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IN VITRO STUDIES OF MICROWAVE-INDUCED CATARACT III: *IN VITRO* LENTICULAR EFFECT ENHANCEMENT BY L-BAND PULSED VS CONTINUOUS WAVE MICROWAVE IRRADIATION OF RAT LENS

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Abstract

Damage to rat lenses *in vitro* caused by continuous (CW) and 24 kW_{pk} microwave pulsed (PW) wave radiation fits a model correlating damage to specific absorption (SA), a product of the specific absorption rate (SAR) and exposure time. This model was extended to include new exposure conditions: higher peak powers of PW radiation at additional pulse durations. The overall model related damage to the SA/dose of radiation. Increasing damage occurred at low doses as the pulse duration increased from 2 to 20 μsec or the peak power of the pulses increased from 24 to 600 kW_{pk}. The enhancement relative to CW irradiation was about 80 times greater at the lowest dose tested when 20 μsec pulses of 600 kW_{pk} microwaves were used. At a 10 μm deep layer of damage in the periphery of the lens, the SAR values for damage were significantly below currently accepted SAR values for the American National Standard Institute (ANSI) safety standard. Consistent with increased damage, experiments using a virtual cathode transmitter delivering 100-200 MW peak power pulses of similar total energy per pulse showed damage after only 10 pulses. The relative biological effect is analogous to that of heavy ion bombardment of the eye lens in experimental cataracts. This new data suggest the need for further exploration into the possibility and the mechanism of accumulative damage from chronic exposure to pulsed microwaves.

Key Words: Eye lens, pulsed microwaves, continuous wave microwaves, damage, thermoelastic expansion, thresholds, oxidative damage, free radicals, rat.

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Introduction

The induction of cataracts by microwaves has traditionally been thought to depend only on the temperature elevation occurring in the microwave-exposed eye lens as a function of the average power absorbed (Cleary, 1980). The rate of microwave energy deposition or specific absorption rate (SAR) in the lens is related but not identical to the elevation of lens temperature during exposure. Different viewpoints on this concept have been expressed. Kramar *et al*. (1975) found that the microwave-induced lens pathology occurred at a threshold temperature of 41°C (SAR 150 W/kg, for 100 minutes exposure). Additionally, Carpenter *et al*. (1977) concluded that elevation of lens temperature *in vivo* is a necessary condition for the production of microwave cataracts but that microwave exposure increased the degree of damage markedly.

The initial papers by our group discussed *in vitro* results (Stewart-DeHaan *et al*., 1981, 1983) which indicated damage, equivalent to that caused by a 30 minute temperature elevation, was caused by continuous wave (CW) power alone at 0.915 GHz in the absence of any lens temperature elevation. For exposure, the lens was placed in a 44.5 cc glass cell filled with standard (STD) physiological saline. Temperature elevation was limited to less than 0.5°C by circulating thermostatically regulated saline solution around the exposed lens. In the next paper, first in a series, our further experiments (Stewart-DeHaan *et al*., 1985) indicated that damage from pulsed or CW microwaves was related to total dose (SA) or energy deposited in the lens. Since the dose rate and the time required to produce a defined amount of damage were reciprocally related, the question raised was whether microwave damage might be cumulative over a longer period of time if the reciprocity extended beyond one hour, the longest period studied. The observations also suggested that pulsed exposure caused more damage than continuous wave (CW) exposure when given at the same average power and for identical times and temperatures. Although the question of reciprocity for longer periods of time is still unanswered, further

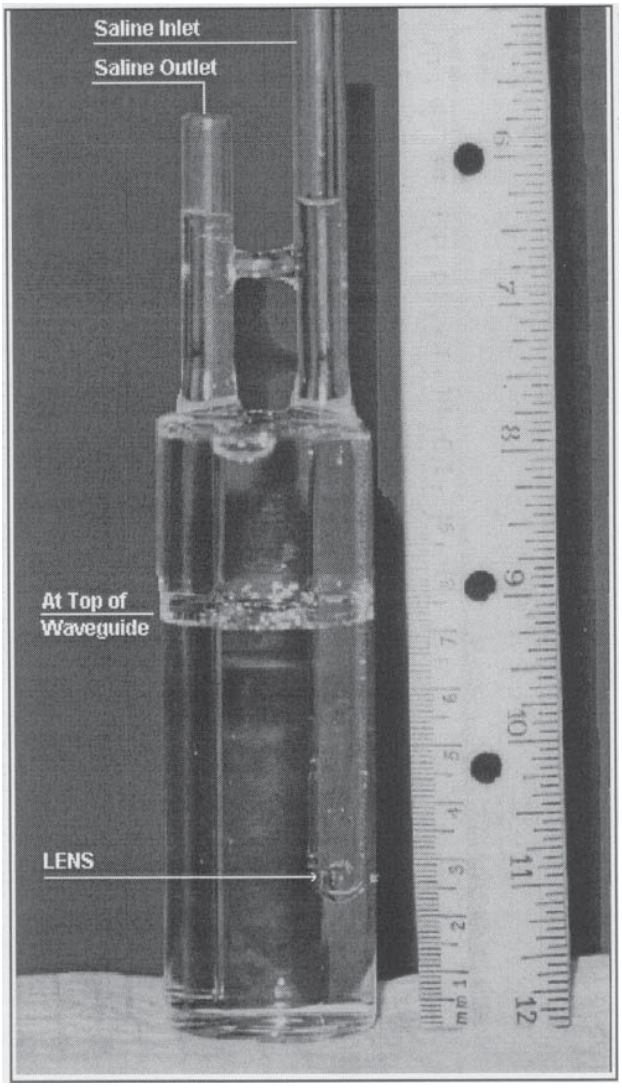


Figure 1. Illustration of wave guide irradiation cell for 600 kW irradiation in waveguide by Cober.

comparisons of damage described in the second paper (Creighton *et al*., 1987) indicated that two additional factors influencing the amount of damage observed by scanning electron microscopy were; (1) the ratio of peak to average power of the pulses and (2) the pulse duration in µsec. A first evaluation of the damage data indicated that repetitive, 10 µsec (sec = seconds) duration pulsed irradiation at 24 kW_{pk} (net peak power) is 4.8 times as damaging as CW irradiation for the same average power level. Comparisons were made for a number of different reciprocally related combinations of average power and exposure duration, all involving the same total microwave energy delivered to the lens under study. This damage was tentatively ascribed to microwave induced thermoacoustic effects (Guo *et al*., 1984) caused by thermoelastic expansion (TEE).

In this paper, to explore further the effects of different peak power and pulse durations, experimentation and modelling were extended to investigate pulse durations of 2, 10, and 20 µsec and net peak powers of 48 and 600 kW at the same average power levels, as well as a brief series of tests of a virtual cathode transmitter (Transformer Engaged Megavolt Pulsed Output, TEMPO; Raslan *et al*., 1993) with a peak power approximately 100 times greater than that possible with the most powerful transmitter used for the other exposures. These experiments indicated that at the same average power level, the relative damage caused by certain pulsed high-peak power microwaves was as much as 80 times more extensive than CW irradiation with the same average power, and the TEMPO exposures were even more damaging. An additional concern is that the ratio of relative damage level increases as the total energy in the lens decreases, i.e., the relative biological effect increases as the dose is lowered. This paper integrates the new microwave results and data obtained in the course of these latest investigations into a greatly extended overall model.

Materials and Methods

Biological

Lenses of Sprague-Dawley (Walter Reed strain) rats, 180-200 g average weight, were dissected and exposed to microwave energy *in vitro* as described previously (Stewart-DeHaan *et al*., 1983). Immediately following irradiation, lenses were fixed using Karnovsky's fixative (Graham and Karnovsky, 1960) and prepared for scanning electron microscopy (Stewart-DeHaan *et al*., 1983) or semi-thin plastic sectioning (Creighton *et al*., 1987) as described. The extent of damage was evaluated by measuring the depth of granular degeneration and foam and changed morphology occurring in the subcapsular region. For semi-thin plastic sections, the morphology was compared to normal morphology observed in unirradiated controls and sham-irradiated controls to allow for any experimental variation associated with the conditions used in the irradiation chamber, and in the preparation of the sample for scanning microscopy. Thus the only experimental variation between sham-exposed lenses and the experimentally irradiated lenses was the irradiation given. The unirradiated controls were used to assess any possible damage caused to the sham-irradiated controls by the lens exposure system. When abnormalities were visible, these would be selected for further examination and photography or storage as a digital image.

For irradiation at 600 kW_{pk} peak power, a circular glass cell was inserted into a waveguide irradiation port in WR 650 waveguide similar to that described previously (Creighton *et al*., 1987), except that the cell was circular and of larger volume (Fig. 1). The design of the waveguide

exposure system permitted tuning to maximum microwave absorption by the lens and saline-filled cell. The volume of phosphate-buffered saline in the cell when filled to the top of the inlet and exit tubes was 87.7 ml.

Specific absorption rates for the lens samples located in the cell were determined as previously described (Creighton *et al*., 1987) except that instead of the fiber optic temperature probe (Luxtron, Santa Clara, CA; response time 0.01 seconds) being placed in saline at the position of the lens, it was surgically inserted to a depth of 1-2 mm in the cortex of the lens. Using the actual experimental conditions for SAR determination minimizes theoretical concerns regarding possible changes in SAR distribution raised by Moten *et al*. (1989, 1991) for short pulses in disperse planar dielectrics. The time-averaged SAR values in the lens were determined at 37°C, with the circulating pump shut off, after temperature equilibration at two different frequencies using the following exposure conditions: (a) at 915 MHz, 20 kW peak power, pulse duration 20 μs, repetition frequency 50 Hz and average power 20 W, which was the same average power used for the con-tinuous wave (CW) dosimetry; (b) at 1250 MHz, 10 kW peak power, pulse duration 20 μs, repetition frequency 100 Hz, and average power 20 W, which was the same average power used for the continuous wave dosimetry at this frequency (details in Appendix 1). Each lens was irradiated four times with pulsed microwaves followed by four CW microwave exposures, and for the next lens, this order was reversed. A total of 6 lenses were exposed, and the six average values of the four determinations for pulsed or for CW exposures were analysed statistically to obtain the mean SAR ± SE (standard error). Only small differences, which were not statistically significant, were seen between the SAR values determined using pulsed or CW exposures. The resulting SAR values stated in W/kg per W incident power were: (a) at 1.250 Ghz (Cober, Norwalk, CT, transmitter source, 600 kW peak power), 6.46 ± 0.98 W/kg per W incident pulsed power and 6.26 ± 1.1 W/kg per W incident CW power, and (b) at 0.915 Ghz (Lucas Epsco Inc., Hopkinton, MA, transmitter source, 24 and 48 kW peak power, and for CW), 10.01 ± 1.50 W/kg per W incident pulsed power and 9.81 ± 2.10 W/kg per W incident CW power. These values are slightly less than those we determined previously at a frequency of 918 MHz with the probe immersed in the saline at the position of the lens in the irradiation cell (Creighton *et al*., 1987): 11.5 W/kg per W incident power. They compare favorably to the previous data, which assumed the specific heat of the tissue to be equal to that of water. Since that time, Foster *et al*. (1982a,b) pointed out that the lens water content varies greatly (Philipson, 1969), since protein distributions within the normal adult rat lens vary from 0.30 (cortex) to 0.90 (center of the nucleus) g/cm^3, mostly water forming the remainder. Taking 1.3 g/cm^3 for the density of protein, water weight fractions range from 0.72 (cortex) to 0.26 (nucleus), yielding heat capacities of 0.83 to 0.55 cal/g°C respectively. The appropriate value to use in these experiments is that for the lens cortex "average" value, since the pathology observed is all measured from the capsular surface inward as a depth of penetration of damage. This is C_p = 0.83 cal/g°C, being the best choice short of measuring the lens thermal properties directly (details in Appendix 2).

The formula used for the specific absorption rate calculations by both methods is:

$$SAR_n(W/kg) = k*4.186\ J/cal*(\Delta T\ in\ °C)/(\Delta t\ in\ s)*C_p\ cal/g°C$$

where k = the conversion factor (W/kg = 10^3 mW/g)/ (incident P_{ave}) and the SAR_{pk}(peak) W/kg = SAR_n * P_{pk}(peak power).

The average powers and exposure times were varied, as previously reported (Creighton *et al*., 1987), to give a number of reciprocal combinations which resulted in the same total dose being absorbed by the lens. The temporal peak SAR for 600 kW_{pk} irradiation was 3.876 MW/kg in the lens cortical region.

Irradiation apparatus

The irradiation apparatus and conditions for irradiation with CW and pulsed irradiation at 24 and 48 kW peak power levels at 0.918 GHz were described previously (Creighton *et al*., 1987). Separate series were run for each pulse duration (2, 10 or 20 μsec) since pulse duration was used as one of the variables in the overall model. For each pulse duration, as reported previously (Creighton *et al*., 1987), the dose rate was varied by changing the pulse repetition rate, since the average power per pulse was known.

For the 600 kW_{pk} peak power system, pulsed microwaves were generated by a modified Cober transmitter with a pulse modulated 1 MW klystron amplifier. A frequency of 1.25 GHz was used for the pulse widths of 2, 10, and 20 μsec. The volume of saline contained in the portion of the cell below the top of the 1.25 GHz waveguide was 58.4 ml (Fig. 1). This differed from the 44.5 ml saline volume of the previous cell used in the CW, 24 kW_{pk} and 48 kW_{pk} irradiations at 0.915 GHz (Creighton *et al*., 1987). Virtually all of the power incident on the cell was absorbed due to careful impedance matching in the exposure system. During all irradiations, the lens sample was cooled by circulating phosphate-buffered saline through the cell using the same specially built thermo-regulated system (Fig. 1).

To test the hypothesis that extremely high peak power pulses, even of short duration, could cause observable damage to the lens at nonthermal average power levels, an experiment was performed using a unique new transmitter. The transmitter, TEMPO (Raslear *et al*., 1993) used a special virtual cathode oscillator which generated incident peak power levels of 100-200 MW and 10-20 nsec

Table 1. Mean depth of damage at 600 kW peak power by SAR, Time and PW.
Table 1a. Depth of damage for pulse width = 10 μsec.

	SAR (W/kg)	O (Sham)	3.23	6.46	16.15	32.3	64.6	193.8
Exposure Duration	Power (W)	0	0.5	1	2.5	5	10	30
6 minutes	Mean	11.7	10.3	22.0	14.0	175.0	53.0	35.3
	Count	2	3	6	2	1	2	1
	SD	9.4	21.6	22.4	7.1	0	6.6	0
20 minutes	Mean	10.7	20.5	18.2	9.8	85.3	84.3	53.9
	Count	2	2	4	2	2	1	1
	SD	4.5	17.2	18.9	1.1	6.7	0	0
60 minutes	Mean	7.3	79.2	55.0	10.4	83.8	119.4	104.4
	Count	9	2	7	2	2	2	2
	SD	4.3	34.1	58.4	0.9	8.8	22.5	37.2

Table 1b. Depth of damage for pulse width = 20 μsec.

	SAR (W/kg)	O (Sham)	3.23	6.46	16.15	32.3	64.6	193.8
Exposure Duration	Power (W)	O	0.5	1	2.5	5	10	30
6 minutes	Mean	5.9	26.1	5.5	60.8	67.5	16.7	28.6
	Count	3	2	4	4	1	2	2
	SD	2.6	3.0	4.0	22.1	0	6.5	7.4
20 minutes	Mean	20.9	34.5	32.1	65.7	59.8	35.3	41.9
	Count	6	1	4	4	2	2	2
	SD	10.6	0	18.2	38.6	11.7	7.5	2.7
60 minutes	Mean	22.0	38.3	27.6	67.3	50.0	41.9	54.2
	Count	6	1	4	4	1	2	2
	SD	9.2	0	17.7	33.3	0	20.4	8.3

duration at a frequency range of 3000 ± 200 MHz. For these experiments, lenses were placed in phosphate buffered saline for irradiation at a distance of 2 m from the antenna source. The total duration of exposure for the lenses varied from approximately 1 minute for the lens exposed to 10 pulses to less than 10 minutes for the lens exposed to 80 pulses, following which the lenses were fixed immediately. The peak SAR found for rat eye at the position at which the lenses were exposed was 5 MW/kg for rat and 15 MW/kg for exposure of a monkey head. If this result was applied as an approximate indication of the average dose per pulse, it could be calculated to be 0.1 to 0.3 J/kg per pulse respectively, or for a series of 10 pulses, 1 to 3 J/kg. The corresponding SAR would be (for 10 pulses in one minute) 0.0167 W/kg or (for 80 pulses in 10 minutes) 0.013 W/kg.

Although 15 kev ionizing radiation was also present at the site of irradiation at a level of 1 milliroentgen per pulse, the total energy absorbed from this source would not be damaging based on previous experience (Ross *et al*., 1983, 1990) in which rat lenses were exposed to higher energy γ-irradiation from ^{60}Co.

Statistical analysis of SEM data

To find the lowest SAR that produces lens damage

Table 2. P-values from 3-way ANOVA on the 600 kW series.

Factor	P <
SAR	0.001
TIME	0.005
Pulse Width (PW)	0.26
SAR x TIME	0.05
SAR x PW	0.001
TIME x PW	0.22
SAR x TIME x PW	0.69

significantly different from that observed in "sham-exposed" lenses, lenses were placed in the standard waveguide/saline cell system without microwave irradiation. The mean depth of damage was measured in samples which had received time averaged SAR's of 3.23, 6.46, 16.15, 32.3, 64.0, and 193.8 W/kg (600 kW_{pk} peak power). Damage was compared with that observed in "fresh" and "sham" exposed lenses prepared during this series. The comparisons were made using the modified Tukey method of multiple comparisons (Winer, 1971).

Effects of SAR, time and pulse duration in the 600 kW_{pk} series When the observations from the 600 kW_{pk} peak power series were included for each SAR level, data were available at the six levels of average SAR cited above. For each SAR level three exposure times (6, 20, 60 minutes) and three pulse widths (2, 10, and 20 µsec), were used. Also data were obtained for sham exposed lenses. A three factor analysis of variance (ANOVA) was applied to the 7 x 3 x 2 factorial design with unequal numbers of observations at the various combinations of seven specific absorption rates (including 0 W/kg shams), at the three exposure durations (TIME), and two pulse widths (PW). In this analysis, the main effects of each factor were assessed with the other two factors held constant. Similarly, the two and three-way interactions were assessed with all main effects and interactions of lower order held constant. This means, for example, that the test for the main effect of SAR was based on the variation attributable only to SAR after the variation attributable to TIME and PW had been removed.

ANCOVA (Analysis of covariance) analysis of data for 24, 48, and 600 kW peak This analysis included exposure times (6, 20, and 60 minutes, pulse widths (2, 10, and 20 µsec) and peak powers (24, 48, and 600 kW peak) as factors. The SAR factor was treated as a covariant. The data are unbalanced, because for pulse peak power 24 kW only data for 10 µsec pulses were available. The software used (SAS/GLM, SAS Institute, 1984) is unable to estimate means adjusted for SAR levels when all interactions are included in the model. For this reason, a model with no interactions between pulse width and other factors was fit to the data.

Table 3. Interactions ANCOVA analysis.

Factor	DF (Degrees of freedom)	Significance levels (P-values)
SAR	1	0.0001
SAR*RPP	2	0.0001
SAR*RTIME	2	0.0250
PP	2	0.0010
TIME	2	0.0001
PW	2	0.0001
PP*RTIME	4	0.0006

General model As described in our previous paper (Creighton *et al*., 1987), the data were fit to a log-linear model. A general model based on our earlier study was developed to express the depth of damage (DEP) as a function of power (P) and SAR x TIME (specific absorption, SA) for CW and pulses (at 24, 48, and 600 kW_{pk}). The model was obtained by fitting all data by multiple regression. The estimates for the coefficients were obtained by transforming the model to its log-linear form with log (P) set equal to zero for CW data (Fig. 3). This model allowed for the possibility of interaction between the reciprocal effect of SAR x TIME and peak pulse power (PP_{pk}): that is, the hypothesis, that the effect of SAR x TIME was the same for each level of PP_{pk}, was tested in the process of fitting the model.

Results

For lenses fixed immediately after exposure, the damage observed by SEM was similar to that previously described (Creighton *et al*., 1987), which was of several types: (1) holes within the fiber cells, especially in the equatorial region of the lens; (2) capsular effects of surface granulation or pitting; (3) globular degeneration starting first in the equatorial region and sometimes in the anterior and posterior subcapsular regions; (4) foam or bladder cells (swollen cells of the lens epithelium often observed in cataractous lenses; Yanoff and Fine, 1989) located subcapsularly; and (5) granular appearance of fibre cell surfaces. The damage noted in lenses fixed immediately is less than the damage developing in lenses that were fixed after 48 hours incubation of the lens; in the latter, more extensive globular degeneration and foam were seen.

Typical patterns of damage produced by the 600 kW_{pk} exposures (Fig. 2) are similar to that observed with 24 kW_{pk} 10 µsec pulses shown previously (Creighton *et al*., 1987). *In vitro* exposure at 48 kW_{pk} (not shown) caused

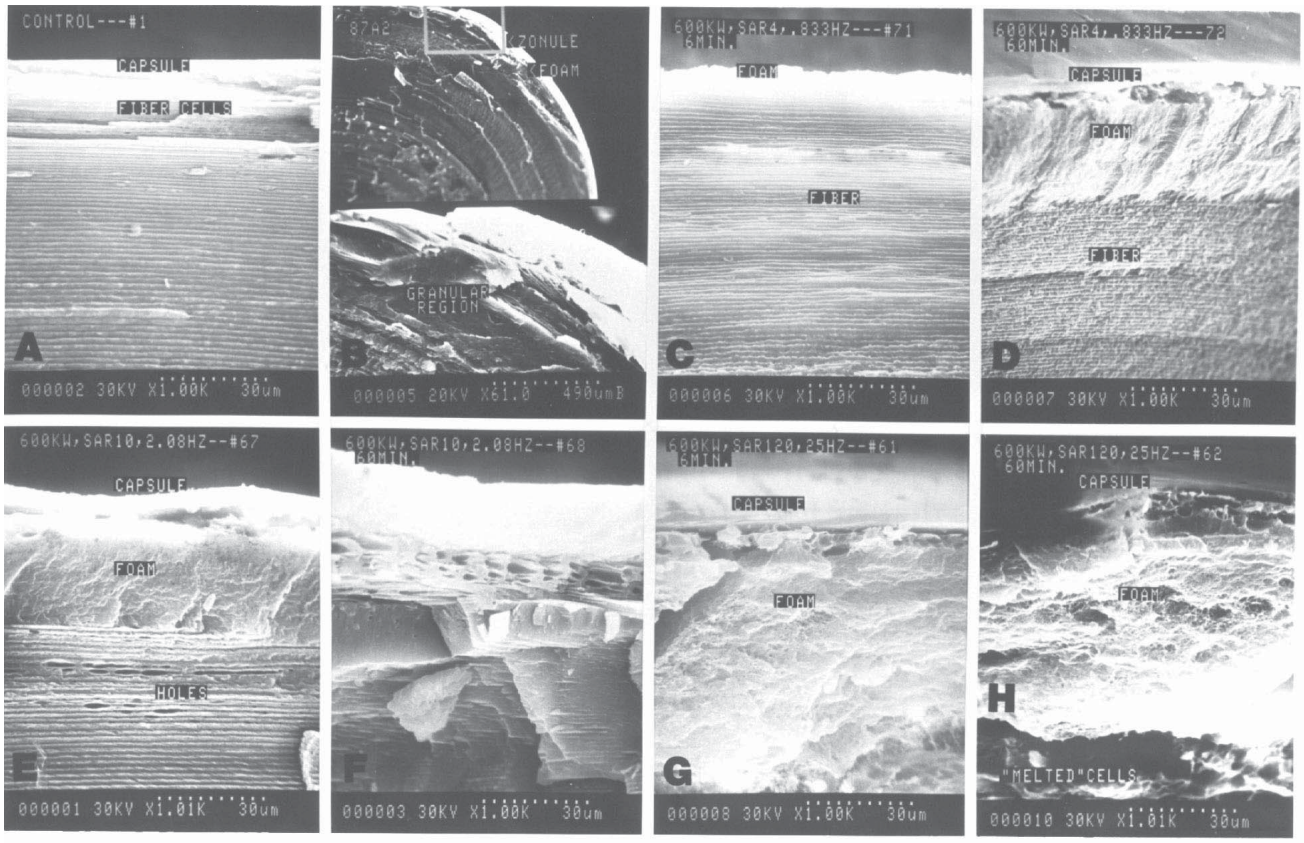


Figure 2. Scanning electron micrographs of lenses exposed *in vitro* to 0.6 MW peak power pulsed microwaves: 10 μsec pulse duration was used. Irradiation conditions to obtain SAR's indicated are described in the text. All lenses were fixed immediately after exposure in Karnovsky's fixative and prepared for SEM as described. Lenses were exposed as follows: (**A**) Unirradiated control showing lens capsule subcapsular region and fiber cells. (**B**) SAR 6.5 W/kg for 60 minutes, showing zonular attachment, subcapsular foam (bladder cells), and at higher magnification (inset), the granular cellular surface. (**C**) SAR 6.5 W/kg for 6 minutes, showing subcapsular foam. (**D**) SAR 6.5 W/kg for 60 minutes, showing capsule, subcapsular foam, and underlying fiber cells. (**E**) SAR 16.2 W/kg for 6 minutes, showing capsule, subcapsular foam bladder cells, and holes and vacuole in underlying fiber cells. (**F**) SAR 16.2 W/kg for 60 minutes, illustrating subcapsular foam and holes in exposed cell surfaces of underlying fiber cells. (**G**) SAR 193.8 W/kg for 6 minutes, showing capsule and subcapsular foam. (**H**) SAR 193.8 W/kg for 60 minutes, showing capsule, subcapsular foam and "melted" cells, underlying which appear to have fused as a result of the irradiation.

similar damage.

Effects of SAR, TIME and pulse width in the 600 kW_{pk} series

The data for the 600 kW_{pk} exposures are tabulated in Table 1. For the major effects and interactions of ANOVA analysis, the p-values (Table 2) suggest that effects of pulse width (PW, the common factor), TIME x PW, and SAR x TIME x PW are not statistically significant. SAR had a significant effect, however it appears that there is no increase in DEP beyond a time-averaged SAR of 32.3 W/kg. The significant interaction between SAR and TIME is due to the large value of DEP at a SAR = 32.3 W/kg and TIME = 6 minutes. Until the SAR equals or exceeds 32.3 W/kg, there is no consistent difference between depth of damage at the two pulse widths (10 and 20 μsec), while above this level these pulse widths show consistent differences.

ANCOVA using model without interactions between pulse width and other factors

As can be seen from the analysis of variance table (Table 3), all factors have significant p-values ($p < 0.05$), however the adjusted means suggest that the dependence on peak power is less significant when the level of SAR is taken into account. The SAR values (Table 4) were positively associated with the depth of damage (i.e., the higher the

Table 4a. Microwave reciprocity study (depth of damage) at 24 kW peak power.

	SAR (W/kg)	20	60	200	650
Exposure Duration	Power (W)	2 watts	6 watts	20 watts	65 watts
6 minutes	Mean	0.625	21.433	42.475	51.560
	Count	4	3	4	5
	Sum	2.5	64.3	169.9	257.8
	SD	0.750	4.704	1.394	6.140
20 minutes	Mean	33.500	39.813	54.638	180.000
	Count	4	4	4	4
	Sum	134.000	159.250	218.550	720.000
	SD	2.656	2.095	1.775	8.416
60 minutes	Mean	41.620	51.112	185.625	203.750
	Count	5	4	4	2
	Sum	208.100	204.450	742.500	407.500
	SD	1.702	1.288	8.985	1.768

Table 4b. Microwave reciprocity study (depth of damage) at 48 kW peak power.

	SAR (W/kg)	20	60	200	650
Exposure Duration	Power (W)	2 watts	6 watts	20 watts	65 watts
6 minutes	Mean	9.775	45.750	49.750	76.600
	Count	4	4	4	4
	Sum	39.100	183.000	199.000	306.400
	SD	4.862	22.456	7.377	38.066
20 minutes	Mean	17.625	60.000	75.625	91.875
	Count	4	3	4	4
	Sum	70.500	180.000	302.500	367.500
	SD	6.028	6.614	17.452	9.331
60 minutes	Mean	51.625	75.000	133.667	82.500
	Count	4	4	3	1
	Sum	206.500	300.000	401.000	82.500
	SD	6.625	10.206	5.132	0

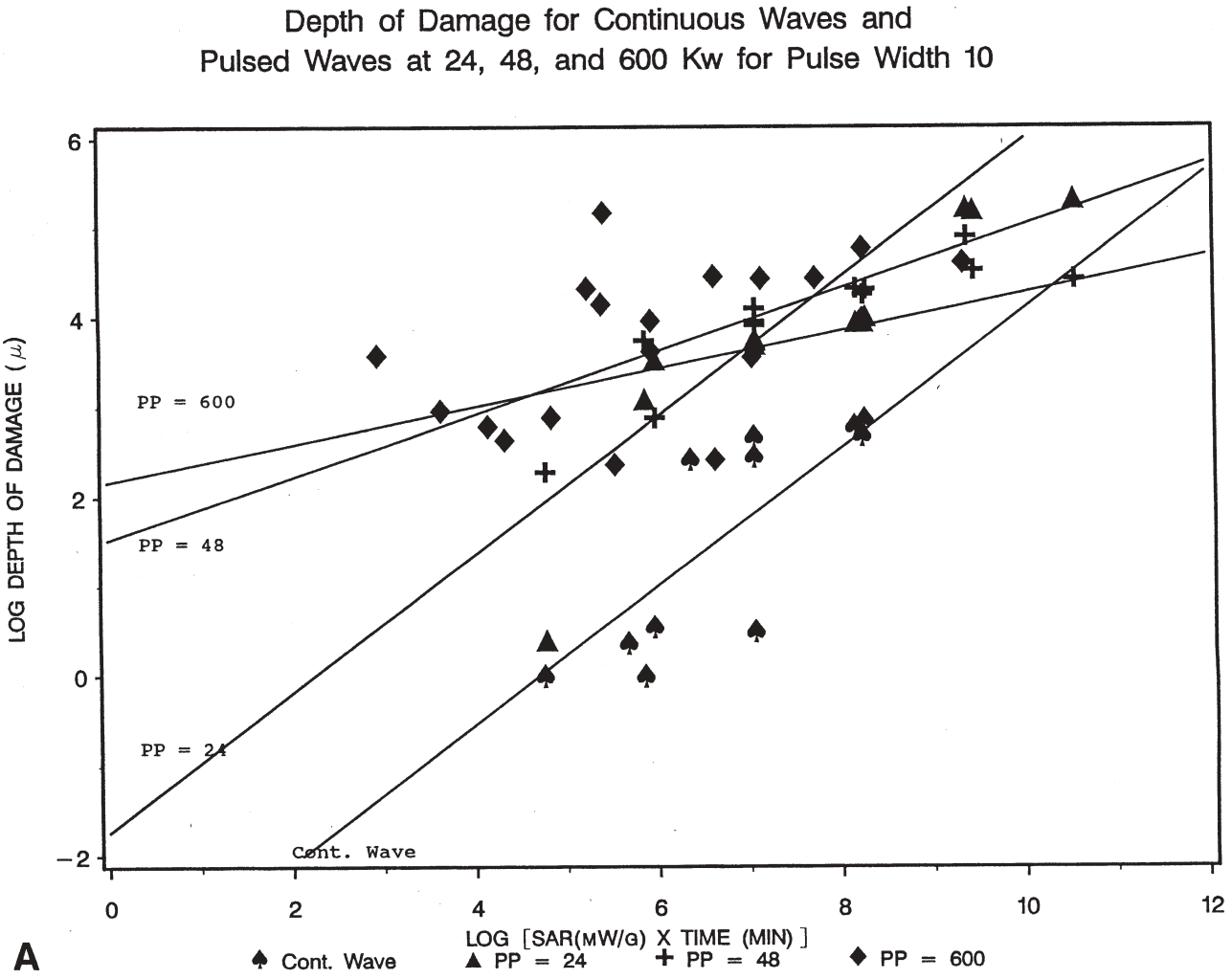


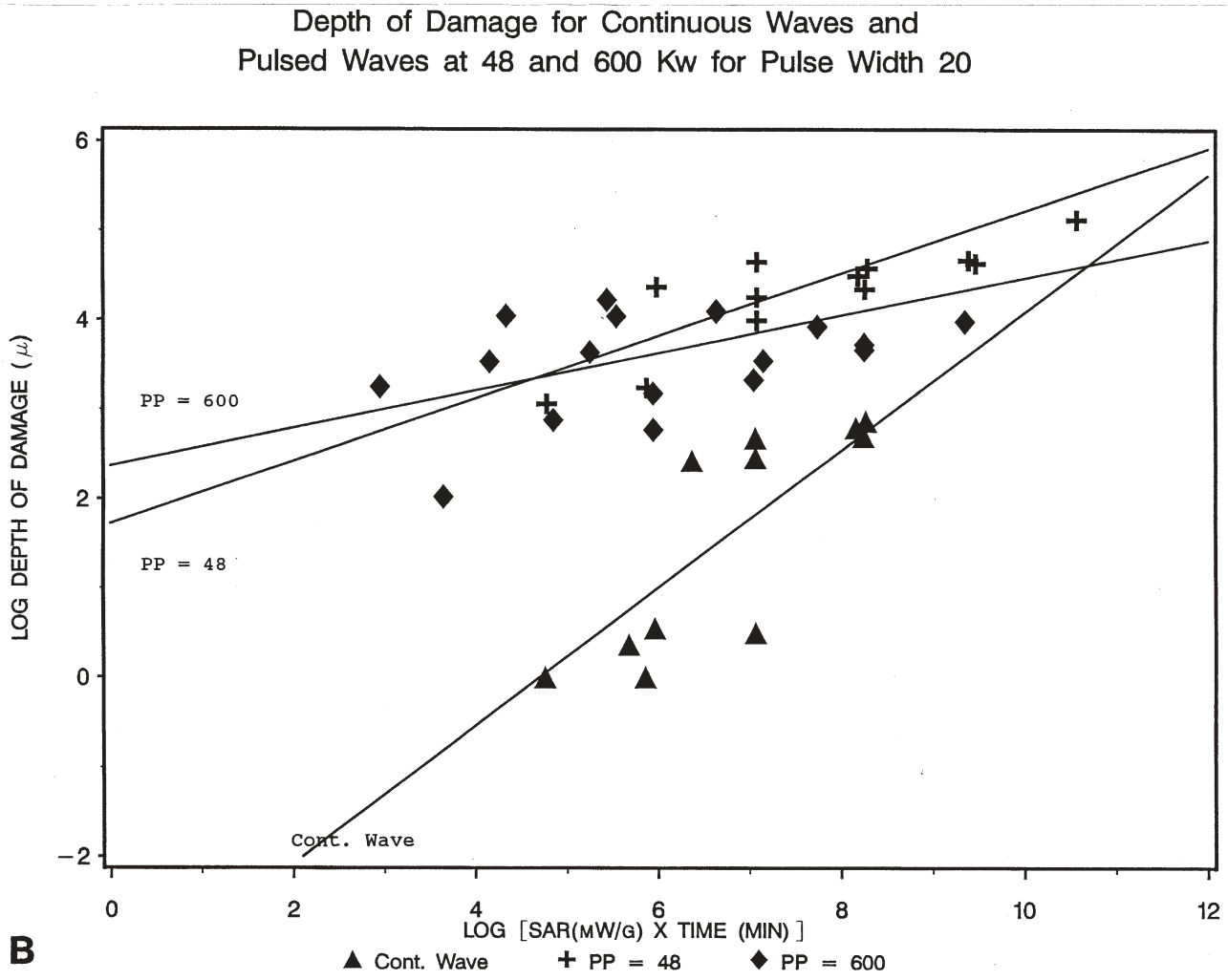
Figure 3. Overall equations for the depth of damage at different pulse durations and peak pulse powers fit to the model at pulse peak powers CW, 24, 48 and 600 kW as discussed in the text. (**A**) Curves for 10 μsec pulses; (**B**) (*on facing page*) curves for 20 μsec pulses.

SAR, the greater the depth of damage, regression coefficient = 0.115, or the depth of damage increases by 0.115 μm for each unit increase in the SAR). This is an overall estimate of the effect of the SAR, however, this effect varies with respect to peak power and time, as indicated by the significant interactions. The overall effect of adjustment for SAR with respect to the other factors would be to decrease the mean depth of damage for a group that was run at higher than average SAR, and increase the mean for a group that was run at lower than average SAR.

General model fitting depth of damage

The general model was fit to the complete data for CW, and for pulsed 24, 48 and 600 kW_{pk} exposures (Table 5). Four data points out of 309 were found to be outliers, points greater than 3.5 standard errors from the fitted surface. These observations were all from the 600 kW_{pk} series and all with DEP = 0. It was decided to fit the model to a reduced data set of 305 observations (i.e., with the four outliers removed). The results are shown in Table 5 and are illustrated graphically in Figures 3A and 3B.

The model accounted for 74% of the total variation in DEP. The enhancement ratio of pulsed vs CW can be calculated. This ratio is defined as the ratio of the depth of damage for a specific pulsed exposure of a lens vs the depth of damage induced by a CW exposure at a given average SAR. The pulsed vs CW enhancement ratio was shown to



be a function of pulse width. In the 48 and 600 kW_{pk} series, the enhancement ratio also varied with (SAR x TIME), the product of SAR and TIME. The general model equations were used to determine the fit of the model to the statistical mean depth of damage for each set of conditions (i.e., each cell mean depth of damage) for the four series and each PW in logarithmic form. The cell means for the full set of data used for constructing the model are given in Table 6. Formulas for the logarithmic form of the model are given in Figures 3A and 3B, and Table 7. Table 8 contains estimates of the pulsed vs CW enhancement ratio as a function of PP_{pk}, PW and SAR x TIME.

Higher ratios of depth of damage are observed as the total energy deposited in the lens decreases. The highest pulsed to CW enhancement ratio (Table 8) occurs at 20 μsec pulse duration and SAR x TIME (minutes) of 20 W-mm/kg. The ratio of damage calculated at PW 20 μsec is 80.9, when compared to CW at the same average power.

Statistical analysis: Threshold values

(1) Using ANOVA analysis to determine 600 kW_{pk} thresholds at which damage is first observed:

Pooling the data for the group containing sham and controls together gave a mean which differed from that of any of the exposed groups (Table 9). The mean depth of damage for lenses irradiated at an SAR of 32.3 W/kg was significantly higher than those receiving SAR 3.2, 6.4 and 16.2 W/kg but not different from those receiving SAR 64.6 or 194 W/kg.

(2) Extrapolation of curves to defined levels of damage: 0, 10, 20, 50, 100 μm (corresponding to depths of (0, 3-4, 6-8, 15-20, and 30-40 cells underlying the capsule-epithelium).

As an alternative method of threshold comparison of radiation levels at which finite damage is predicted from the equations based on the statistical model, the point of intersection of the damage curve with the lines corresponding to 10, 20, 50 and 100 mm damage, proceeding

Table 5. Estimates of parameters for the general model.

MODEL: DEP =
$a\ (SAR \times TIME)^f$ for continuous wave
$a \times b\ (PW)^e\ (SAR \times TIME)^f$ for PP = 24
$a \times c\ (PW)^e\ (SAR \times TIME)^{f+g}$ for PP = 48
$a \times d\ (PW)^e\ (SAR \times TIME)^{f+h}$ for PP = 600

Parameter	Estimate	SE
a	0.03	0.003*
b	2.49	0.50*
c	107.12	7.92*
d	227.47	15.68*
e	0.28	0.05
f	0.77	0.05
g	-0.42	0.06
h	-0.56	0.06
	R^2 - 0.74	

*These standard errors have been converted from the log scale to give the same t-value for significance testing and should not be used in the construction of confidence intervals.

inward from the capsule-epithelium were compared for the sets of various irradiation conditions. The overall equation calculated for each of CW, 24 kW, 48 kW and 600 kW peak power was used. The average powers at which each curve showed defined levels of damage were calculated (Table 10). This gives an alternative way of calculating damage thresholds, using the model. Average energy levels in the model (SAR x TIME) at which a defined depth of damage occurs or a defined number of cell layers is affected, may be compared. For 10 mm (3-4 cell depth) and 60 minutes exposure, the average total dose: SAR (mW/g) x TIME (sec) varied from 149 J/g (CW) to 0.044 J/g (0.6 MW, 20 ms pulses). For 20 mm depth (6-8 cells), the doses ranged from 345 J/g to 1.01 J/g for 0.6 MW, 20 ms pulses. The corresponding SAR values for a 60 minute exposure to cause 10 mm deep damage are 41.3 W/kg (confidence limits, CL, 31 -54.4 W/kg) for CW or 0.0123 W/kg (CL 0.00417 - 0.247 W/kg) for 20 ms 600 kW pulses. The ratio of these SAR values causing comparable damage is 3357, which is also found if equivalent damage after 6 minutes is compared. For 20 mm deep damage, the CW SAR value for 60 minutes exposure is 95.7 W/kg (CL 68.3 - 134.0 W/kg), as compared to 20 ms 0.60 MW pulses, an SAR of 0.28 W/kg (CL 0.076 - 1.03 W/kg). The ratio of these SAR values is 342.

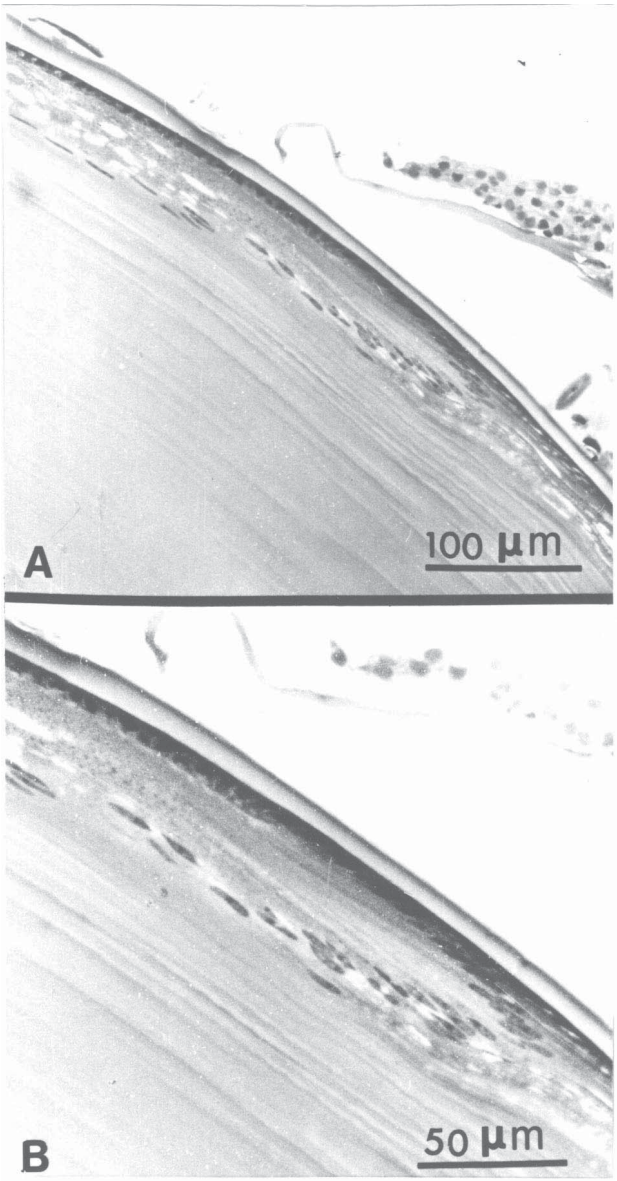


Figure 4. Thick plastic section showing bow region of a 60 minute Sham-irradiated lens. (**A**) The sham-irradiated lens looks very much like the fresh control lens. The bow area nuclei are elongated and show the characteristic configuration. The fibers also show a very orderly arrangement. (**B**) Higher magnification of (A). Note the typical elongated nuclei, regular capsule and small extent of vacuolation.

Semi-thin plastic sections

A more sensitive analysis of thresholds of damage was performed by examination of histopathology of thick plastic sections of the exposed lenses. Compared to

Table 6. Mean depth of damage by exposure (expo.) time (in minutes) and SAR (in W/kg) for continuous waves and pulsed waves at 24, 28 and 600 kW.

Table 6a. Mean depth for continuous waves.

	SAR	5	10	20	60	200	650
Expo. (min)	**Power (W)**	**0.5**	**1**	**2**	**6**	**20**	**65**
6	Mean			0	0	0.75	14.44
	n			4	3	3	4
	SD			0	0	0.66	4.18
20	Mean			0.81	11.50	17.25	
	n			4	4	4	
	SD			0.55	4.82	5.06	
60	Mean	0.5	10.69	14.00	15.63		
	n	3	4	2	4		
	SD	0.5	2.84	4.24	2.81		

Table 6b. Mean depth for PP = 24 kW.

	SAR	20	60	200	650
Expo-sure (min.)	Power (W)	2	6	20	65
6	**Mean**	**0.63**	**21.43**	**42.48**	**51.56**
	n	4	3	4	5
	SD	0.75	4.70	1.39	6.14
20	Mean	33.50	39.81	54.64	180.00
	n	4	4	4	4
	SD	2.66	2.10	1.78	8.42
60	Mean	41.62	51.11	185.62	203.75
	n	5	4	4	2
	SD	1.70	1.29	8.99	1.77

Table 6c. Mean depth for PP = 48 kW.

	SAR	10	30	100	300
Expo-sure (min.)	Power (W)	1	3	10	30
6	Mean	14.60	30.58	42.03	64.88
	n	12	12	12	12
	SD	6.95	15.69	20.24	26.84
20	Mean	40.86	59.93	71.50	88.21
	n	12	7	12	12
	SD	34.58	14.25	31.63	22.31
60	Mean	67.63	73.38	99.00	93.00
	n	12	12	11	6
	SD	40.52	16.01	36.36	39.00

Table 6d. Mean depth for PP = 600 kW.

	SAR	**1.62**	**3.23**	**6.46**	**16.2**	**32.3**	**64.6**	**193.8**
Expo-sure (min)	Power (W)	0.25	0.5	1	2.5	5	10	30
6	Mean	0	34.60	15.39	45.18	121.25	34.83	30.80
	n	1	5	10	6	2	4	3
	SD	0	17.33	18.87	29.78	76.01	21.60	6.54
20	Mean		25.13	20.66	47.07	72.50	51.63	45.90
	n	3	8	6	4	3	3	
	SD		14.61	17.34	41.61	16.65	28.78	7.18
35	Mean			63.80				
	n			2				
	SD			8.91				
60	Mean		65.57	45.04	48.29	72.50	80.65	79.28
	n		3	11	6	3	4	4
	SD		33.74	48.23	39.08	20.46	48.05	36.41

controls of Figure 3, these lenses (Figs. 4-6) showed irregular pycnotic nuclei in the equatorial area, along with increased vacuolation of the equatorial fiber cells at average SAR values of 0.646 W/kg at a peak power of 600 kW_{pk} after exposure to as few as 8 pulses in 6 minutes (0.25 W average power).

At a higher average SAR 1.615 W/kg (Figs. 6 and 7), 600 kW_{pk} pulsed irradiation resulted in more extensive vacuolation, disorganization of the equatorial lens fiber cells, and production of a pronounced equatorial pucker in the capsule.

Similar damage was observed in preliminary experiments at high peak powers using a virtual cathode oscillator (TEMPO) (Figs. 8 and 9) which generated energies per pulse of similar magnitude to that generated by the transmitter emitting 0.6 MW peak power pulses, but at peak powers of 100-200 MW, almost two orders of magnitude more. The damage observed included formation of many vacuoles in the epithelial and fiber cells, which were not seen in control lenses. Separation of epithelial cells from the capsule (Fig. 8), and of fiber cells from the epithelial layer and from each other (Fig. 9) was seen, along with distinctly darker staining of the nuclei in irradiated lenses,

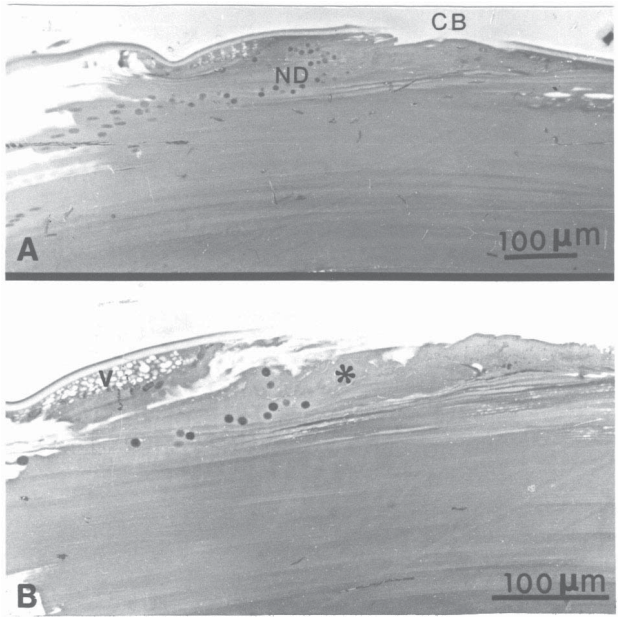


Figure 5. Thick plastic section showing bow region of a lens exposed at 0.6 W/kg (0.1 W average power) for 60 minutes: 30 pulses of peak SAR 2.4 MW/kg (peak power 600 kW). The capsule becomes very fragile and breaks can be seen (CB). (**A**) Lower magnification view: the nuclear arrangement in the bow region is disturbed (ND) and the nuclei become rounded, darker and pyknotic. (**B**) Higher magnification. There is obvious vacuolation (V) of the epithelial layer not seen in sham-irradiated controls. The regular arrangement of fibers is also absent on the posterior side of the bow (*).

Table 7. Logarithmic form of general model.

Logarithmic form
For continuous waves:
-3.62 + 0.77log (SAR x TIME)
For PP = 24 kW:
-2.71 + 0.28log (PW) + 0.77log (SAR x TIME)
log (DEP) =
For PP = 48 kW:
0.72 + 0.28log (PW) + 0.35log (SAR x TIME)
For PP = 600 kW:
1.61 + 0.28 log (PW) + 0.21 log (SAR x TIME)

consistent with damage to the nuclei of the fiber cells.

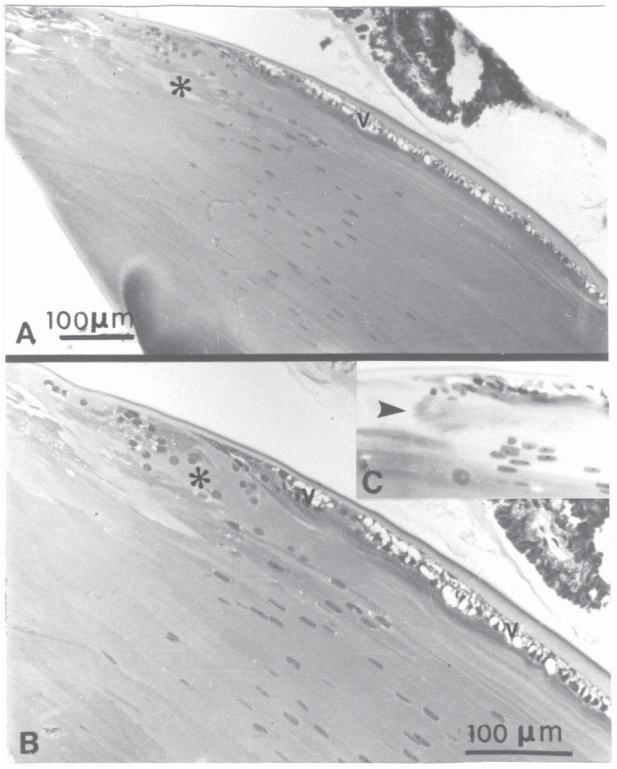


Figure 6. Thick plastic section showing bow region of a lens exposed to SAR 3.2 W/kg (average power 0.25 W) for 6 minutes: 8 pulses of peak SAR 2.4 MW/kg (peak power 600 kW). (**A** General view showing disturbance of nuclear and fiber arrangement especially in the outer fiber cells (*). Note marked vacuolation in the epithelial cells (V). (**B**) Part of (A), enlarged to show the changes more clearly, same magnification as (C), (**C**) Another section from the same lens showing a group of lens fibers unattached to the capsule (arrowhead).

Discussion

As our previous work suggested, the possibility of enhanced damage to the lens exists, arising from exposure by high peak power pulsed microwave fields versus CW fields with the same average power density. However, the generally accepted view of microwave radiation is that the hazard potential may depend only on the average power of the field. A newer appreciation for the pulsed phenomena, i.e., thermoelastic expansion effects, may result in added hazards. The results reported here confirm our previous findings that, at low SAR, additional *in vitro* damage occurs in isolated lenses as peak power increases for a constant average power. Also, at low values of average power, the ratio of pulsed to CW damage is higher. At a constant average power, the ratio of pulsed to CW damage increases

Table 8. 95% confidence intervals for pulsed to CW enhancement ratio, for pulsed waves of various widths, peak power and combinations of SAR by exposure time.

Table 8a. Peak Power = 24 kW.

Pulse Width	Pulsed to CW Enhancement Ratio (Estimate)	Confidence Limits
2	3.01	2.20 - 4.11
10	4.74	3.63 - 6.18
20	5.76	4.37 - 7.59

Table 8b. Peak Power = 48 kW.

		Pulse Width (μsec)					
		2 μsec		10 μsec		20 μsec	
Energy Absorbed kJ/kg	SAR x TIME	Pulse vs CW	Confidence Limits	EST	Confidence Limits	EST	Confidence Limits
1.2	20	26.4	15.3 - 45.3	41.5	24.7 - 69.7	50.4	29.8 - 85.23
4.8	80	14.7	9.75 - 22.0	23.1	15.8 - 33.7	28.0	19.0 - 41.8
12	200	9.94	7.13 - 13.86	15.7	11.7 - 21.1	19.0	13.9 - 26.0
36	600	6.24	4.7 - 8.19	9.83	7.78 - 12.42	12.0	9.30 - 15.4
72	1200	4.66	3.60 - 6.03	7.33	5.88 - 9.14	8.91	7.0 - 11.4
144	2400	3.47	2.65 - 4.55	5.47	4.31 - 6.93	6.64	5.1 - 8.59
432	7200	2.18	1.57 - 3.03	3.43	2.52 - 4.67	4.17	3.02 - 5.8

Table 8c. Peak Power = 600 kW.

		Pulse Width (μsec)					
		2 μsec		10 μsec		20 μsec	
Energy Absorbed kJ/kg	SAR x TIME	Pulse vs CW	Confidence Limits	EST	Confidence Limits	EST	Confidence Limits
1.2	20	42.2	25.2 - 70.9	66.5	41.1 - 107.6	80.9	50.0 - 130.9
4.8	80	19.4	13.1 - 28.8	30.5	21.6 - 43.0	37.1	26.3 - 52.3
12	200	11.6	8.3 - 16.2	18.2	13.9 - 24.0	22.2	16.8 - 29.1
36	600	6.2	4.6 - 8.58	9.83	7.7 - 12.5	11.9	9.4 - 15.1
72	1200	4.2	3.1 - 5.8	6.66	5.17- 8.57	8.09	6.28 - 10.4
144	2400	2.9	2.0 - 4.1	4.51	3.36- 6.04	5.48	4.09- 7.34
432	7200	1.54	1.0 - 2.4	2.43	1.65- 3.58	3.0	2.00- 4.35

Table 9. Comparison of fresh and sham lenses with irradiated lenses at various levels of SAR in the 600 kW series.

	SAR (W/kg)							
SAR(W/kg)	Control	Sham	6.46	3.23	16.15	193.8	64.6	32.3
Power (W)			1	0.5	2.5	30	10	5
Mean	3.2*	12.2	30.4	40.5	46.8	54.7	56.1	83.3
SD	3.8	9.2	34.8	25.8	34.9	30.8	37.8	37.3
n	6	40	31	11	18	10	11	9

Groups connected by the same underline are not different at the 0.05 significance level.
*This mean was also not significantly different from those for SAR 3.23 and 6.46 because of the small sample size.

as the pulse duration increases up to 20 µsec. For example, 2 µsec pulses produce a depth of damage of one-third or less than that observed for 20 µsec pulses deposited in the lens. At the highest levels of deposited energy which were studied, the ratio of pulsed to CW damage for 2 µsec pulses was approximately one-half of the ratio for 20 µsec pulses at the same average power. This data, obtained for two microwave frequencies (918 and 1250 MHz), each with a different irradiation cell, confirms our earlier hypothesis that low total doses of pulsed radiation at low duty factors cause very large increases in the relative biological effect, resulting in massive additional damage compared to CW irradiation at the same average power. The SAR values for 600 kW peak power pulses sufficient to cause damage at the 10 µm level (SAR 0.01 W/kg) and 20 µm level (SAR 0.28 W/kg) are both below the threshold of 0.4 W/kg assumed in the accepted safety standard of 4 W/kg (this includes the factor of 10 usually used for safety above the minimum biological effect).

This type of enhanced relative biological effectiveness shown by high peak power pulsed microwaves, has also been shown by Worgul's group (Worgul, 1988), for cataracts caused by irradiation by heavy ions, or more recently, by neutron irradiation (Worgul *et al*., 1996). In our studies, a similar relative biological effect enhancement has been shown, suggesting the possibility of similar mechanisms being involved. As a crude physical model, it might be suggested that if one hit by a heavy ion or neutron would be enough to damage a cell, multiple hits would not cause more damage, and thus higher doses would only result in lower relative biological effect (RBE). Therefore, 10 or 100 hits per cell would still result in cell damage but the RBE would be reduced by factors of 10 and 100, resulting in an apparent increase in RBE at lower doses.

Lenses exposed to 30 pulses in 6 minutes (0.5 W incident, time averaged SAR = 3.23 W/kg) sustained

(Figures 7 and 8 on facing page)

Figure 7. Thick plastic section showing bow region of a lens exposed to SAR 1.6 W/kg (average power, 0.25 W) for 60 minutes: 75 pulses of peak SAR 2.4 MW/kg (peak power 600 kW). (**A**) General view showing changes in the subcapsular bow area. Some superficial nuclei are rounded and darkly stained with a gradual transition to the normal elongated appearance of the nuclei occurring towards the center of the lens. (**B**) Higher magnification view of equatorial area of (A). The epithelial nuclei are also darker than normal. At the equatorial depression (ED), the epithelial cells show disorganization and vacuolation.

Figure 8. Lenses exposed to TEMPO source *in vitro* as described in the text. (**A**) Fresh lens showing intact lens capsule (c) and a regular arrangement of the epithelium (ep) and the lens fibers below. (**B**) Even the lens exposed to only 10 pulses shows detachment of capsule (c) from the lens epithelium below. Also note the presence of holes (h) in outermost lens fibers.

significant damage as observed with the scanning electron microscope. The examination of semi-thin plastic sections offered a slightly more sensitive measure of damage. Using this technique, it was possible to visualize pathological changes in lenses exposed at an average SAR of 0.172 W/kg (incident power 0.026 W), which received in 6 minutes as few as eight 20 µsec pulses of 600 kW_{pk} peak power microwaves. This SAR also is significantly below the SAR (0.4 W/kg) assumed in the safety standard. More extensive changes were observed in the lenses exposed to 75 pulses of 20 µsec in 60 minutes. These changes to epithelial cells and subcapsular fiber cells are similar in location and type to those we have observed in precataractous damage

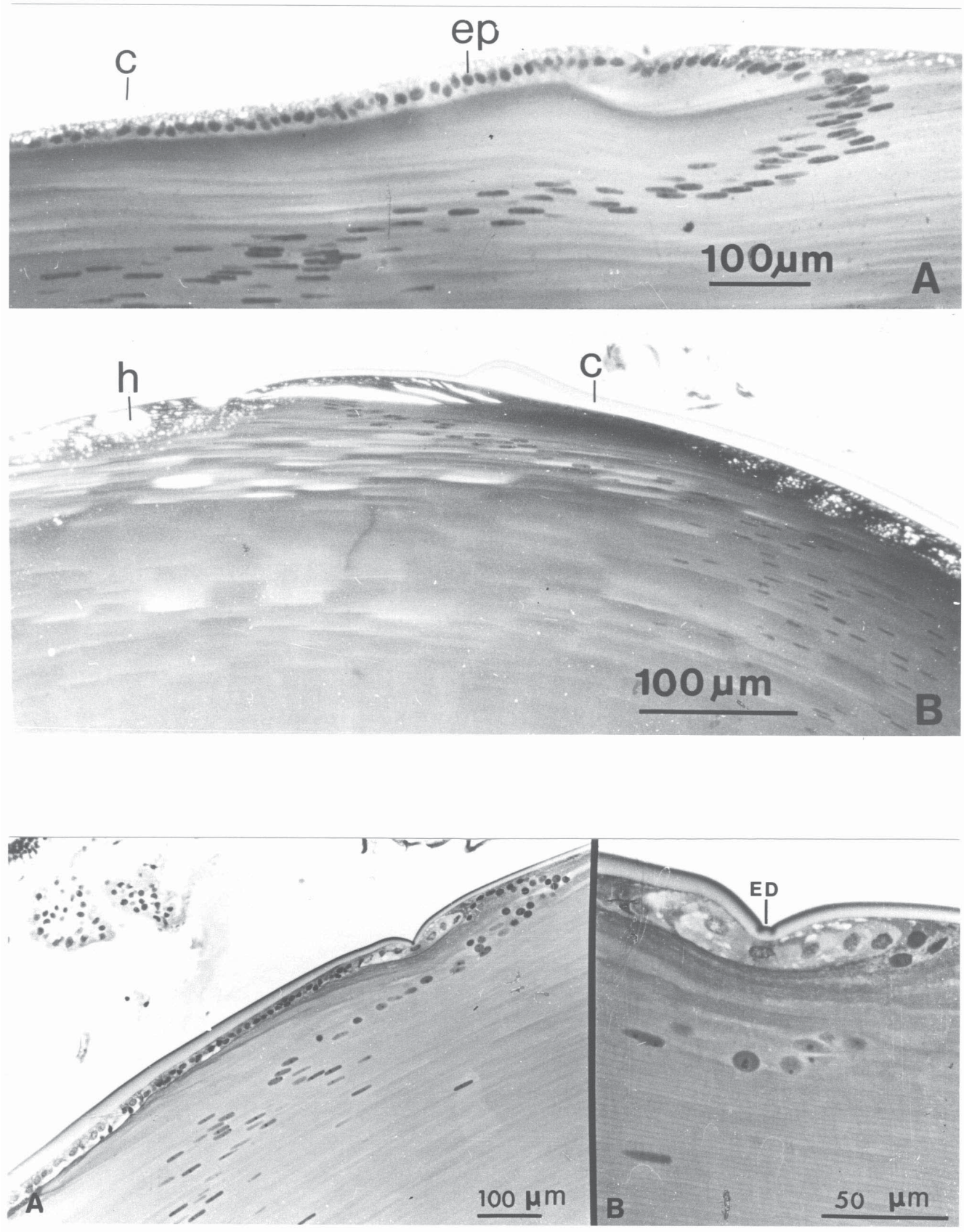

c
ep
100µm
A
h
c
100 µm
B
A
100 µm
ED
B
50 µm

Table 10. Levels of SAR(W/kg) predicted to cause defined depth of damage.

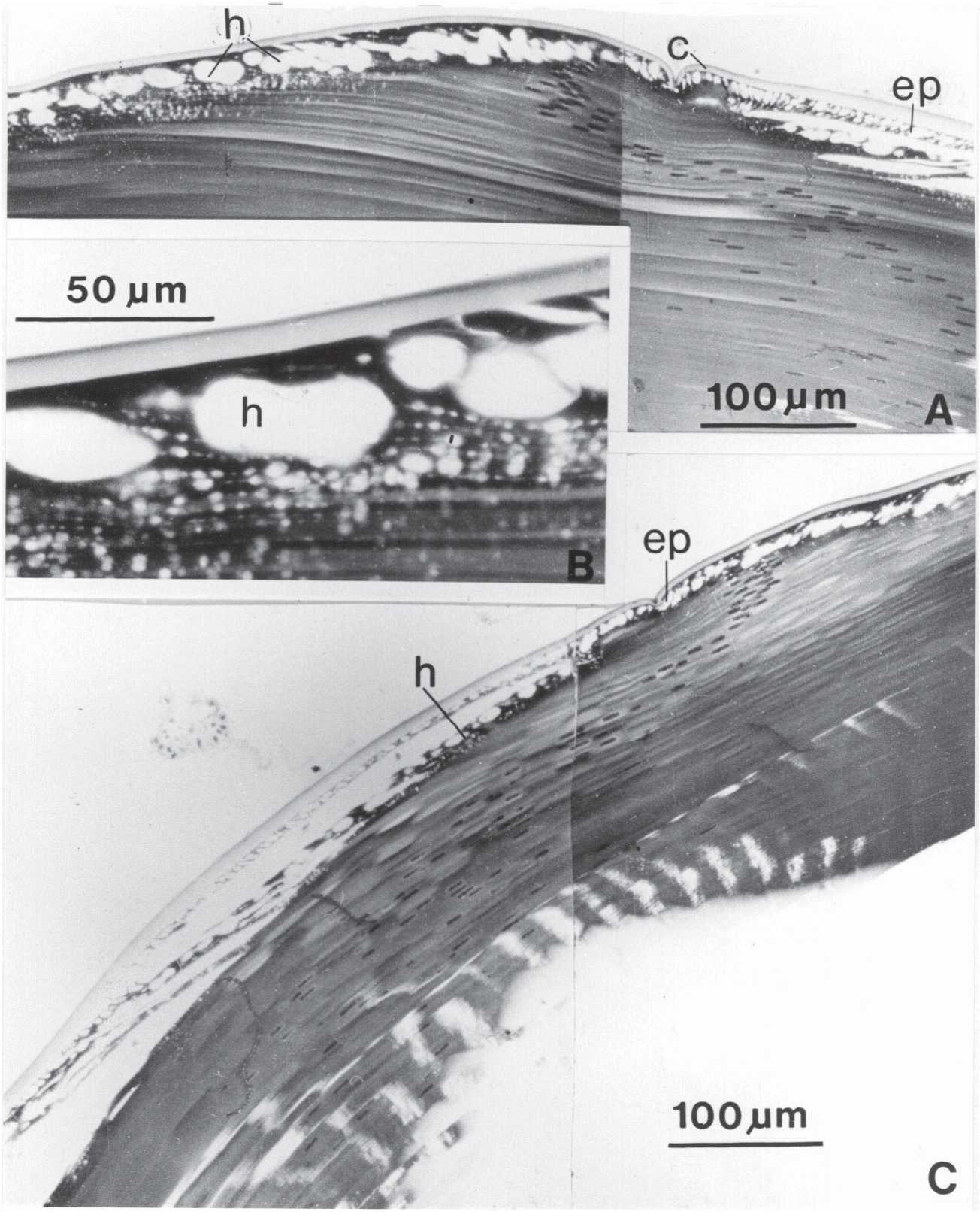
Peak Power	Depth of Damage (μm) or (number of cells)	6 minutes SAR (W/kg)	60 minutes SAR (W/kg)	Confidence Limits 6 minutes	60 minutes	
CW	10 (3-4 cells)	413	41.3	313 - 544	31 - 54.4	
	20 (6-8 cells)	957	95.7	683 - 1339	68.3 - 134	
	50 (15-20 cells)	3031	303.1	1942 - 4730	194.2 - 473	
	100 (32-40 cells)	7364	736.4	4292 - 12636	429.2 - 126.4	
24 kW Depth (μm)	2 μsec, 60 minutes SAR (W/kg)	Confidence Limits	10 μsec SAR (W/kg)	Confidence Limits	20 μsec SAR (W/kg)	Confidence Limits
10	9.88	7.00 - 13.9	5.48	4.00 - 7.51	4.25	2.99 - 6.03
20	22.88	16.7 - 31.3	12.68	9.05 - 16.4	9.85	7.40 - 13.1
50	72.47	52.3 - 100.4	40.2	32.1 - 50.4	31.2	24.5 - 39.8
100	176.1	121 - 255.8	97.65	75.2 - 126.1	76.8	58.2 - 98.6
48 kW Depth (μm)	2 μsec SAR (W/kg)	Confidence Limits	10 μsec SAR (W/kg)	Confidence Limits	20 μsec SAR (W/kg)	Confidence Limits
10	1.18	0.52 - 2.7	0.319	0.125 - 0.814	.181	0.062 - 0.526
20	7.637	4.29 - 13.6	2.06	1.12 - 36.5	1.17	0555 - 2.47
50	99	58.61 - 167.4	26.7	19.6 - 36.5	15.2	9.82 - 23.5
100	712.1	335 - 1513	192.1	119 - 308	109	66.8 - 170
600 kW	2 μsec SAR (W/kg)	Confidence Limits	10 μsec SAR (W/kg)	Confidence Limits	20 μsec SAR (W/kg)	Confidence Limits
10 μm	0.283	0.058 - 1.39	0.032	0.00417 - 0.247	0.0123	0.00117 - 0.136
20 μm	6.38	2.03 - 20.1	0.717	0.264 - 1.95	0.28	0.076 - 1.03
50 μm	459.5	59.5 - 3551	51.6	17.83 - 149.2	20.12	9.02 - 44.86
100 μm	12372	526 - 290,617	1389	155 - 12438	541.7	85.4 - 3437

induced by ionizing radiation (Ross *et al*., 1983, 1990), suggesting the possibility of an oxidative component in cataractogenesis induced by microwaves.

Several explanations of the damage observed in cataracts may be correct, and in fact several different mechanisms may all contribute to the damage.

The most probable explanation of this damage is the mechanism of TEE (Lin, 1978). In this mechanism, the incoming microwave pulse induces a transient heating which results in expansion of the water leading to a pressure wave in the biological tissue. If TEE is involved as a possible cause of the damage, what mechanism could produce such oxidative damage? Pressure pulses associated with ultrasound have been shown (Crum and Fowlkes, 1986) experimentally to cause cavitation and free radical production. Microwave pulses were shown to result in

Figure 9 *(on facing page)*. Lenses exposed to TEMPO source *in vitro* described in the text. (**A**) The lens exposed to 30 pulses shows greater damage than the lens which received 10 pulses. Note the greater number of larger holes (h) in the outer lens fibers. There is also a separation of lens fiber bundles from the epithelium c (ep). (**B**) Part of 7A, magnified to show the variety in size and shape of the holes in the lens fibers. (**C**) Lens exposed to 80 pulses shows changes which are similar to the lens receiving 30 pulses, with greater separation of the lens fiber bundles. Note also the darker degenerating nuclei in the epithelium and the lens fibers.


h
c
ep
50 µm
h
100 µm
A
B
ep
h
100 µm
C

acoustic pressure waves (Brown and Wyeth, 1983; Guo *et al*., 1984). Each pulse (up to 48 kW peak power corresponding to peak SAR 480 kW/kg) caused 5 µm physical displacement of an irradiated lens (Wyeth, 1987). This physical displacement is indicative of a strong pressure wave associated with each individual pulse. Just as in the production of free radicals during cavitation induced by ultrasound pressure pulses, it is possible that free radical formation may also occur as a result of cavitation during irradiation by microwave pulses. Such free radicals could act as initiators of the cascade of oxidative events, causing the biological damage observed, as also suggested by Liburdy and Vanek (1985) for continuous microwave exposures. Consistent with this, Somosy *et al*. (1991b) have shown damage similar to that reported here, in 3T3 cells exposed to pulsed microwaves, but not in cells exposed to CW irradiation at the same average power. This group previously reported damage similar to that found for PW in 3T3 cells exposed to ionizing radiation (Somosy *et al*., 1991a), supporting a possible role for free radicals in damage caused by pulsed microwaves.

Recent studies by Phelan *et al*. (1992) and Liburdy and Vanek (1985) support the involvement of free radicals in some phase of microwave-induced alterations in membrane properties. The former study showed superoxide dismutase protection against the changes in membrane ordering, while the latter study indicated that microwave irradiation of rabbit erythrocytes increased permeability, resulting in sodium influx, and this was prevented by antioxidants such as ascorbate or mercaptoethanol. The effect of antioxidants is similar to that found in model diabetic and radiation cataracts in our studies (Ross *et al*., 1982, 1983) in preventing opacity, which recent studies have related to calcium influx (Kilic, 1995); calcium influx probably occurs coincident with the sodium influx, and also appears to be prevented by ascorbate. These data suggest that antioxidant prophylaxis of microwave-induced damage may be possible.

In addition to this damage induced by free radicals, the pressure wave (capable of causing a 5 µm lens displacement; Brown and Wyeth, 1983) could be responsible for physical damage to the lens cells by breaking, stressing, stretching or fracturing the cell membrane (Webber *et al*., 1980). We previously reported damage to the lens capsule consistent with stretching and breaking of the lens capsule basement membrane fibers (Stewart-DeHaan *et al*., 1985). Such disruption, even if much smaller in magnitude than the damage we observed, could lead to stretch-activation of ion channels which would admit calcium or sodium to the lens epithelial and fiber cells (Strange *et al*., 1996); calcium in particular could lead to the formation of globular degeneration (Fagerholm, 1979; Srivastava *et al*., 1994; Bhatnagar *et al*., 1995). Blackman (1991) has also noted calcium changes induced in biological systems by low doses of modulated microwave irradiation, and oxidative stress may influence such alterations in calcium concentration. Calcium-induced disruption of the actin microfilament structure could lead to supercontraction of actin microfilaments and globular degeneration. Calcium activation of calpain could result in proteolytic digestion of the essential cytoskeletal protein fodrin (Kilic, 1995). Disruption of membrane structure either mechanically or electromagnetically could form or release lipoperoxides by exposing polyunsaturated fatty acids in the disrupted membrane to oxygen. Release of iron from mito-chondria or disruption of cellular membrane structure would potentiate free radical reactions by the Haber- Weiss and Fenton reactions, leading to production of the dangerous hydroxyl radicals from the iron-catalysed reaction of superoxide anion and hydrogen peroxide (Fee and Valentine, 1977). We previously illustrated, using the inhibitor cytochalasin D (Mousa *et al*., 1979), that disruption of actin microfilament structure could lead to formation of globular degeneration and cataracts. The process was reversible up to 2 hours but after this time other processes prevented its reversal. It is intriguing to suppose that the irreversibility was due to the processes set in motion early after cytochalasin treatment, which could be reversed, if the damage caused were not too severe.

Thus both free radical production associated with the pressure waves and physical stretching, deformation, and tearing of the membranes of the lens cells may contribute to the initiation of a cascade of damaging events, or to the damage itself.

Acknowledgements

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This work was performed under protocols approved by the WRAIR Laboratory Animal Care and Use Committee and guidelines of the "Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources National Research Council."

Appendix 1: Lens SAR Computation Using Foster Approximation

Experimental results of the Cober *in vitro* exposure of rat lens are recalculated by two methods and compared to previously recorded data. Lens extracted from 180-200 g

rats are placed in a 58.4 ml cylindrical holder filled with standard physiological saline which is circulated for thermal regulation under normal (non-dosimetric) exposures. The rates of temperature change (_T) are the slopes taken the first 5 to 10 seconds after the application of RF. These values are utilized in two approximations of the experimental medium comprised of saline solution and the rat lens. The first assumes the lens dielectric properties are similar enough to the physiologic saline (> 99% water, $C_p(H_2O)$ = 0.997 cal/g°C) that it makes an effective model of the lens complex aqueous tissue. An improved model, provided by K. Foster references a correlation function (Cooper and Trezek, Aerospace Medicine, Jan. 1971):

$$C_p = w(H_2O) + 0.4(1 - w(H_2O)$$

where C_p = the heat capacity at constant pressure and $w(H_2O)$ is the mass fraction of water. This is based on an "average" value:

$$C_p \text{ (non-water content tissue)} = 0.4 \text{ cal/g°C}$$

using the C_p(fat) = 0.55, the C_p(protein) = 0.26 and the C_p(water) = 1.0 cal/g°C, comparatively. Various tissue specimens were shown to fit the model quite well with less than 5% scatter in their data points.

Composition of ocular tissue varies greatly in water content (Philipson, 1969), while protein distributions within the normal adult rat lens vary from 0.30 (cortex) to 0.90 (center of the nucleus) g/cm^3, mostly water forming the remainder. Taking 1.3 g/cm^3 for the density of protein, water weight fractions range from 0.72 (cortex) to 0.26 (nucleus), yielding heat capacities of 0.83 to 0.55 cal/g°C respectively. The "average" value C_p = 0.69 cal/g°C is the best choice short of measuring the lens thermal properties directly.

The formula used for the specific absorption rate (SAR) calculations by both methods:

$$SAR_n(W/kg/W) = k*4.186 \text{ J/cal}*(\Delta T \text{ in °C})/(\Delta t \text{ in sec})*C_p \text{ cal/g°C}$$

where k = the conversion factor (W/kg = 10^3 W/g)/(incident P_{ave}) and the SAR_{pk}(peak) W/kg = SAR_n * P_{pk}(peak power).

Appendix 2: Experimental Methods

Rat lens SAR dosimetry involved the measurement of the rate of temperature change in the saline-lens medium using the Luxtron (Santa Clara, CA) model 2000 fiber-optic thermometer. The exposure cell (Fig. 1) with the lens placed in the bottom of the inlet tube and filled (58.4 ml) to the top of the tubes with STD physiologic saline. This is inserted in a WR650 waveguide fixture with a RF transparent block for positioning the top of the cell just inside the guide and the bottom of the inlet tube at the mid-plane. All temperatures were taken within the inlet tube at Luxtron probe sensor positions referenced (inches above) that of the lens and at the lens location without the lens. For the actual lens measurements, the probe was surgically implanted in the lens before placing it in the cell.

Pulsed microwave energy was produced by a modified Cober L-band (1.25 Ghz), klystron output transmitter, delivering a 600 kW_{pk} 10 μsec pulse at 38 Hz to the waveguide fixture.

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Discussion with Reviewer

Z. Somosy: Describe the general model more clearly. Is one

equation fit to each set of data (i.e., one equation for CW SAR vs DEP, a second equation for 40 kW_{pk} SAR vs DEP?
Authors: Damage was ranked as the depth of visible damage, granular or globular degeneration (holes in cells or cell surfaces), in the scanning electron microscope (SEM). This measurement was the maximum depth of penetration at the apex, the wedge-shaped area of damage, usually seen at the lens equator. The depth of damage for various pulsed regimens and CW irradiation was compared using two techniques: (1) by one-way analysis of variance for the CW and pulsed samples, and (2) by using an overall model to fit the data. Prediction of the SAR at which identical depths of damage would occur for the different peak power pulses and CW irradiation was also possible using the model. Several depths of damage were compared using this strategy.

The similarities in damage observed for heavy ion-induced cataracts and our previous studies of radiation-induced cataracts, suggest the possibility that high energy pulsed microwaves may result in oxidative damage to tissue similar to that observed for ionizing radiation. Such oxidative damage could be a result of production of the superoxide anion which may occur when thermoelastic expansion in biological tissues induces high pressure pulses. These pulses may cause cavitation, similar to that observed for ultrasound pulses.

Z. Somosy: Is there any practical reason why the pulse modulation chosen was used from the point of environmental radiation hygiene or for theoretical reasons?
Authors: The Cober radar transmitter used for the high peak power pulsed work at peak powers of 600 kW was modified from a military unit available to the Walter Reed Army Institute of Research with whom we were collaborating. The frequency used was that used in the previous radar applications although the pulse durations used were chosen theoretically based on the work of Lin (1978) to have maximum thermoacoustic effects. The extrapolation of these effects to shorter pulse durations usually found in radars is possible using the overall model because the pulse durations were varied over a ten-fold range.