISEC	ND 24 MILLISEC	SINGLE PULSE REPETITION RATE 1/2 PULSE REPETITION RATE  7.4 MILLISEC
CW 24 KW 10 MICROSEC		ND		



CONSTANT FACTORS 37°, QUICK FIX

TIME	PULSE				CW	
	2W	6W	20W	65W	2W	6W
20 MINS						
60 MINS		ND	ND	ND		ND

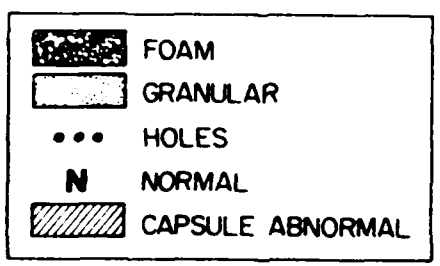


Figure 3. Variation in Depth of Globular Degeneration as a Function of Dosage of Irradiation, for Different Modes of Irradiation (Pulsed, with Varying Energy per Pulse, and CW).

Rat lenses were irradiated, fixed immediately, dehydrated and critical-point dried and examined by SEM as described in Figure 1. The types of damage found are: foam in equatorial fiber cells in the immediate subcapsular region, and granulation of the fiber cells below the foam. The granulation resulted in a roughened, granular appearance of the cell surfaces and cytoplasm, where visible, when breaks or cuts in the cells occurred.

The maximum depth of both types of damage occurring was measured and is plotted as function of dosage of the irradiation. Several single points illustrate the effect of doubling the energy per pulse by doubling the peak power to 48 kW (instead of 24 kW) for the same pulse width (10 μ sec). These correspond to 240 mJ and 480 mJ per pulse, respectively; in either case, the total dose delivered to the lens remained constant. These points always show significant increases in damage, although not quite doubling.

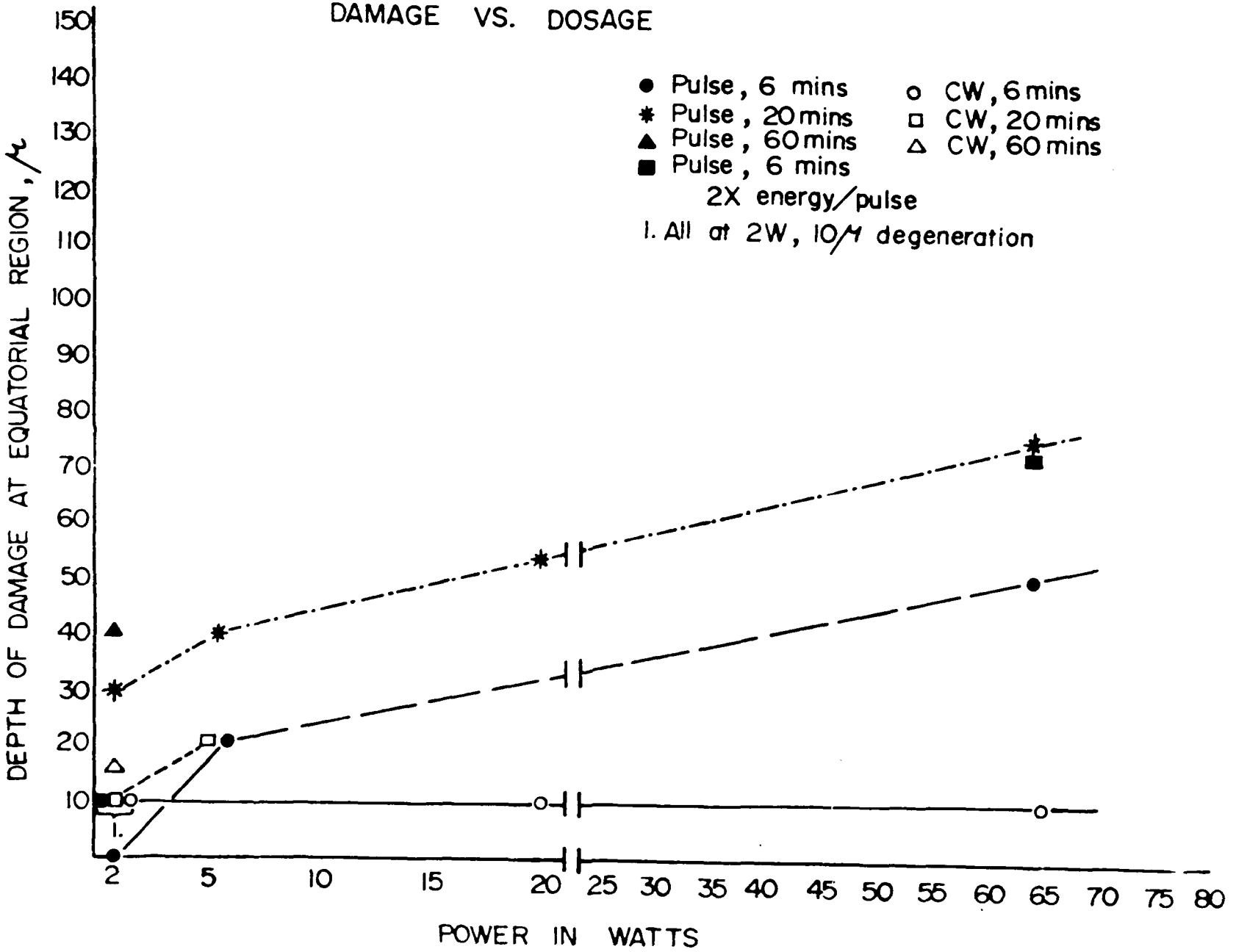

DAMAGE VS. DOSAGE
● Pulse, 6 mins
✱ Pulse, 20 mins
▲ Pulse, 60 mins
■ Pulse, 6 mins
2X energy/pulse
o CW, 6 mins
□ CW, 20 mins
△ CW, 60 mins
1. All at 2W, 10/μ degeneration
DEPTH OF DAMAGE AT EQUATORIAL REGION, μ
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1.
2
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
POWER IN WATTS

Figure 4. Variation in Depth of Globular Degeneration as a Function of Time of Irradiation, for Different Modes of Irradiation (Pulsed, with Varying Energy per Pulse, and CW).

Rat lenses were irradiated, fixed immediately, dehydrated and critical-point dried and examined by SEM as described in Figure 1. The types of damage found are: foam in equatorial fiber cells in the immediate subcapsular region, and granulation of the fiber cells below the foam. The granulation resulted in a roughened, granular appearance of the cell surfaces and cytoplasm, where visible, when breaks or cuts in the cells occurred.

The maximum depth of both types of damage occurring was measured and is plotted as function of time of the irradiation. Several single points illustrate the effect of doubling the energy per pulse by doubling the peak power to 48 kW (instead of 24 kW) for the same pulse width (10 μsec). These points always show significant increases in damage, although not quite doubling.

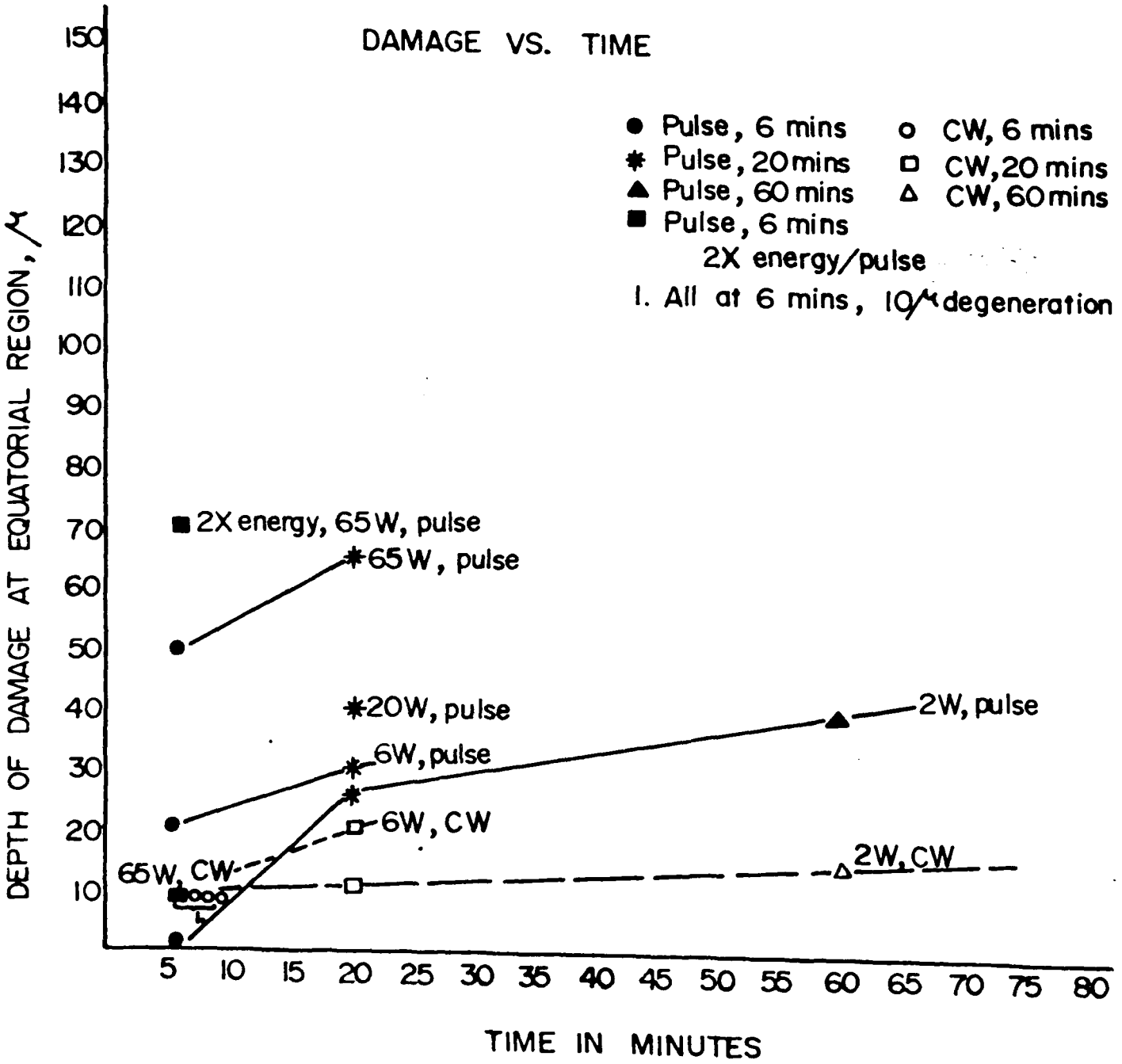

DAMAGE VS. TIME
● Pulse, 6 mins
✱ Pulse, 20 mins
▲ Pulse, 60 mins
■ Pulse, 6 mins
2X energy/pulse
o CW, 6 mins
□ CW, 20 mins
△ CW, 60 mins
1. All at 6 mins, 10% degeneration
DEPTH OF DAMAGE AT EQUATORIAL REGION, μ
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
■ 2X energy, 65 W, pulse
✱ 65 W, pulse
✱ 20W, pulse
6W, pulse
2W, pulse
6W, CW
65W, CW
2W, CW
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
TIME IN MINUTES

Figure 5. Representation of Different Doses (Shown by Numbers in Boxes), Which Were Given at Different Dose Rates (Watts) and Times (Min).

A reciprocal relationship between time and dose rate for the same total dose was tested by different dose rates and times which resulted in diagonally related boxes having same dose of energy in joules.

INTENSITY-TIME RECIPROCITY

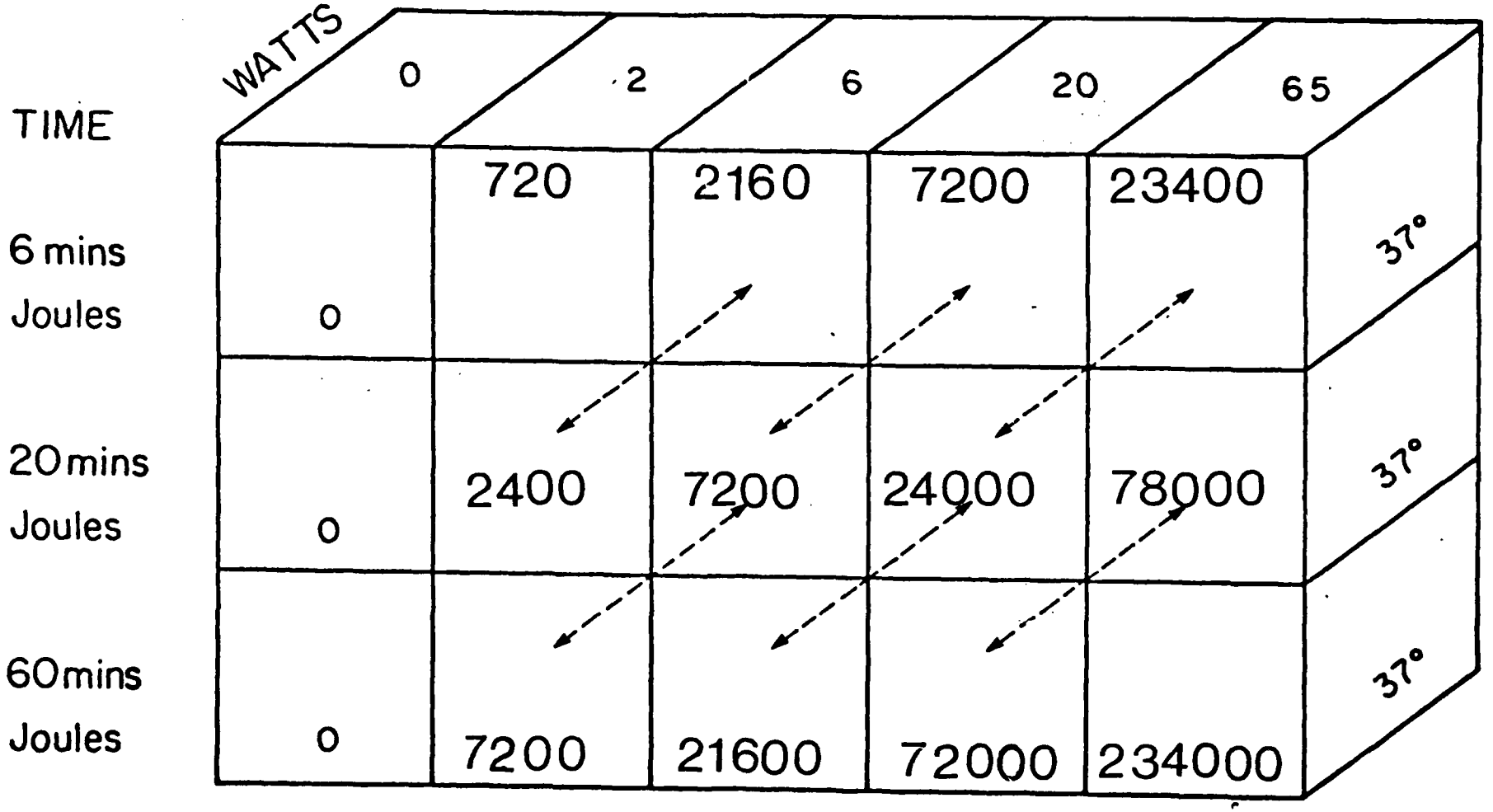


Figure 6. Comparison of Depth of Damage and Frequency of Knobs at Cell Interstices in Undamaged Areas after Varying Dose and Time Reciprocally to Give Similar Total Dose of Microwave Irradiation to Lens.

All lenses were fixed as described in Figure 1. The figure illustrates the effect of several dose-time regimens for irradiation of lens which result in similar total doses (see Fig. 5, for doses). In each case, increasing total dose resulted in increased depth and severity of damage (granulation of cells, vacuole and holes). In no case did numerical values of depth of damage overlap between lower doses and higher doses, indicating a progressive degree of dose-related damage. In no case was any significant difference in frequency of knobs found when normal controls and the undamaged area of irradiated lenses were compared.

RECIPROCITY

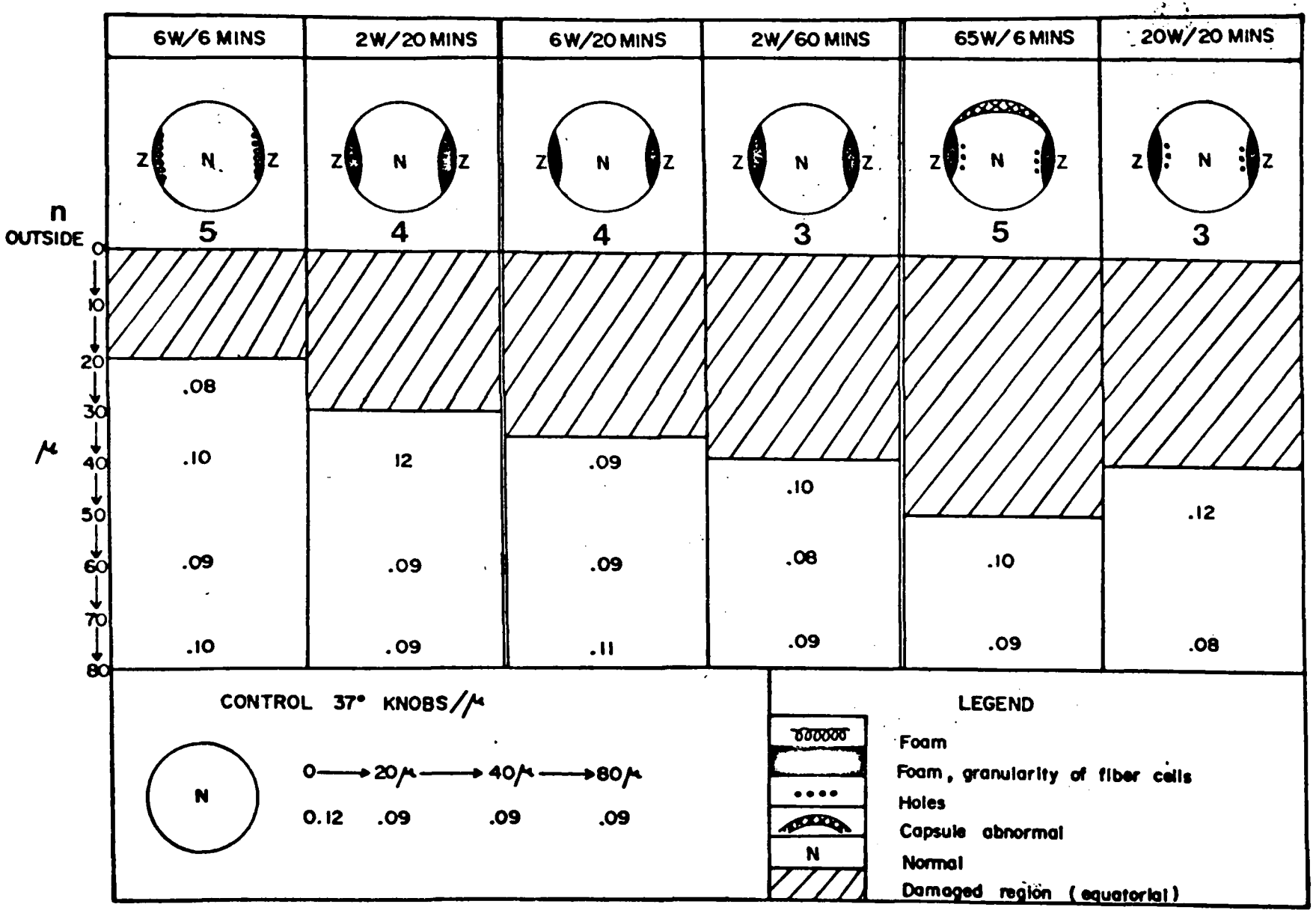


Figure 7. Effect of Pulsed Microwaves on Cortical Lens Fiber Cells, Illustrating Different Types of Damage Observed: Lenses were fixed and prepared for SEM as described in Fig. 1. The photographs illustrate: (a) normal control, showing equatorial cortical lens fibers; (b) equatorial area of lens exposed to pulsed microwaves 915 MHz at 65 W for 20 min at 37°C illustrating equatorial subcapsular foam (types 1 and 2), and holes in the area of fiber cells immediately under the foam; (c) normal control, lens fiber cells at higher magnification than shown in (a); (d) equatorial subcapsular cortical cells exposed to 65 watts for 20 min at 37°C, illustrating granular appearance of cells; (e) subcapsular foam (type 1) illustrating cells reminiscent of goblet cells in lens irradiated at 2 W for 6 min at 37°C; (f) subcapsular foam (type 2) illustrating fibrous area of subcapsular foam in lens irradiated at 65 W for 20 min at 37°C.

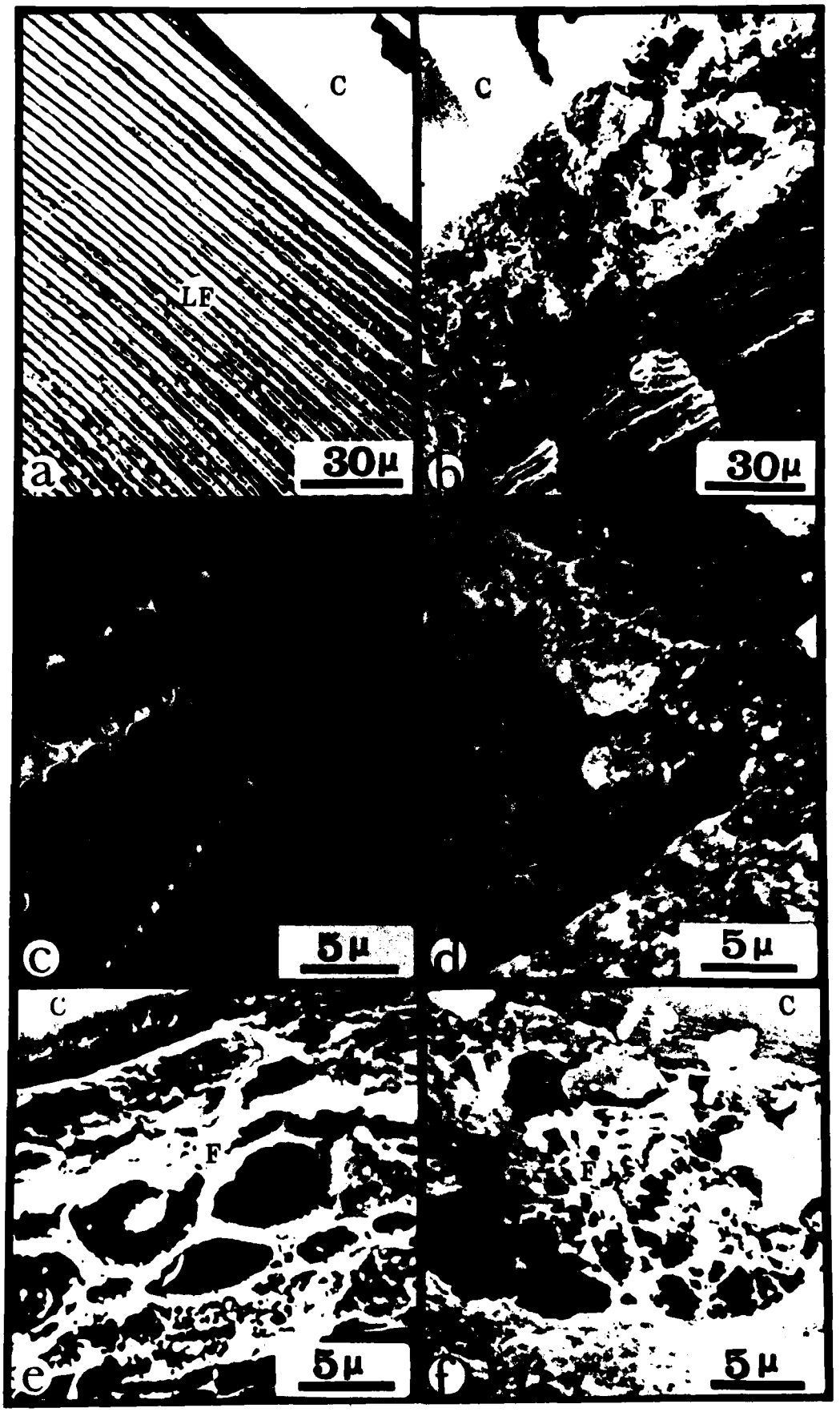

C
LF
30μ
a
C
F
30μ
b
5μ
c
5μ
d
C
F
5μ
e
C
F
5μ
f

Figure 8. Capsular Damage and Different Degrees of Damage to Lens Fiber Cells: (a-c) changes in capsule of rat lens prepared for SEM as described in Fig. 1. (a) normal capsule (note smooth surface); (b) capsule of rat lens exposed to continuous wave (CW) microwave irradiation at 6 W for 20 min at 37 C (note pitted granular appearance of surface); (c) capsule of rat lens after exposure to 915 MHz pulsed irradiation 65 W for 20 min at 37 C; (d-e) subcapsular fiber cells, illustrating gradation of damage (type I-III) occuring as a result of pulsed microwave irradiation: after exposure 20 915 MHz irradiation at 20 W for 20 min at 37 C, fixed immediately at depths shown. (d) type I damage: subcapsular vacuolar damage to lens fiber cells: the vacuoles do not appear to have resulted in holes penetrating the cell membrane which still has a smooth surface.; (e) type II damage: (10-20 deep, below form) larger vacuolar holes in cells which are still intact with slightly granular cell surfaces; (f) type III damage: immediately below capsule and foam many holes penetrating granular cell membranes. Similar damage has been seen in lenses fixed immediately after irradiation at other dose rates, as follows: Type 1-2 W/20 min/37 C /pulsed; Type II 6 W 20 min 37 C pulsed; Type III 65 W/20min/37 C pulsed.

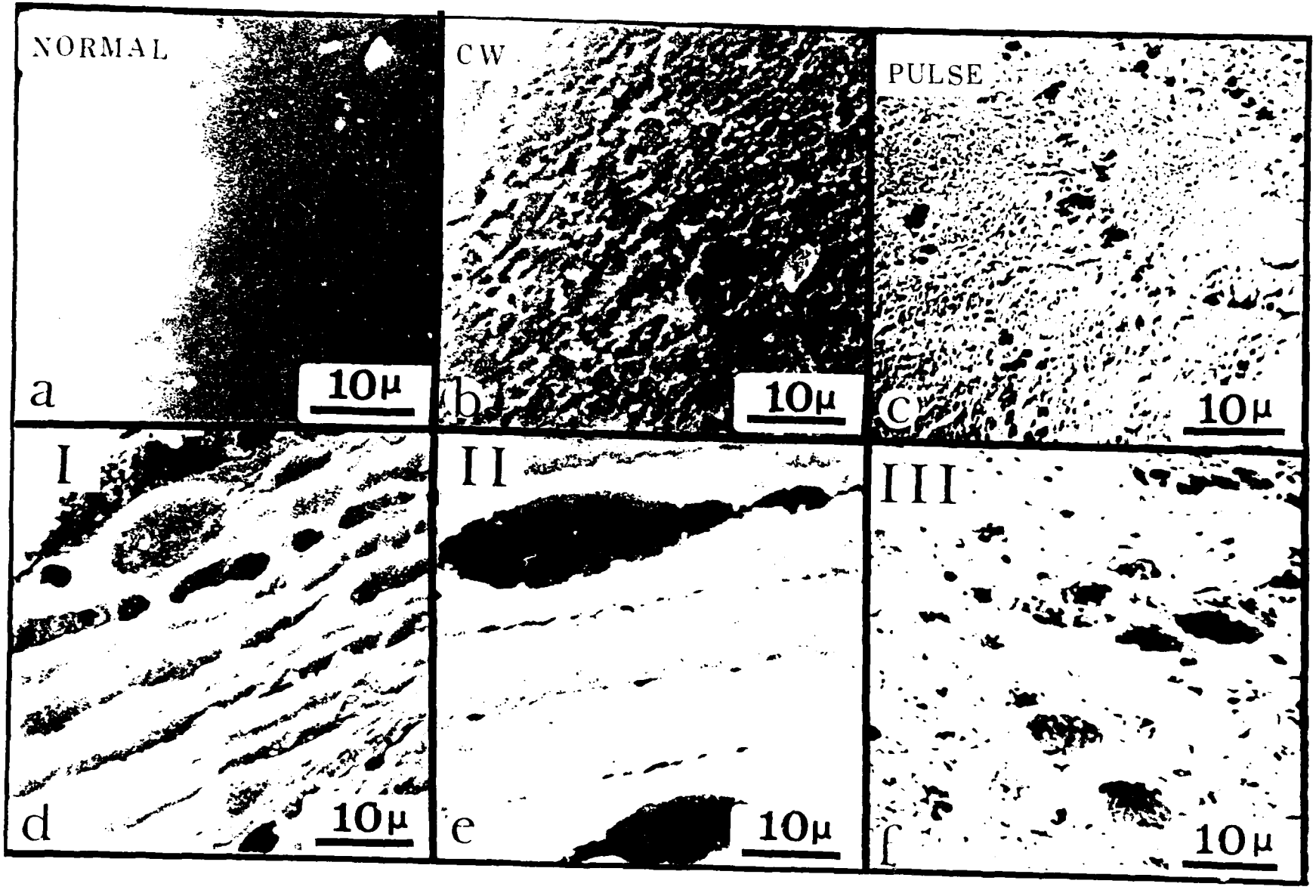

NORMAL
CW
PULSE
a
10μ
b
10μ
c
10μ
I
II
III
d
10μ
e
10μ
f
10μ

Figure 9. Effect of Changing Pulse Parameters at Same Total Energy Delivered to Lens. All lenses were fixed immediately after pulsed irradiation at 2 W for 6 min (720 joules total) illustrating Z (zonules), C (capsule), F (foam); LF (lens fibers), H (holes). (a) pulse parameters: peak power (24 kW), pulse width (10 μsec), interpulse interval (120 msec): energy/pulse 0.24 joules, energy delivered to 3 mm diameter lens ($0.15 \times 4/3 \times \pi$ ml = 13.2 μl), of 50 ml irradiated $.24 \times \frac{13.2 \times 10^{-3}}{50} = 63.4 \times 10^{-3}$ joules/pulse

(b) pulse parameters: peak power (48 kW), pulse width (10 μsec), interpulse interval (240 msec), energy per pulse 0.48 joules energy delivered to lens 126.8×10^{-3} joules/pulse

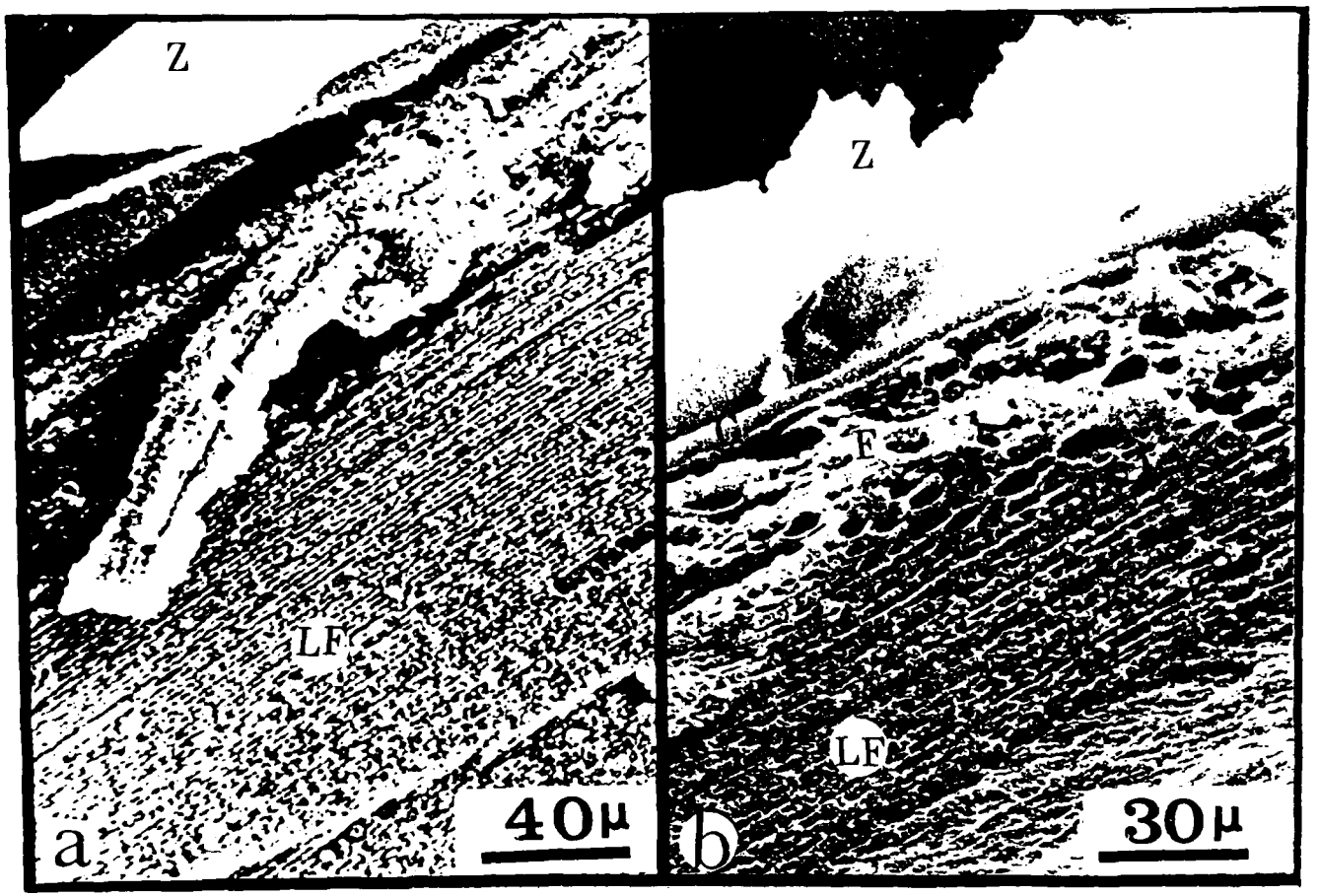

Z
LF
40μ
a
Z
E
LF
30μ
b

Figure 10. Damage to Lens at 39°C by CW and Pulsed Irradiation.
Pictures illustrating equatorial areas of lenses: C (capsule), LF (lens fibers), F (foam), H (holes).
(a) control.
(b) CW irradiation 2 W for 60 min at 39°C.
(c) Pulsed irradiation 2 W for 60 min at 39°C.
Please note that no examples permitting this comparisons were available at 37°C. These should be obtained to permit comparison at a physiologically normal temperature.

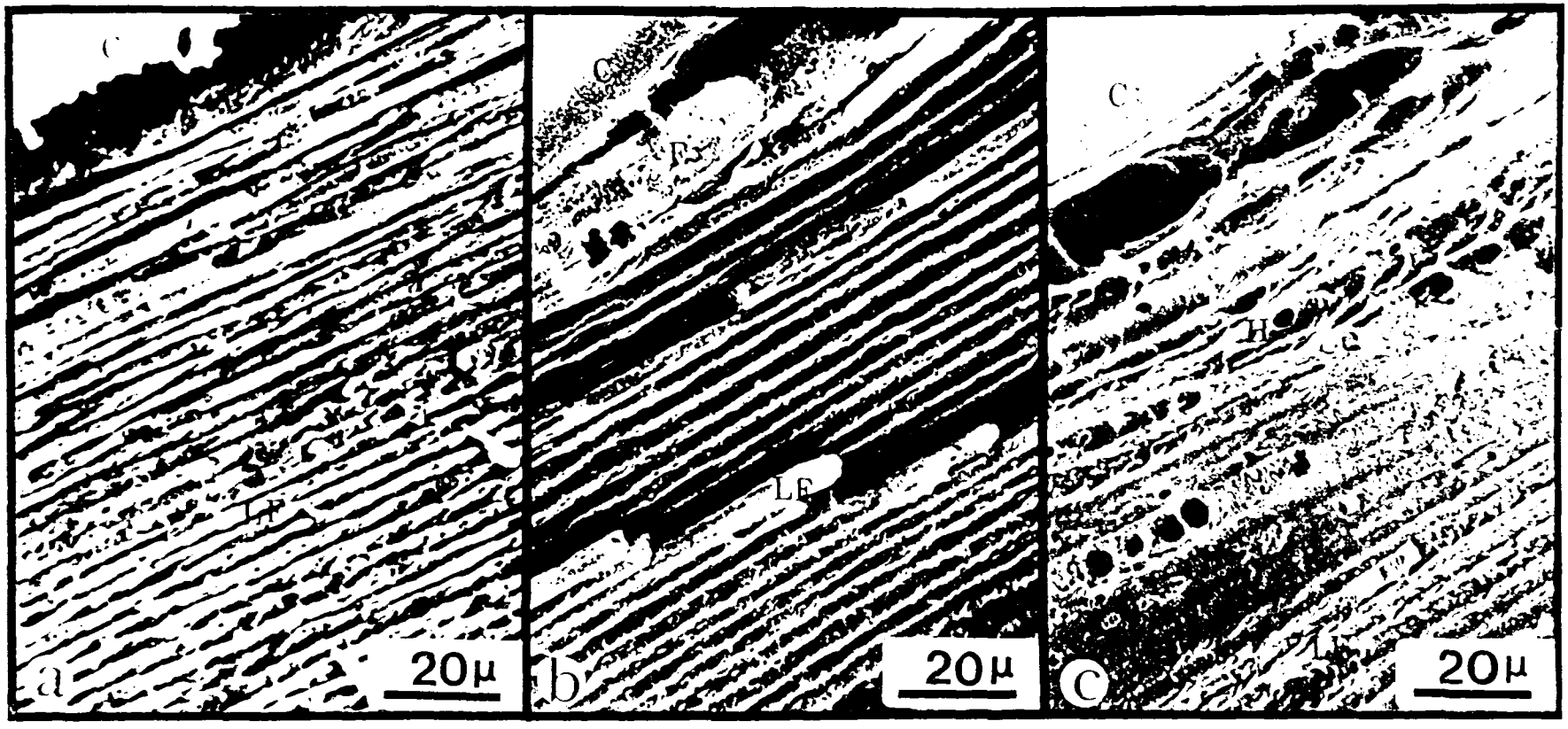

a
20μ
b
20μ
c
20μ

Figure 11. Thick Sections of Plastic-Embedded Control Rat Lenses:

(a) Section through the equatorial region showing the wider capsule (C) on the anterior side and a one layered epithelium.
(b) part of (A) magnified to show healthy epithelial cells with a large nucleus (N) and a few vacuoles. The lens fibers (LF) are arranged in regular rows.

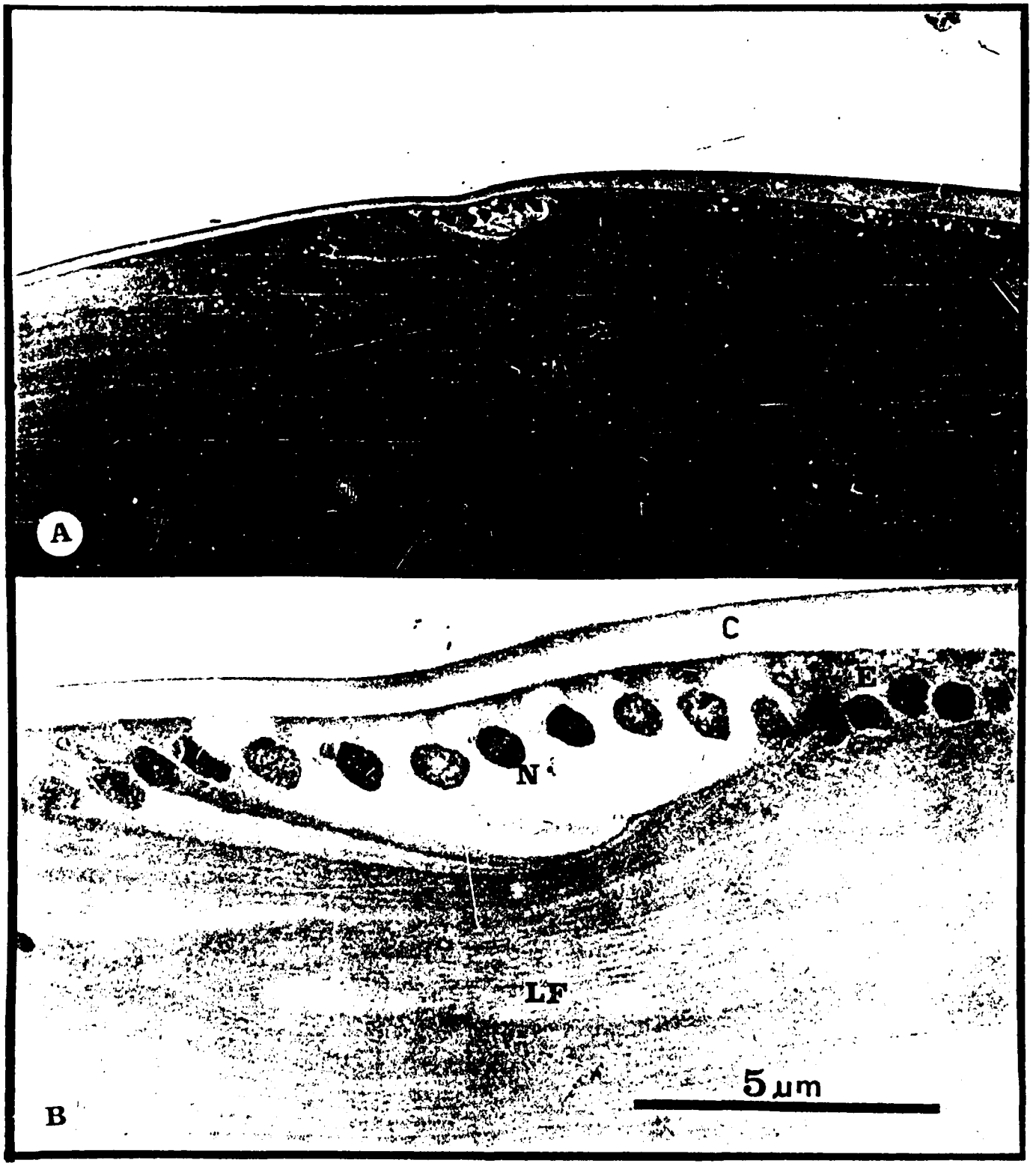

A
C
E
N
LF
5 µm
B

Figure 12. Thick Sections of Plastic Embedded Rat Lenses: (A) Equatorial region from a lens exposed to 2 W, 6 minutes pulsed (24 kW, 10 μsec) microwave irradiation at 37°C and then fixed immediately. The lens looks quite normal except for some large vacuoles in the epithelial cells. (B) Same area from a lens exposed to 2 w, 6 minutes of CW microwave irradiation at 37°C and fixed immediately. The epithelial cells and lens fibers are quite normal.


C
PU
5 µm
A
CW
B

Figure 13. Thick Sections of Plastic-Embedded Rat Lenses: Equatorial region of lenses exposed to 2 W/ 20minutes/37°C microwave irradiation and then fixed immediately.
Lens exposed to pulsed microwave irradiation (A) shows a lot more 'holes' and vacuoles than the lens, (B) Exposed to CW microwave irradiation for the same period.

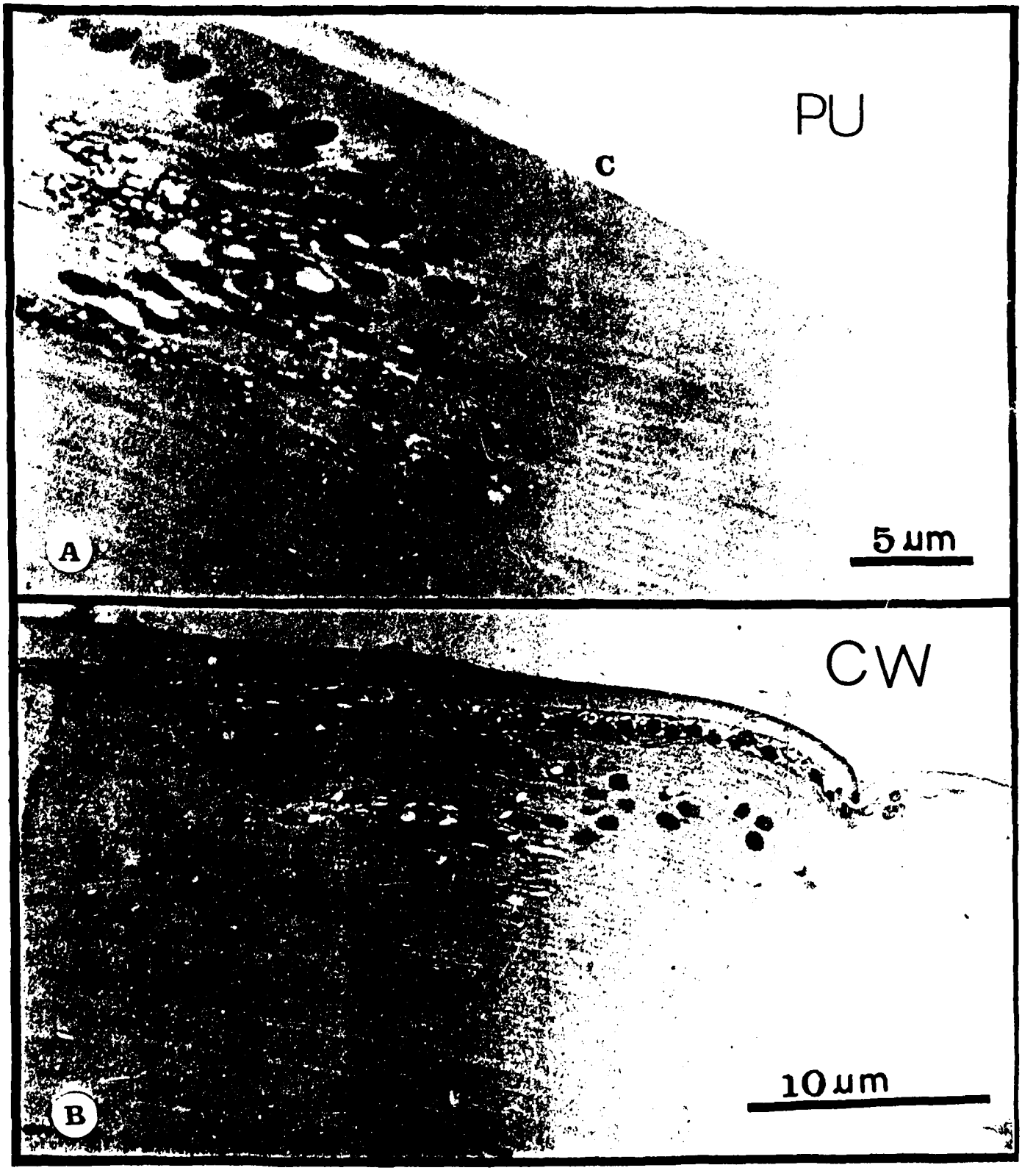

PU
C
5 µm
A
CW
10 µm
B

Figure 14. Thick Sections of Plastic-Embedded Rat Lenses: Lens exposed to 2 W/60 minuts/37°C/pulsed (24 kW, 10 μsec.) microwave irradiation and then fixed immedately.
(A) General view of the equatorial region shows a large clump of degenerated nuclei instead of a regular arrangement.
(B) The equatorial region magnified to show some degeneration of nuclei.
(C) Enlarged view of the clump of nuclei and distorted fibers associated with holes and vacuoles as seen in (A). The fibers below this region appear well organised.

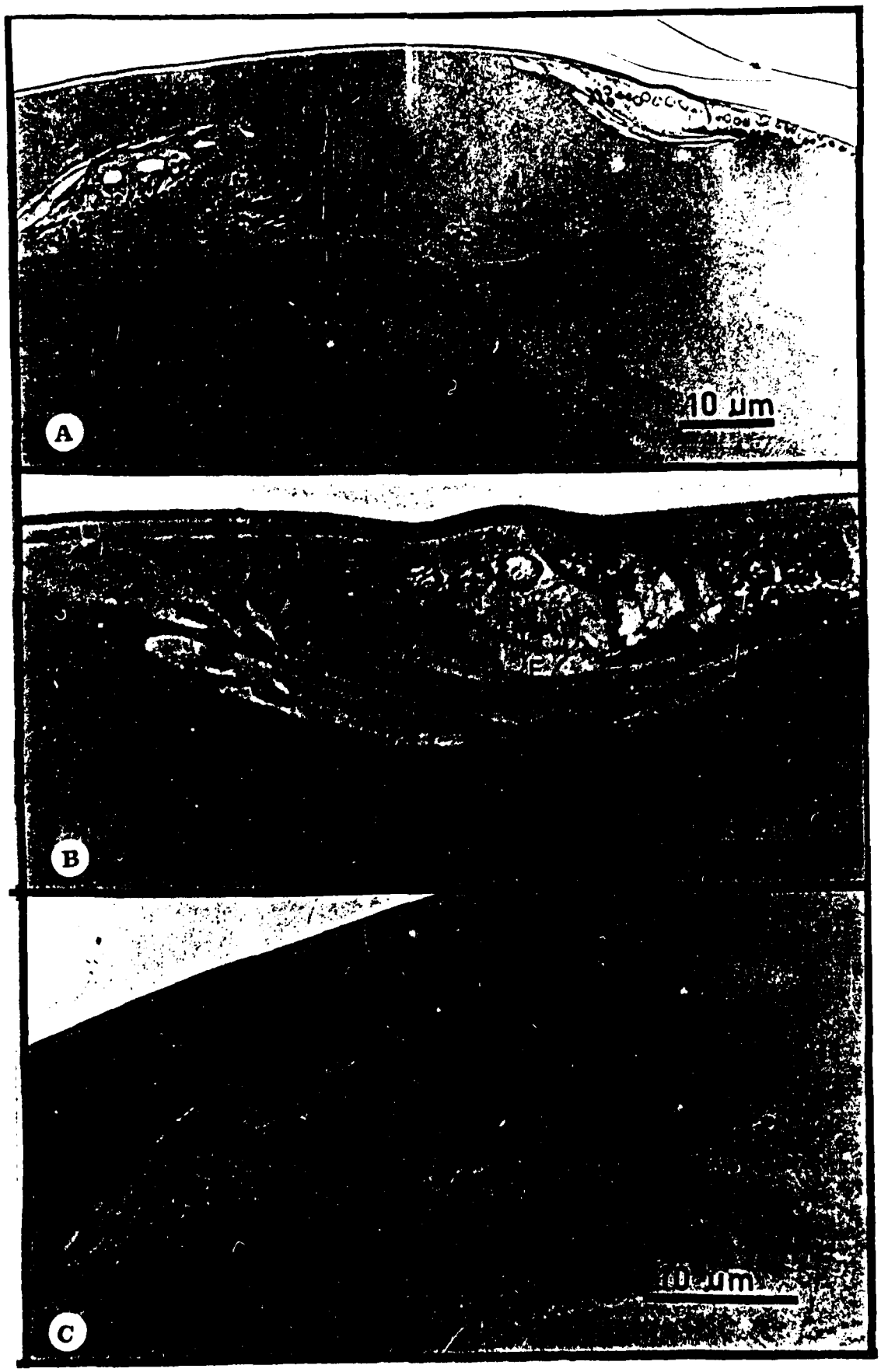

10 µm
A
B
10 µm
C

Figure 15. Thick Sections of Plastic-Embedded Rat Lenses: Lenses exposed to 2 w/60 minutes of CW microwave exposure at 37°C and then fixed immediately.
(A) General view of lens showing some degeneration in the equatorial region and the epithelium.
(B) Anterior region of the lens showing a vacuolated epithelium corresponding to the 'foam' seen in SEM observations.
(C) Posterior region of the lens showing a 'foamy' appearance.

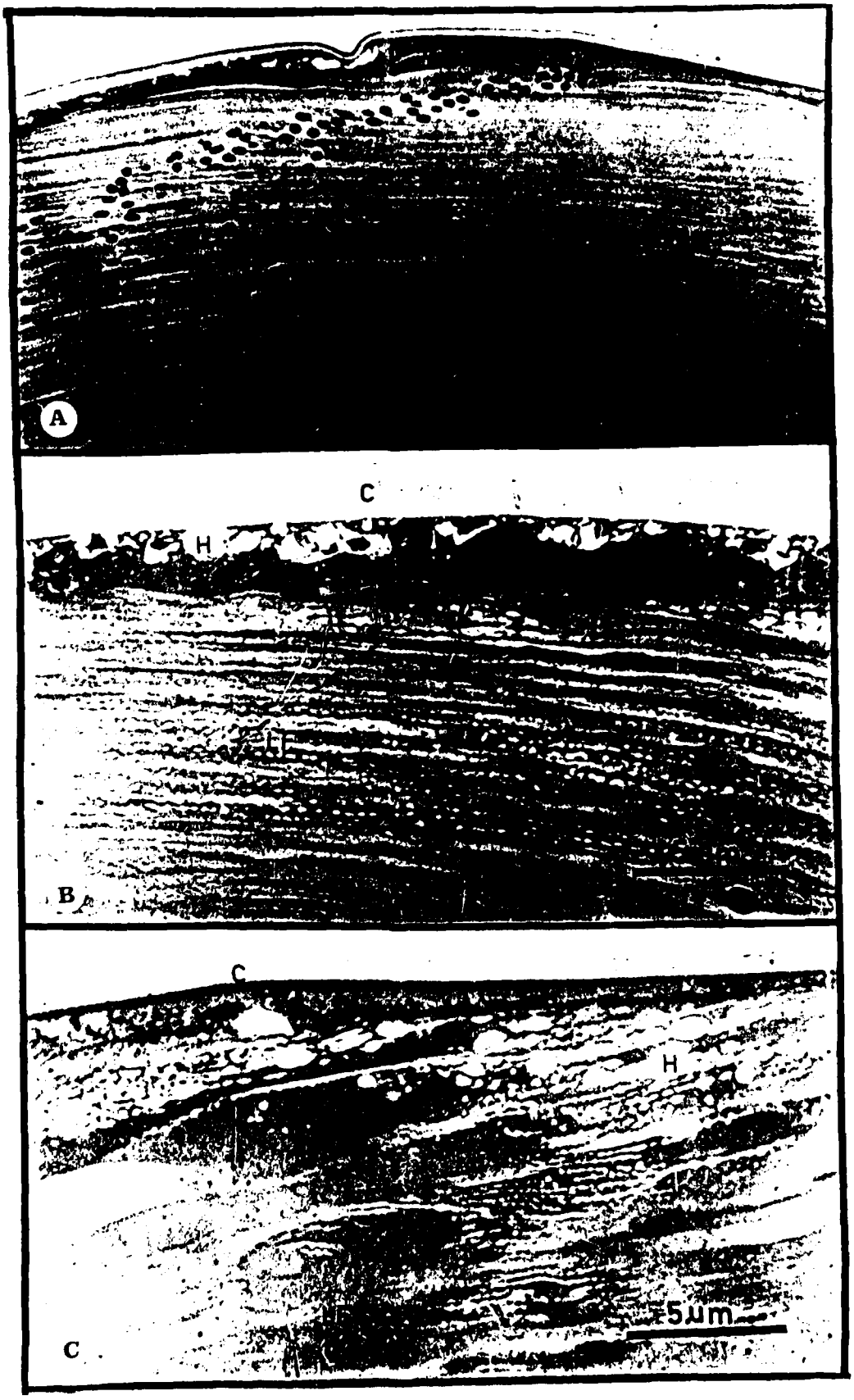

A
C
H
B
C
H
5µm
C

Figure 16. Thick Sections of Plastic-Embedded Rat Lenses: Lens was exposed to 2 W for 6 minutes pulsed (48 kW, 5 μsec) microwave irradiation (915 MHz). (A , B) General views of the lens showing abnormal orientation of the nuclei and lens fibers in the equatorial zone. (C) The epithelial from (A) shows degnerating nuclei and many vacuoles.

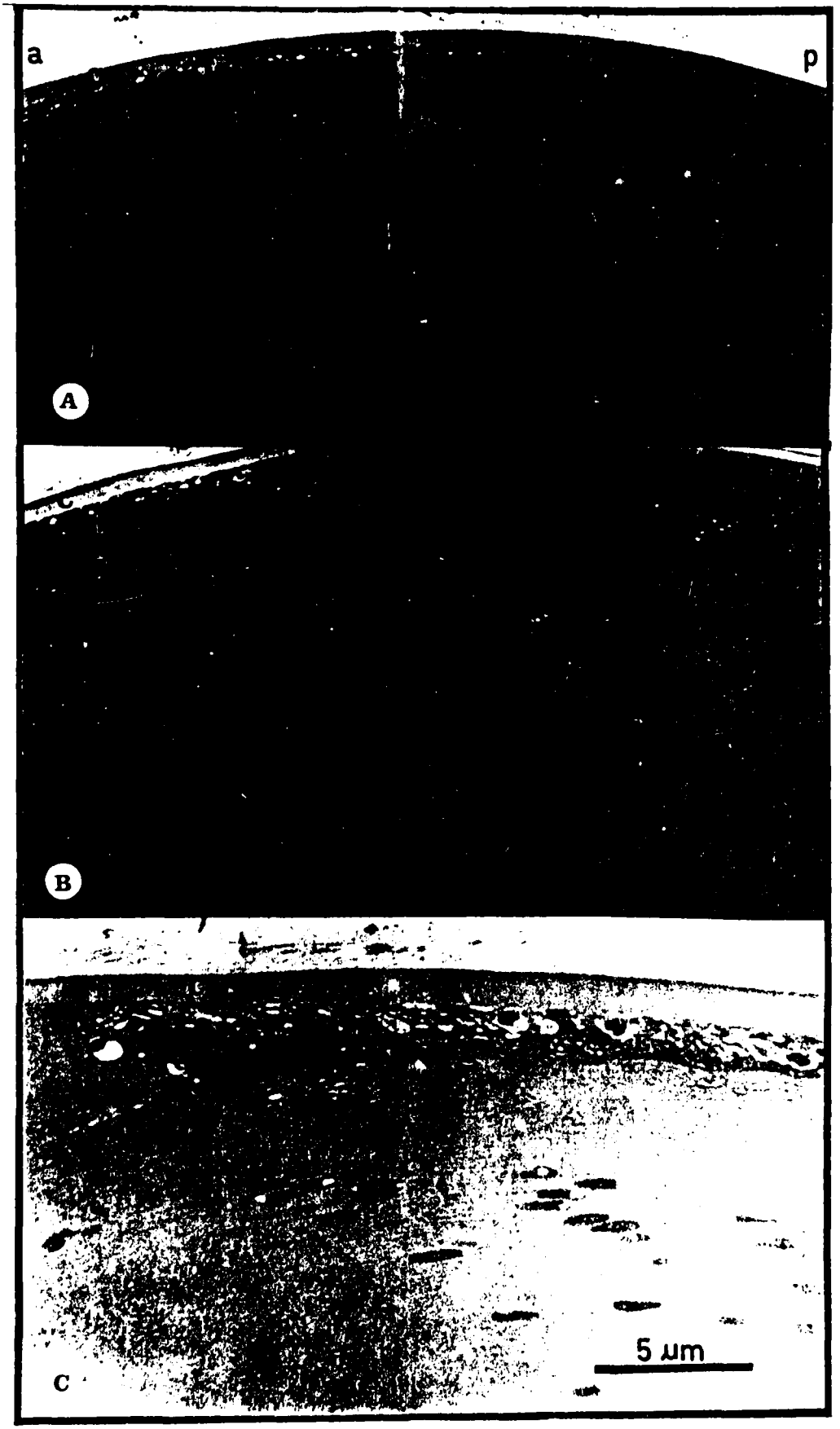

a
p
A
B
5 µm
C

Figure 17. Thick Sections of Plastic-Embedded Rat Lenses: Lens was exposed to 2 w for 6 minutes pulsed (48 kW, 10 μsec) microwave irradiation (915 MHz). (A) Equatorial region of the lens showing an abnormal capsule (C) which is dissociating from the underlying cells. The epithelial is bilayered with degenerating nuclei. (B) Epithelial layer from the anterior region showing many holes and degenerating nuclei.

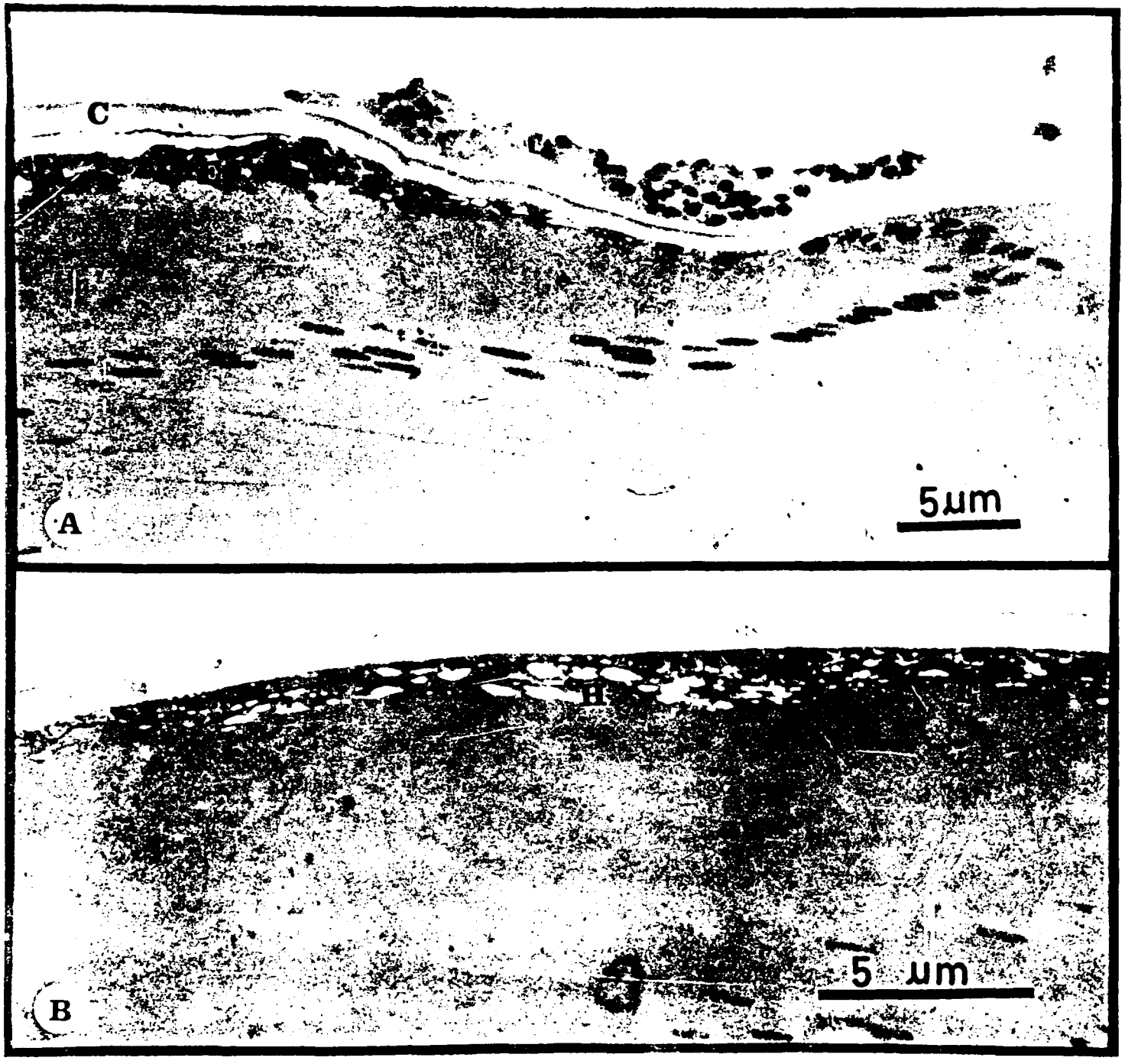

C
A
5µm
H
B
5 µm

Figure 18. Thick Sections of Plastic-Embedded Rat Lenses: Lens was exposed to 65 w for 6 minutes pulsed (48 kW, 5 μsec) microwave irradiation (915 MHz).
(A) Section through the equatorial zone showing a broken capsule (C), disorganized epithelium with degenerating nuclei.
(B) Part of the anterior region with disrupted capsule (C) and degnerating epithelial layer.
(C) Part of the equatorial region from (A) enlarged, showing disorganization of lens bow nuclei.

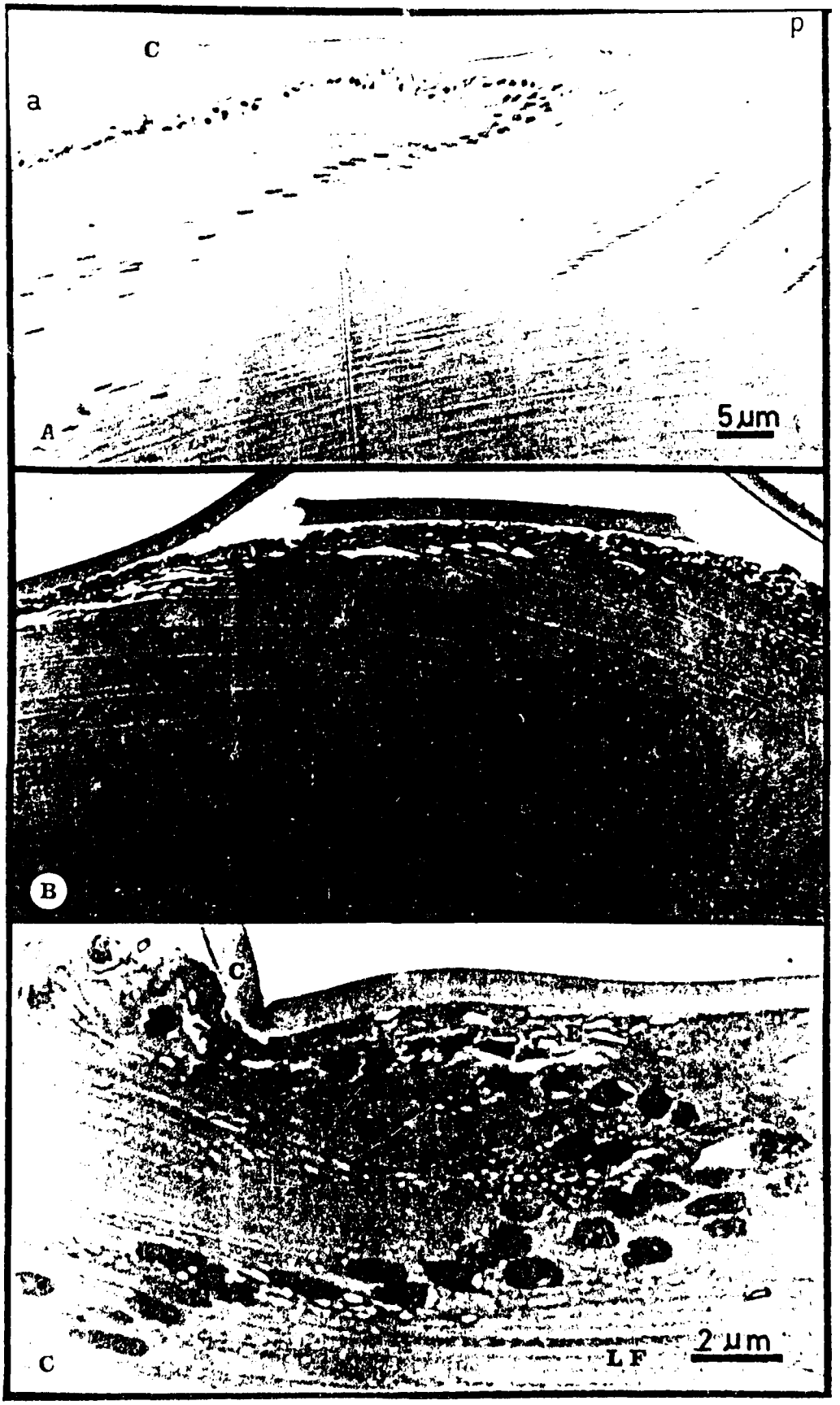

C
a
p
A
5 µm
B
C
C
LF
2 µm

Figure 19. Thick Sections of Plastic-Embedded Rat Lenses: Lens was exposed to 65 W for 6 minutes pulsed (48 kW, 5 μsec) microwave irradiation (915 MHz) showing various type of 'holes' and vacuoles for comparison see SEM results.
(A) 'Holes' (H) between the epithelial and lens fibers below.
(B) 'Holes' (H) seem to be inside the lens fibers as well as in between.
(C) Holes and vacuoles in the posterior region of the capsule.

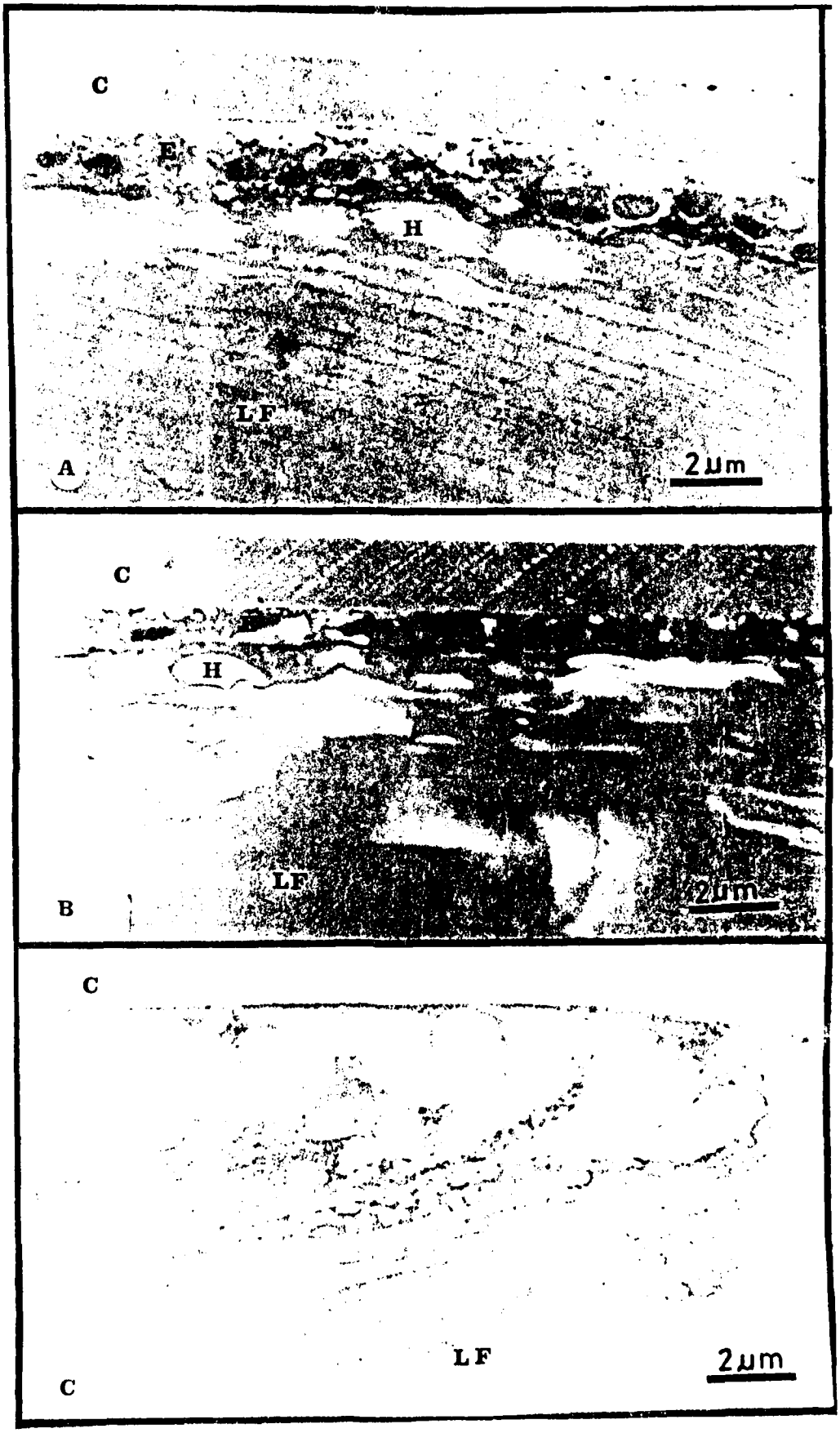

C
E
H
LF
A
2µm
C
H
LF
B
2µm
C
LF
2µm
C

Figure 20. Thick Sections of Plastic-Embedded Rat Lenses: Lens was exposed to 65 W for 6 minutes pulsed (48 kW, 10 μsec) microwave irradiation (915 MHz).
(A) Anterior region of the lens showing an incomplete capsule (C). The epithelial region is multilayered.
(B) Part of (A) enlarged to show multinucleate epithelium cell borders are not demarcated.
(C) Anterior region of the lens showing a lot of 'holes' and vacuoles under a degenerating epithelium.

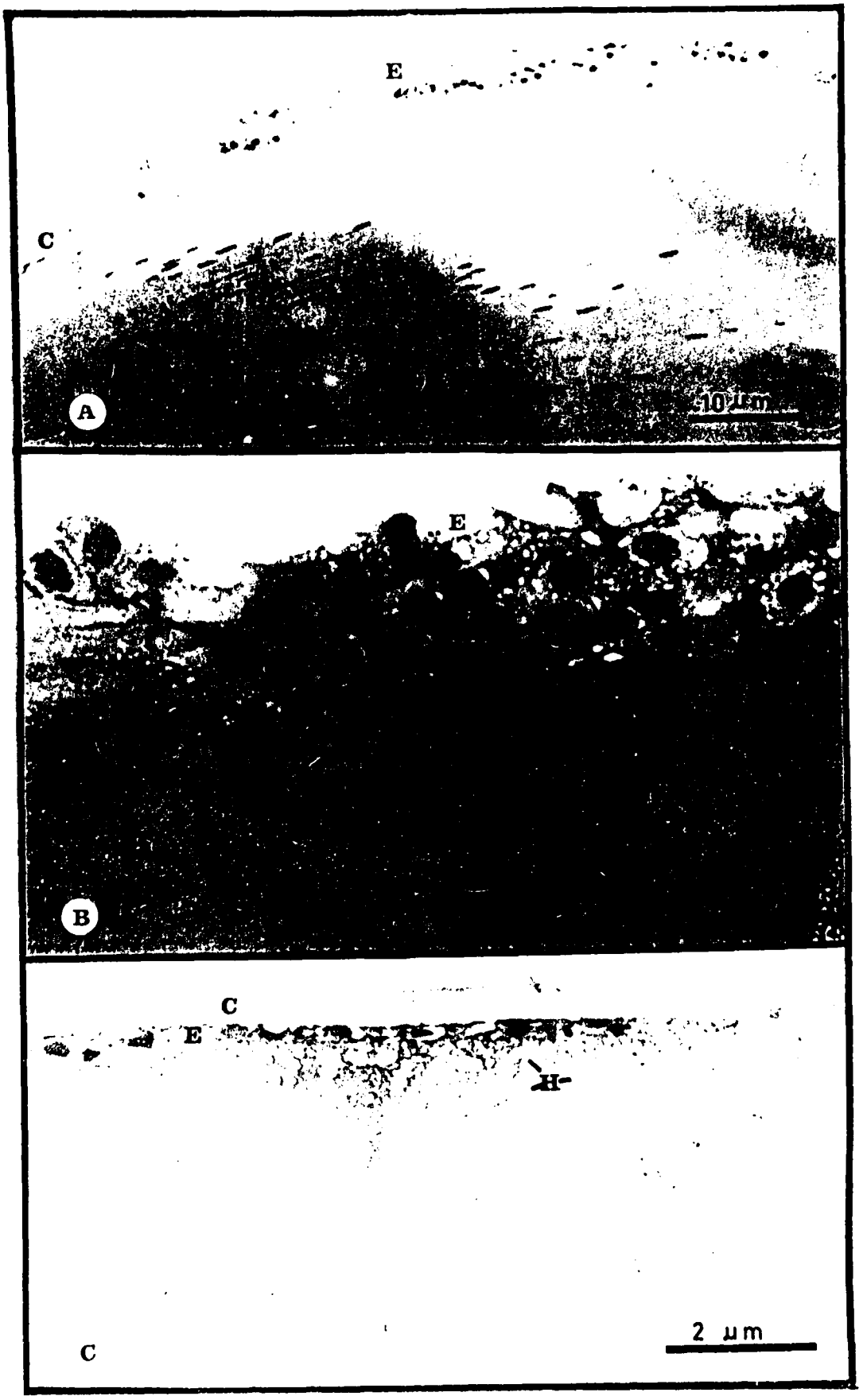

E
C
A
10 µm
E
B
C
E
H
C
2 µm

Fig. 21. Changes in Equatorial Lens Cortex at Low Doses of Microwaves in Rat Lenses Irradiated In Vitro and Fixed Immediately in Karnovsky's Fixative.

After exposure to pulsed microwaves for 6 min at the dose rate shown lenses were fixed, quartered as described previously (Mousa, Creighton and Trevithick, 1979) and examined by scanning electron microscopy. The area shown is the lens equator in the region of the zonules. Total doses of 360 joules and 180 joules respectively correspond approximately to S.A.R. values of 20 mW/g and 10 mW/g (if 6 min instead of 4 min for 3 joule dose is used for the calculation) respectively. Symbols: (C) capsule; (LF) lens fiber, (Z) zonular apparatus; (F) foam. The figure illustrates equatorial lens cortex, after exposure for time shown to pulsed microwave irradiation at 37 C using 20 μsec. pulses and peak power 48 KW and average powers (AP) and interpulse intervals (IPI) shown: (a) 4 min, (IPI) 1290 msec. (maximum possible IPI), total dose, 180 joules (3 per 0.05 g lens, 180 millijoule; (b) 6 min, AP m/W IPI, 960 msec, total dose 360 joules (or per .05 g lens 360 millijoules); (c) conditions as for (a), but at higher magnification, showing almost normal fiber appearance; (d) condition as as for (b), but at higher magnification showing area of subcapsular foam and granular cell surfaces.

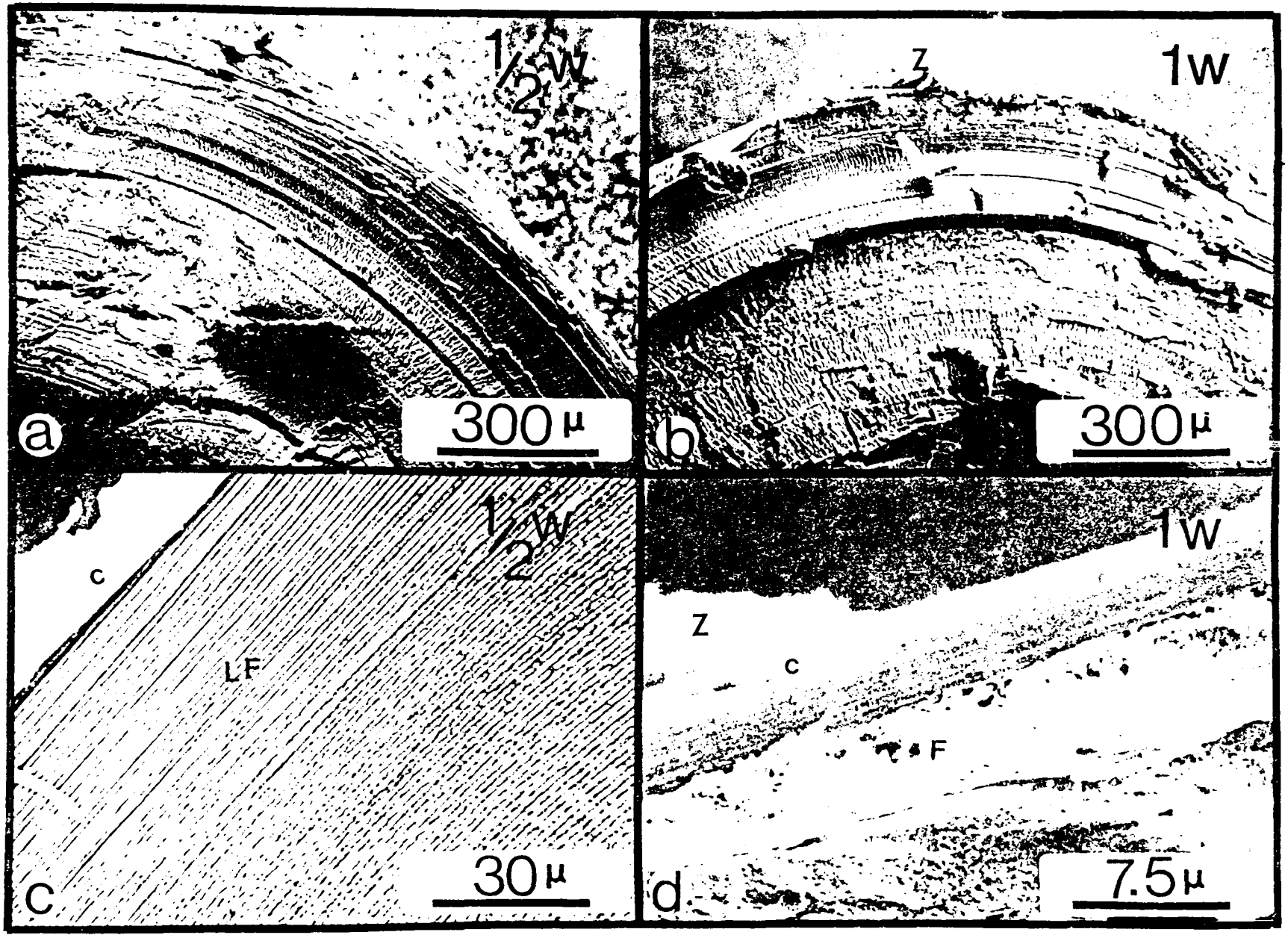

a
1/2W
300 µ
b
Z
1W
300 µ
c
C
LF
1/2W
30 µ
d
1W
Z
C
F
7.5 µ

Fig. 22. Changes in Equatorial Lens Fiber Cells at a Low Dose of Microwaves in Rat Lenses Irradiated In Vitro with 2 W for 6 min and Fixed Immediately in Karnovsky's Fixative.

Rat lenses were exposed to microwaves at total dose 2 W for 6 min, fixed immediately and quatered as described in Fig. 21, then examined by SEM. The subcapsular foam and granularity of the cell surfaces slightly deeper in the cortex may be seen more clearly in magnified areas (c) and (d) taken from areas shown in (b). The dose of 720 joules corresponds to an SAR value of 40 mW/g. Symbols: (c) capsule; (LF) lens fibers; (Z) zonular apparatus; (N) cell nucleus of elongating cortical fiber of bow; (F) foam. The figure illustrates lens fiber cells from equatorial lens cortex fixed after exposure for 6 min at 37 C to microwaves: 20 μ sec pulses at peak power 48 KW, average power 2 W, resulting in a total dose of 720 joules (per 0.05 g lens, 720 millijoules): (a) equatorial cortex just posterior region of zonular attachment, showing area of foam and granular cells surfaces between foam and well-defined lens fibers; (b) equatorial cortex at area of zonular attachment, illustrating similar subcapsular foam and, slightly deeper, granular cell surfaces; areas chosen for viewing at higher magnification in (c) and (d) are indicated; (c) subcapsular area of foamy degeneration shown in (b), illustrating cell nuclei and foamy appearance of cell surfaces; (d) subcapsular area of foam and granular cell surfaces shown in (b).

2w

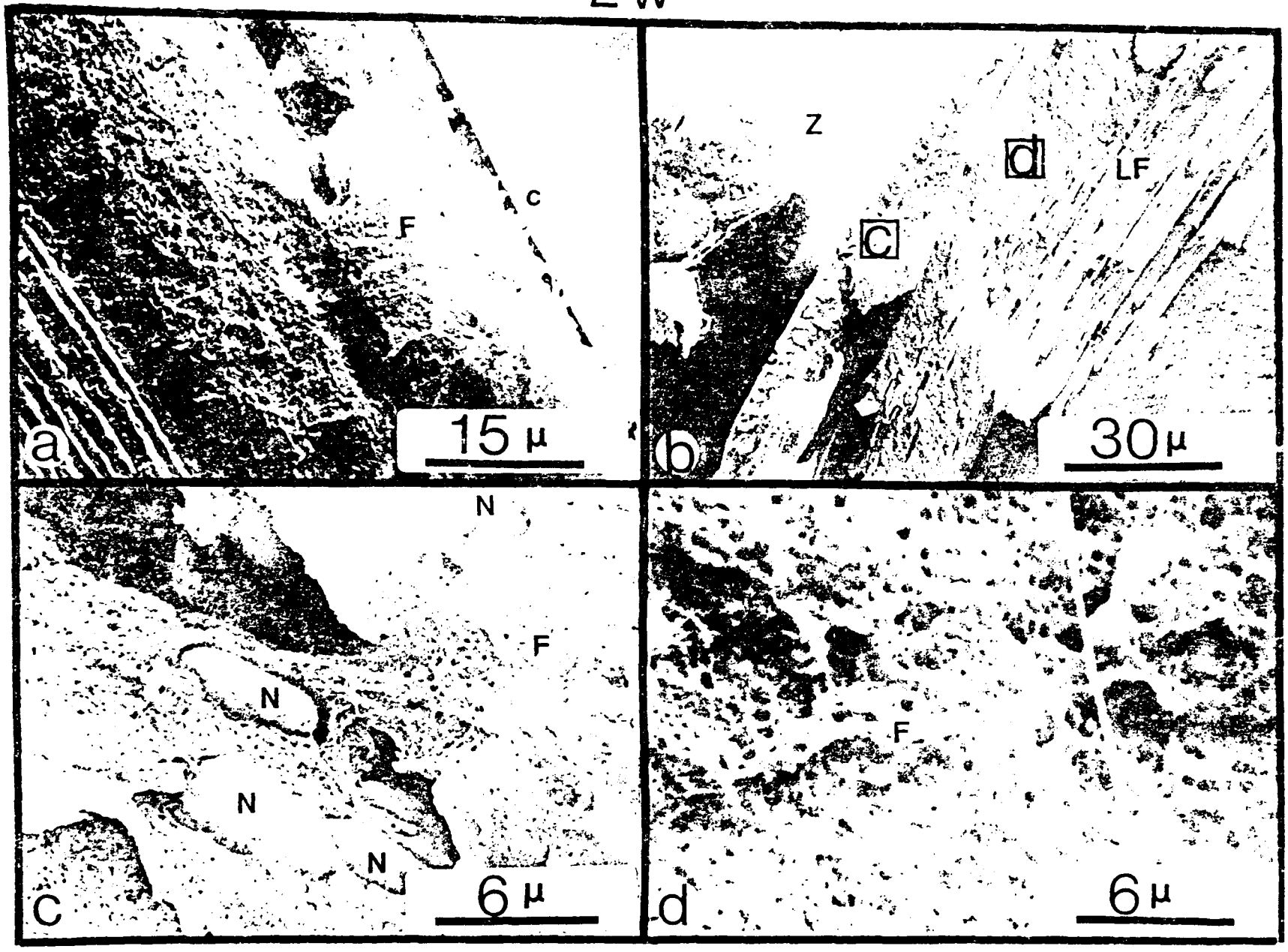


Fig. 23. Changes in Appearance of Capsule of Rat Lenses Irradiated with Pulsed Microwaves In Vitro and Fixed Immediately in Karnovsky's Fixative.

The capsular changes seen were striking and unexpected. The smooth appearance of the equatorial capsule surface seen in controls (not shown) and at 1/2 W becomes sandpaper-rough in appearance after exposure to 6 m microwave irradiation at 1 W and develops small bumps and long threadlike processes after exposure to 2W, offering evidence of mechanical disruption of the capsule. Exposure conditions, as for Figures 21 and 22; pulse width 20 μsec, peak power 48 KW, (a) 4 min average power to give total dose 180 joules (180 mJ/lens) IPI 1290 msec (at limit); (b) 6 min average power 1 W IPI 960 msec total dose 360 joules (360 mJ/lens); (c) 6 min average power 2 W to give total dose 720 joules (720 mJ/lens).

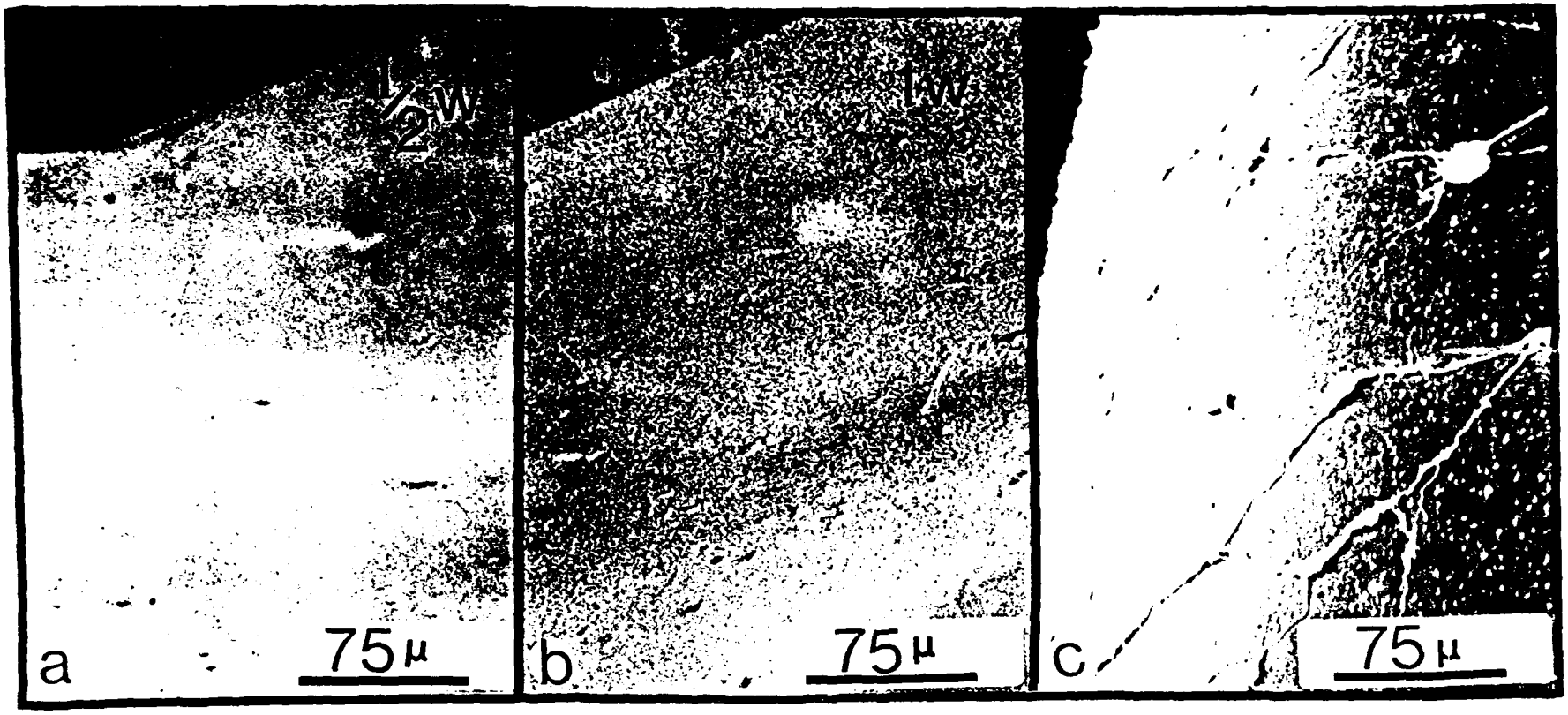
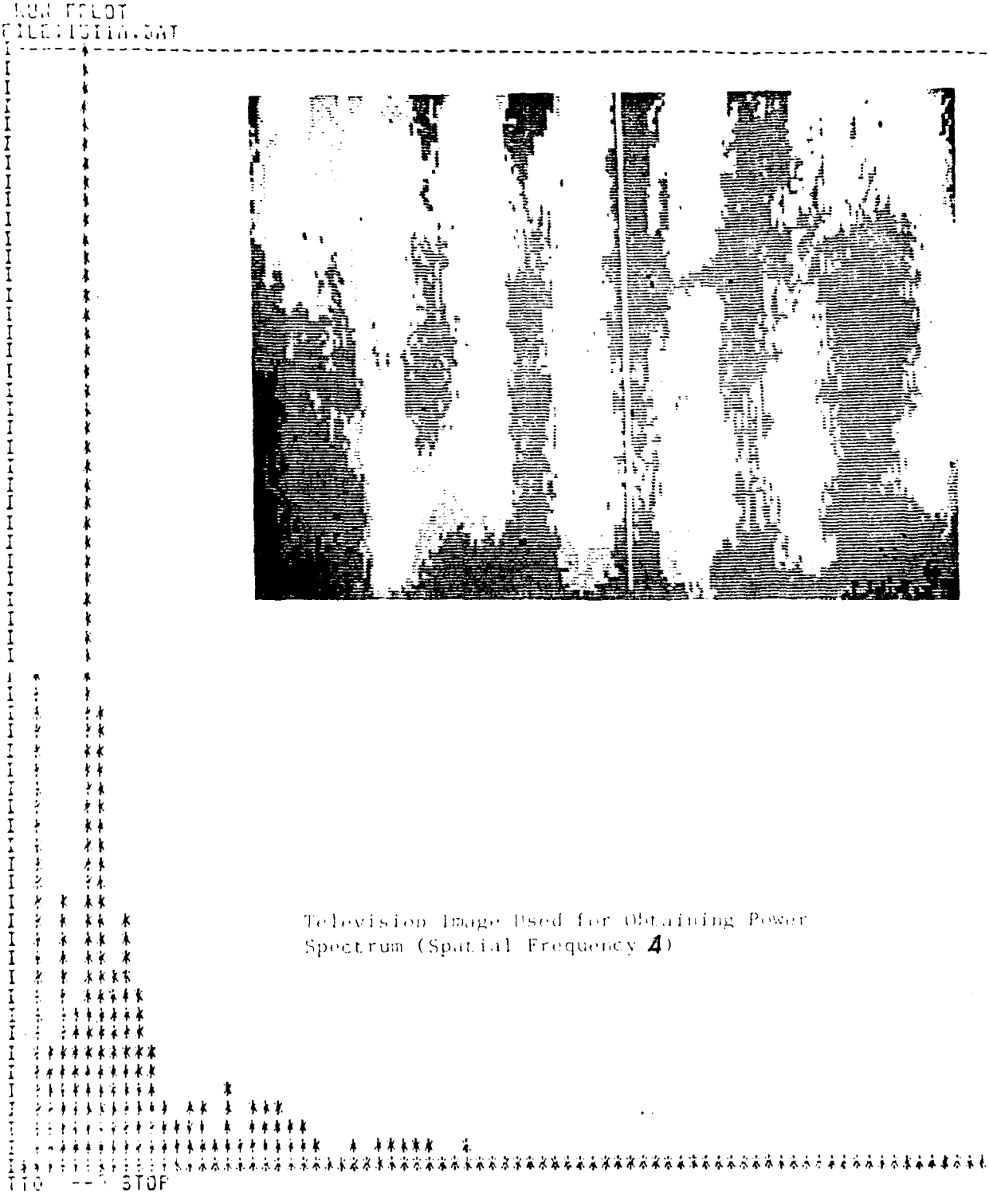

½w
a
75μ
1w
b
75μ
c
75μ

Figure 24. Power Spectrum Obtained Using Fixed Control Rat Lens at Spatial Frequency of Four.
Television images were obtained at different spatial frequencies by imaging different areas of a standard bar grating used to determine lens resolving power. The bar pattern shown in the photograph (upper right corner) was obtained. Using computer techniques, the intensity along each line was treated using the reverse Fourier transform technique to obtain a power spectrum for each horizontal television line. These power spectra were then summed vertically to elminate random noise which can be seen in the photograph. For the power spectrum illustrated in this example, the peak can be seen at a spatial frequency of four. In order to approximate the modulation transfer function, the percent of the total power which is in the peak (compared to total power in the noise peak) is plotted against the spatial frequency (see next figure, Fig. 25). In determining the power in the peak, the noise is assumed to be a logarithmically decreasing function of spatial frequency.



Television Image Used for Obtaining Power Spectrum (Spatial Frequency 4)

Spatial Frequency

Figure 25. Approximate Modulation Transfer Function of Control Fixed Rat Lens.
A close approximation of the modulation transfer function of the lens is obtained by plotting the percent of total power in the peak for different spatial frequencies, against the spatial frequency. A curve is obtained which shows decreasing resolution at increased spatial frequency. Cataractous lenses would be expected to show a more rapidly declining curve, as resolution falls off more rapidly at higher spatial frequencies (closer lines). We expect soon to try this with cataractous lenses.

Fig 25

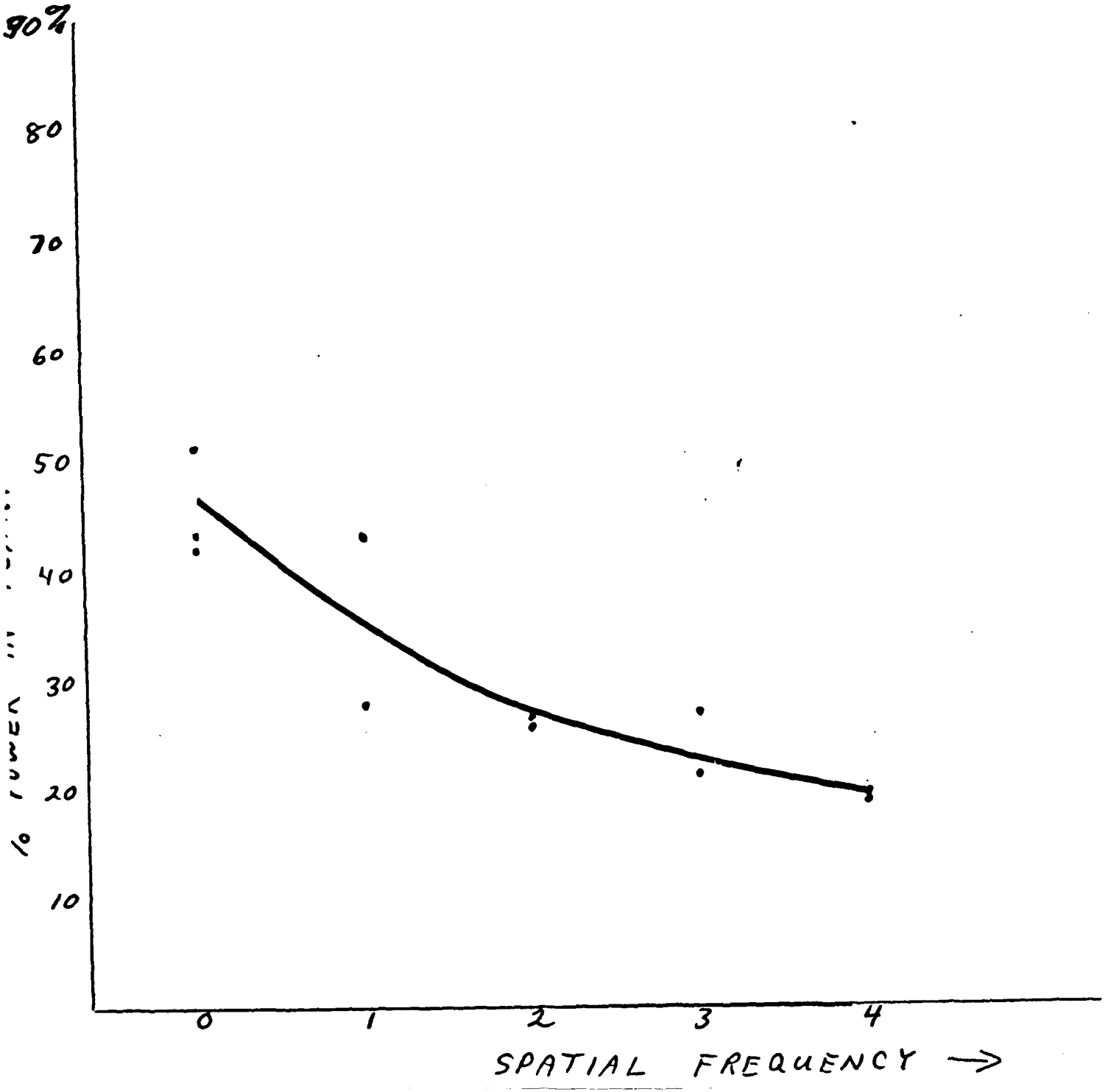


TABLE 1

ATP Levels in Lenses Heated to 42 C for 20 min and Exposed to 55.6 mM Glucose

Incubation Conditions	ATP ng/g wet lens*
Control Zero time	153
Control, Incubated 24 hr.	30.2
42 C (30 Min)	16.3
55.6 mM glucose	15.8

* Except for zero time controls which were frozen immediately, all lenses were incubated in groups of 3 and weighed. ATP levels were determined on guanidinium chloride homogenates of each lens by Lumac ATP kit and triplicates averaged.

TABLE 2

Determination of Protein Leakage From Lenses Undergoing Glucose-Induced Cataractogenesis In Vitro: Protective Effect of Vitamin C and Glutathione.

Additives	Concentration of Additive	Medium 199 Control (n)	Medium 199 + 56 mM Glucose (n)
		γ- Leakage (µg/mg lens) in	
Experiment 1			
-----	-------	18.4(15)	19(21)
C	5 mM	17.6(14)	12.8(22)
Experiment 2			
-----	-----	16.5(15)	17.3(14)
Glutathione	5 mM	10.4(14)	11.6(15)

The University of Western Ontario

Department of Biochemistry
Health Sciences Centre
London, Canada
N6A 5C1

April 26, 1982

Dr. Patrick Whippey
Department of Physics
University of Western Ontario
London, Ontario

Dear Patrick:

In our work we have often performed two dimensional separations of proteins using O'Farrell gels, obtaining patterns similar to those illustrated on pages 74-75 of our paper "Actin in the Lens: Changes in Actin During Differentiation of Lens Epithelial Cells In Vivo", Mousa, G.Y. and Trevithick, J.R. (1979), Exp. Eye Res. 29, 71-81 (Copy attached). I understand that the system you have available in the Physics Department can quantitate the amount of colour in each spot. Such quantitations would be helpful for this type of work and for use with radioactive proteins which are detected after such separations by exposure of the gel (treated with scintillators to enhance detection) to x-ray film.

Would you be so kind as to inform me whether you would be willing to collaborate with our group in quantitating such gels, and letting us know the maximum cost which might be involved? I am currently preparing a grant application and would appreciate receiving a letter giving this information as soon as conveniently possible.

Yours sincerely,

John R. Trevithick
Professor

JRT:blg

The University of Western Ontario

Faculty of Science
Department of Physics
London, Ontario
Canada, N6A 3K7

May 19th, 1982

Dr. John Trevithick
Department of Biochemistry
U.W.O.

Dear John

I shall be glad to collaborate with your group in the quantitative analysis of your gels. We have the necessary facilities available now to do a small number. We shall be able to handle larger numbers within a year or so, when our Norpak Image Display system becomes available.

Initially we shall be glad to work with you without charge. You can perhaps buy us the odd box of paper for the line printer. If your need expands to require us to process gets on a production line basis, we will charge a suitable sum per gel. I would guess that $5.00 per gel might be appropriate.

Yours sincerely

P. W. Whippey
Associate Professor of Physics

PWW/clb

END

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