

## MICROWAVE-INDUCED CHANGES TO THE PRIMATE EYE

The study of the effects of low-level microwave radiation on the primate eye is a cooperative program between the Milton S. Eisenhower Research Center of The Johns Hopkins University Applied Physics Laboratory and the Wilmer Ophthalmological Institute of the Johns Hopkins School of Medicine. Effects observed range from cellular disruption to altered visual function after microwave exposure of the cornea, iris vasculature, and retina. The results obtained may contribute to a scientific basis for the development of safety guidelines for microwave exposure.

### BACKGROUND

Possible adverse health effects in humans exposed to microwave radiation have generated concern in the medical community for more than forty years. With the proliferation of military, industrial, and consumer use of radar, communication equipment, industrial products, and microwave ovens, investigators continue in their efforts to examine possible health hazards associated with electromagnetic radiation. Initially, primary concern focused on the potential effects of tissue heating in organs (e.g., eyes) that have a limited ability to dissipate heat.

A number of experiments<sup>1</sup> have been conducted to investigate the formation of cataracts in the crystalline lens of research animals. These experiments used high-level microwave exposure that generated a significant increase of temperature in the eye. After microwave exposure, slit-lamp examination and histological evaluation of the lens were used to assess the type and degree of changes. These studies concluded that: (1) cataract induction depends on both the microwave frequency and the exposure levels; (2) the minimum exposure level that causes cataracts is 100 mW/cm<sup>2</sup>; and (3) in general, microwave exposure conditions that elevate the temperature of the lens to 41°C will induce cataracts.

Researchers have also conducted studies to examine microwave-induced ocular effects other than cataract formation. Kinoshita et al.<sup>2</sup> demonstrated that the level of ascorbic acid in the rabbit lens is decreased after whole-body microwave exposure. Weiter et al.<sup>3</sup> confirmed these findings using cultured rabbit lenses under the strictly controlled exposure conditions known to generate cataracts. Rosenthal et al.,<sup>4</sup> using electron microscopy and slit-lamp biomicroscopy, examined rabbit eyes following acute microwave exposure to 35 and 107 GHz. They observed epithelial and stromal damage to the cornea at exposure levels below those necessary to produce lenticular damage. Kramar et al.<sup>5</sup> exposed nonhuman primates and rabbits to microwaves (2.45 GHz) at high-power densities and observed distinct differences in the effect on the two species. The induction of cataracts was con-

firmed in rabbits; however, cataracts were not observed in nonhuman primates, even at exposure levels that caused facial burns, lid edema, and changes in the anterior chamber of the eye.

The basis for the APL/Wilmer Ophthalmological Institute collaborative study of ocular effects from microwave radiation dates back to 1982, when the Navy expressed interest in the effects of nonionizing radiation (both light and microwave) on the eye and visual processes. The Laboratory's engineering expertise and facilities and the diagnostic capabilities of the Wilmer Institute were combined to form a research team to address the Navy's questions. Other than those previously noted, few studies had been conducted on the effects of microwaves on ocular structures, or on the question of possible ocular effects produced by low-level microwave exposure.

The APL/Wilmer Institute Microwave Research Program has focused on three specific ocular structures: the cornea, the iris, and the retina. This article describes observed effects of microwave radiation on those structures. After a brief presentation of the anatomy of the eye and research protocols, we describe our initial efforts to examine the corneal endothelium of nonhuman primates following microwave exposure, which was the subject of an earlier *Technical Digest* article.<sup>6</sup> A diagnostic tool new at the time, the wide-field specular microscope, was used in those studies. The instrument permits noninvasive diagnostic examination of the corneal endothelium in the living subject, which allows for the evaluation of morphological changes in the cornea's posterior cellular layer after low-level microwave exposure. We also describe changes in the permeability of the blood vessels of the iris after low-level microwave exposure. These changes were documented using fluorescein iris angiography immediately postexposure. We next present damage threshold data demonstrating the synergy of low-level microwaves and ophthalmic drugs used in the treatment of glaucoma. The data were derived from research efforts investigating the mechanism of interaction between low-

level microwaves and ocular tissue. The final section addresses more recent findings relating low-level microwave exposure to functional and morphological changes within the retina. The use of electroretinography permits noninvasive examination of the electrophysiological response of the retina before and after microwave exposure. With this tool, the functional integrity of various components of the retina can be independently assessed to determine temporary or long-term disruption. Possible implications of these findings for human exposure guidelines are also examined.

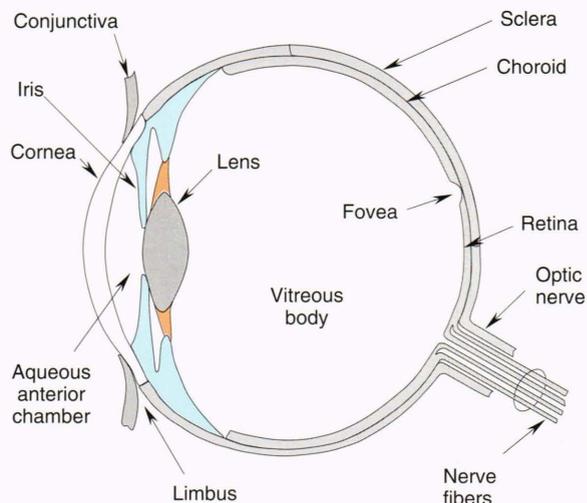
### ANATOMY OF THE PRIMATE EYE

The eye (Fig. 1) is approximately spherical in shape and measures about 25 mm in diameter in humans and primates. Primary structures of the eye include the cornea, iris, lens, vitreous body, retina, and an aqueous anterior chamber. The outermost optical element, the cornea, with its many transparent layers, provides the refractive and light-gathering power essential for unobstructed vision. The lens provides the variable refractive power needed to focus on near and distant objects. The iris, much like the diaphragm of a camera, varies the amount of light incident on the retina. The aqueous humor, composed almost entirely of water, and the vitreous body, a gel-like substance, fill the space between the cornea and lens and the lens and retina, respectively. The innermost and arguably the most important component of the eye is the retina. The retina is a many-layered photosensitive surface composed of nerve cells, photoreceptors, retinal pigment layers, and two separate vascular systems: the outermost choroid and innermost retinal blood vessels.

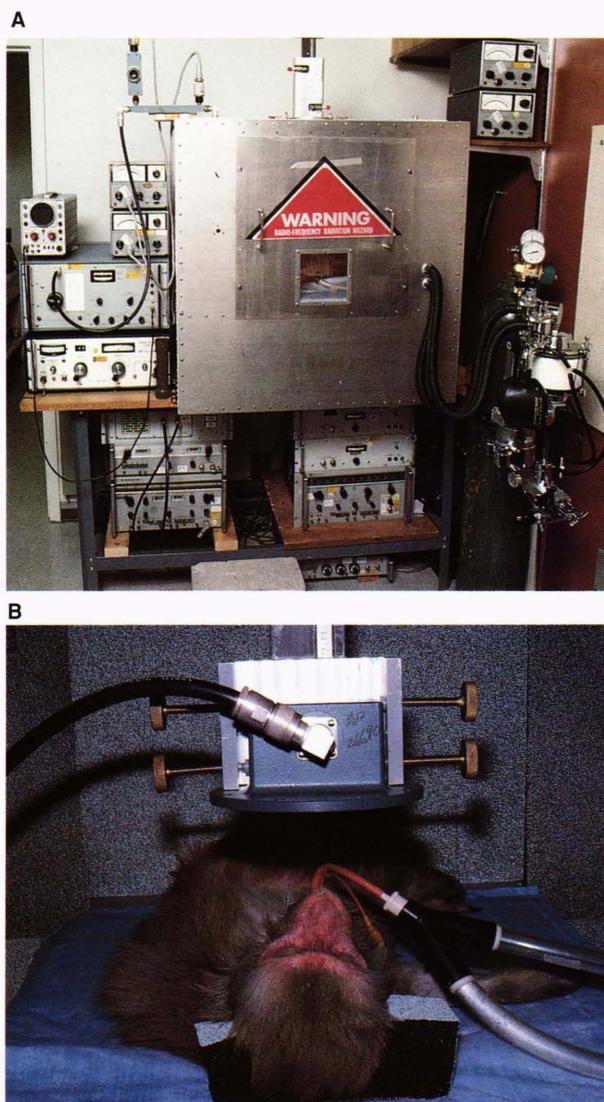
### RESEARCH PROTOCOLS

Nonhuman primates (referred to in this article as primate subjects or primates) are exposed to microwaves in a metal chamber (Fig. 2). Its interior walls are lined with microwave-absorbing material. The chamber door is ventilated, and two air exhaust ports are located at the top of the chamber. Door ventilation and ports have been designed to prevent internal reflection of the microwaves. The internal chamber temperature remains within  $\pm 1.5^\circ\text{C}$  of the  $23$  to  $25^\circ\text{C}$  room temperature during a four-hour exposure session.

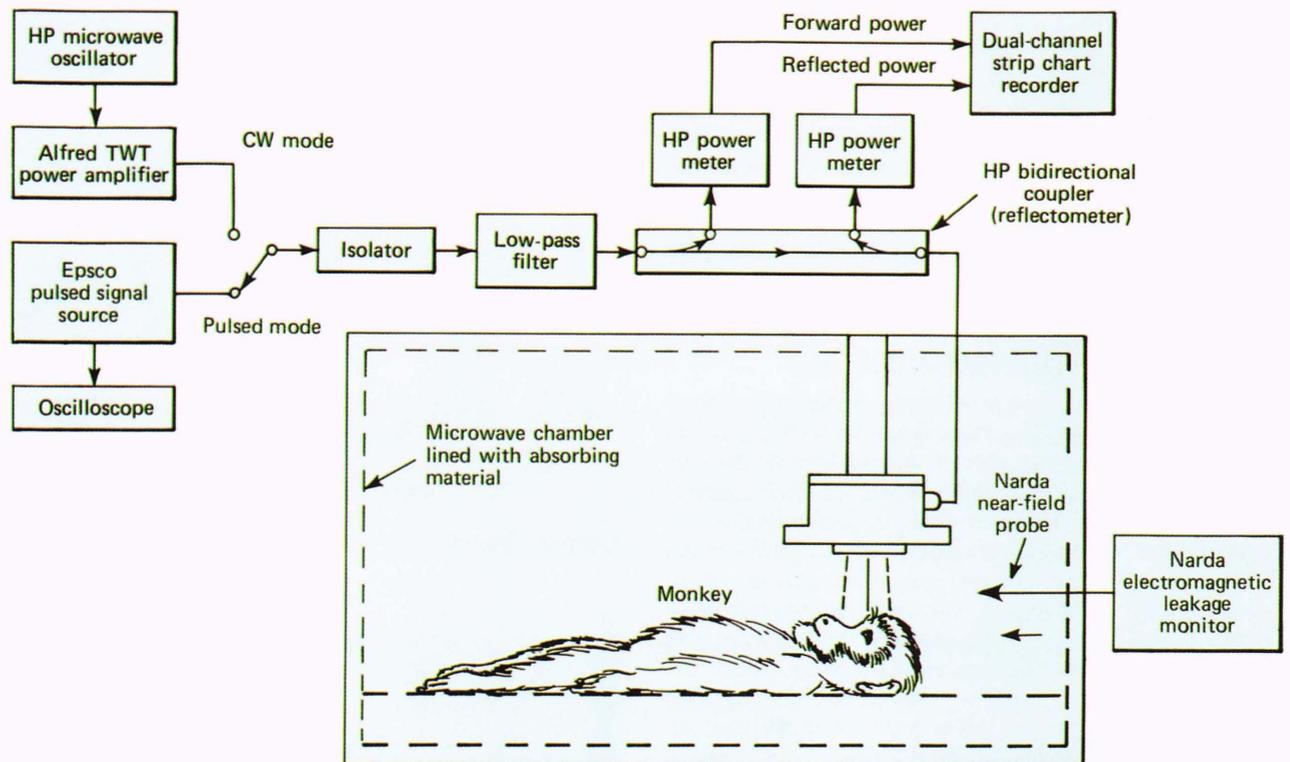
Continuous wave (CW) and pulsed microwaves at 2.45 GHz are used for experiments (Fig. 3). The pulsed microwave signal consists of  $10\text{-}\mu\text{s}$ -wide pulses at a repetition frequency of 100 Hz. The microwaves are transmitted through a coaxial cable to a bidirectional coupler that measures forward and reflected input power. The microwaves from the bidirectional coupler then pass through a coaxial cable to a coaxial-to-waveguide transition source located within the anechoic chamber. For our experiments, the microwave source (with its  $7.2\text{ cm} \times 3.4\text{ cm}$  aperture) is positioned over the ocular area; its long dimension is centered 10 cm above the bridge of the nose. This arrangement provides exposure to the far field and produces equal irradiation of both eyes. Unless indicated otherwise, all exposures were performed with pulsed microwaves.



**Figure 1.** The major components of the eye.



**Figure 2.** Apparatus used for the irradiation of primates. **A.** Anechoic chamber used for microwave exposure of anesthetized primates. **B.** Anesthetized primate positioned for exposure.



**Figure 3.** Microwave system used to expose the ocular area of anesthetized primates. The system can provide 2.45-GHz microwaves in the continuous wave (CW) or pulsed mode. (TWT = traveling wave tube.)

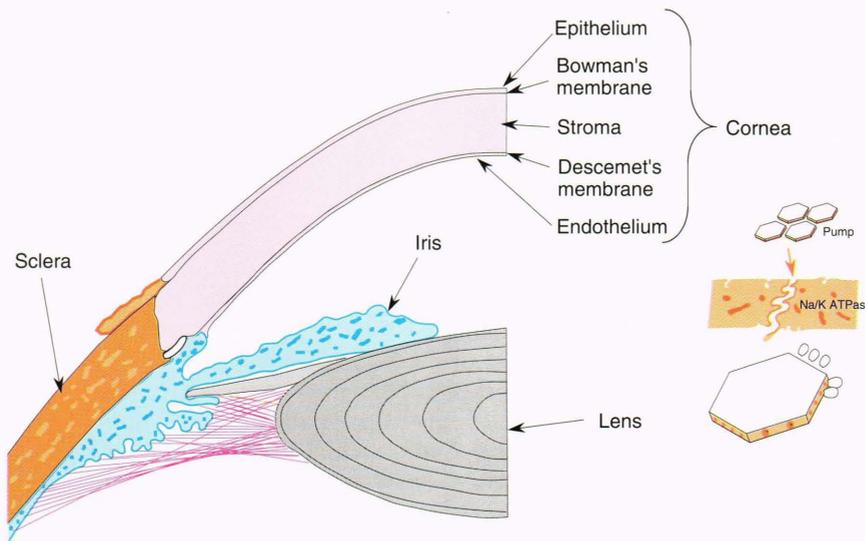
To convert conventional incident power density readings (watts per square centimeter) into a biologically significant means of reporting dosimetry (i.e., specific absorption rate or SAR), *in vivo* temperature measurements were made during a four-hour microwave exposure of a primate to  $20 \text{ mW/cm}^2$ . A nonperturbing thermal probe was surgically implanted in the anterior chamber of the eye abutting the endothelial layer of the cornea (Fig. 4). A baseline temperature of  $34.5^\circ\text{C}$  was recorded immediately before exposure. Microwave radiation ( $20 \text{ mW/cm}^2$ ) was then applied for four hours, as in a normal exposure session. The temperature was recorded every four seconds for the first hour and every minute thereafter for the remaining three hours. A one-minute temperature rise of  $0.09^\circ\text{C}$  was selected from the steepest part, which occurred at the beginning of the recorded four-hour temperature increase of  $0.77^\circ\text{C}$ . The  $0.09^\circ\text{C}/\text{min}$  reading was then used to calculate an SAR of  $0.26 \text{ W/kg}$  per  $\text{mW/cm}^2$ .

### CORNEAL ENDOTHELIAL EFFECTS

The cornea (Fig. 4), with its stratified structure of epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium, received little attention in earlier microwave research efforts. The innermost layer of the cornea, the endothelium, comprises a single (mono) layer of about half a million flat, mostly hexagonal cells (Fig. 5A). This cellular layer, with its anatomic integrity and active cell "pumps," is the most important component,

maintaining corneal dehydration and transparency. The pumps remove excess fluid buildup from the corneal stroma to prevent corneal swelling (inset, Fig. 4).

To determine the effects of low-level microwave exposure on the eye, we first examined the effects of exposure on the corneal endothelium.<sup>7</sup> Corneal examinations were performed on all primate subjects anesthetized with halothane gas or sedated with ketamine. During examination, an eyelid speculum was inserted and the cornea kept moistened with a balanced salt solution. The wide-field specular microscope was used to scan and photograph the central region of the cornea (about 6 mm in diameter). Each photograph represented a microscopic field of the endothelial surface, about  $2 \text{ mm}^2$ . Examination immediately after microwave exposure revealed no damage; however, twenty-four hours after exposure, varying degrees of damage were observed, depending on the exposure level. The degree of endothelial damage was evaluated using the photograph demonstrating the highest number of visible lesions. These lesions have a pock-like appearance in the endothelial monolayer (Fig. 5B). Two researchers evaluated each photograph, assigning a score on the basis of the total number of lesions present. A score of 1 was assigned to a healthy endothelium with fewer than three lesions; 2 represented three to ten lesions; 3 represented eleven to fifty lesions; and 4 was assigned to greater than fifty lesions. Results are presented in Table 1. This work and the research reported in the remainder of the article were performed in collaboration with Salvatore D'Anna and others of the Wilmer Institute.



**Figure 4.** General anatomy of the cornea, showing its multilayers. Inset illustrates the endothelial cellular “pump.”

**Table 1.** Comparison of changes to the corneal endothelium and iris vasculature.

Power density avg. (mW/cm <sup>2</sup> )	Specific absorption rate (W/kg)	Mean ± standard error of the mean	
		Endothelial damage <sup>a</sup>	Vascular leakage <sup>a</sup>
0	0	1.0 ± 0.00 (3)	1.0 ± 0.00 (2)
5	1.3	1.2 ± 0.13 (8)	1.0 ± 0.40 (8)
10	2.6	1.9 ± 0.31 (8) <sup>b</sup>	2.1 ± 0.28 (6) <sup>b</sup>
15	3.9	2.8 ± 0.15 (6) <sup>b</sup>	2.8 ± 0.26 (6) <sup>b</sup>

<sup>a</sup>Number of eyes examined appears in parentheses.  
<sup>b</sup>Probability <0.001 when compared with sham-exposed subjects.

Twenty-four hours after controlled exposure to microwave radiation, abnormalities in the corneal endothelial monolayer were observed clearly with the specular microscope. The lesions, unicellular to multicellular in diameter, were distributed throughout the endothelium. Postmortem histology (including light and scanning electron microscopic analyses) performed immediately after specular microscope evaluation demonstrated that about 3% to 5% of the visible lesions resulted in actual cell death (Figs. 5C and 5D). The extent of microwave-induced lesions appeared to be influenced by the incident power density, the type of microwave mode, and the exposure protocol. Pulsed microwave exposure produced abnormalities at lower power densities (10 mW/cm<sup>2</sup>) than cw exposure (20 to 30 mW/cm<sup>2</sup>). Higher power exposures of both pulsed and cw microwaves yielded an increased number of lesions.

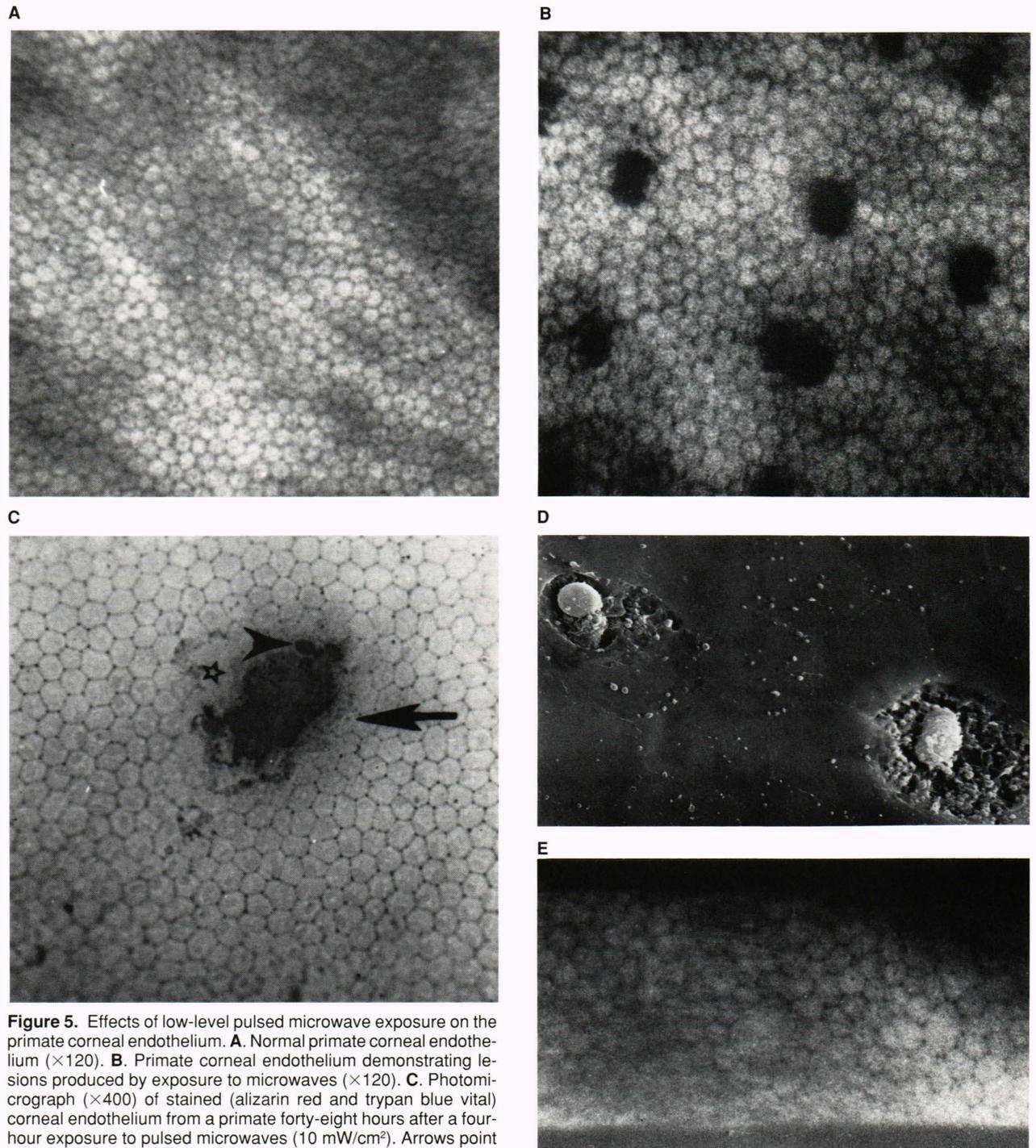
Endothelial cell loss is a serious adverse health effect for humans and other primates, as these cells do not regenerate in primates. Instead, lesions resulting from cell loss are repaired by a process in which surrounding cells increase in size and migrate to fill in the gaps. A significant reduction in the endothelial cell population can result in marked swelling of the cornea and loss in trans-

parency. Examination of microwave-exposed primates four days postexposure revealed a “normal-in-appearance” endothelium, with no detectable lesions present. Cell loss described above was compensated for by enlargement and migration of the surrounding cells (Fig. 5E).

### IRIS LEAKAGE

During our efforts to examine the mechanism by which microwave fields interact with ocular tissue to produce the observed corneal pathology, we also discovered that a microwave-potentiated increase in iris vascular permeability compromised the integrity of the blood–aqueous barrier. This increase in permeability appeared to occur at some point during the four-hour exposure and persisted up to seventy-two hours postexposure. Subsequent histopathological examination of the iris using a horseradish peroxidase (HRP; a glycoprotein 100 times larger than sodium fluorescein), a common tracer technique, confirmed leakage of the iris vasculature. The HRP molecule (molecular weight = 40,000) is representative of certain serum proteins within the blood stream.

Primate subjects were anesthetized with halothane (inhalation anesthetic) and exposed to 2.45-GHz pulsed microwaves (10 μs, 100 pps), at one of the average incident power densities listed in Table 1, four hours a day for three consecutive days. Fluorescein iris angiograms were performed before microwave exposure to provide a baseline and immediately after the final microwave exposure session. Each angiogram entailed the injection of 0.5 cc of ophthalmic sodium fluorescein dye solution into a leg vein. Transit of the dye through the vessels of the iris was visualized and photographed using a modified Zeiss fundus camera<sup>8</sup> during a fifteen-minute period. Vascular integrity of the iris was evaluated by two researchers during this fifteen-minute period. A score of 1 was assigned to an eye exhibiting no leakage (Fig. 6A); 2 was assigned to minor leakage of sodium fluorescein in tissue and the anterior chamber; and 3, or moderate leakage, was assigned to an eye exhibiting partial filling



**Figure 5.** Effects of low-level pulsed microwave exposure on the primate corneal endothelium. **A.** Normal primate corneal endothelium ( $\times 120$ ). **B.** Primate corneal endothelium demonstrating lesions produced by exposure to microwaves ( $\times 120$ ). **C.** Photomicrograph ( $\times 400$ ) of stained (alizerin red and trypan blue vital) corneal endothelium from a primate forty-eight hours after a four-hour exposure to pulsed microwaves ( $10 \text{ mW/cm}^2$ ). Arrows point to missing cells, and the star shows an area of cells that are in the process of dying. **D.** Scanning electron micrograph of a primate corneal endothelium forty-eight hours after a four-hour exposure to pulsed microwaves ( $10 \text{ mW/cm}^2$ ). **E.** Specular photomicrograph ( $\times 120$ ) of a corneal endothelium four days postexposure showing enlargement and migration of cells to compensate for cell loss.

of the anterior chamber more than five minutes after injection (Fig. 6B). Leakage was considered major and assigned a score of 4 if, within the first three minutes following injection, a significant amount of fluorescein leaked into the anterior chamber.

Fluorescein angiographic evidence demonstrates that a breakdown of the ocular blood-aqueous barrier in the iris vasculature can occur during pulsed microwave ex-

posure. Other research efforts have established that environmental stress and some pharmacological agents can induce a similar breakdown under appropriate conditions.<sup>9</sup> The absence of a similar response in our sham-exposed primate eyes, however, tends to rule out the likelihood of this type of artifactual effect. The data, presented in Table 1, indicate a correlation between microwave-induced vascular permeability changes<sup>10</sup> and

the subsequent development and severity of corneal endothelial lesions.

## DRUG EFFECTS

In a continuing effort to understand the effects of microwave radiation on ocular tissue, we undertook another study using two pharmacological agents: timolol maleate and pilocarpine. These drugs, which reduce intraocular pressure, are used clinically in the treatment of glaucoma. To evaluate the possibility of a thermal mechanism, timolol was selected to test its potential as a protective measure against microwave-induced increases in vascular permeability. Timolol has been shown to protect the eye against heat-induced disruption of the blood–aqueous barrier.<sup>11</sup> In contrast, pilocarpine is known to increase the permeability of the iris vasculature to fluorescein<sup>12</sup> when exposed to a thermal insult. Pilocarpine was selected to evaluate its capacity to potentiate the leakage induced by microwave exposure if the microwave-induced damage results from an increase in ocular temperature.

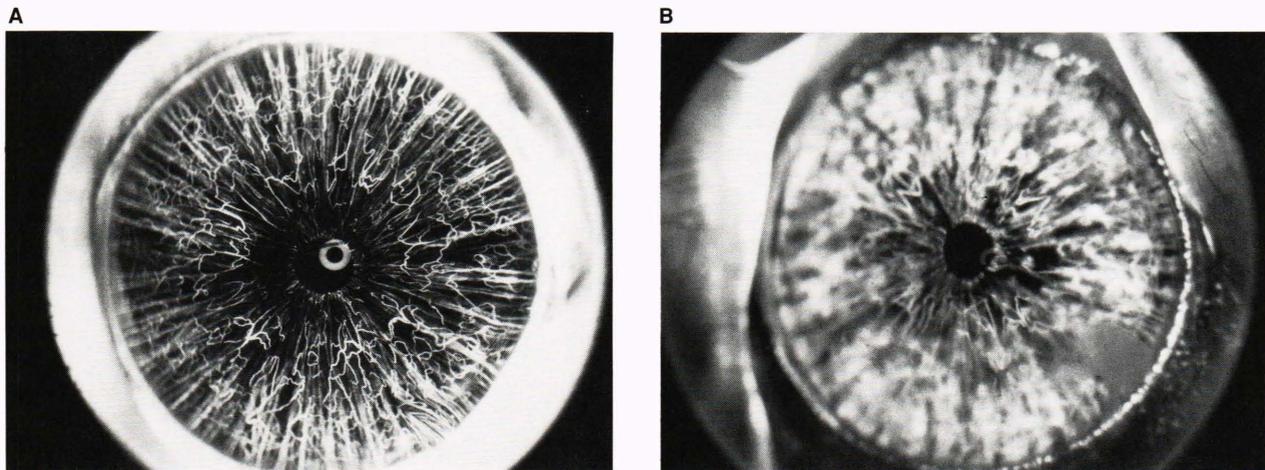
The protocol for this study entailed exposing each primate subject to 2.45-GHz pulsed microwaves (10  $\mu$ s, 100 pps) four hours a day for three consecutive days at a specific power density. Incident power densities ranged from 0 (sham) to 15 mW/cm<sup>2</sup>, measured at the position of the eyes. Because of the high cost and limited availability of primate subjects, these animals are used more than once throughout the course of a planned study. To normalize any animal-to-animal variability in biological susceptibility, each primate subject received, in a random pattern, a range of power densities throughout the study. In drug-pretreated eyes, ophthalmic solutions (one drop of 0.5% timolol or 2.0% pilocarpine) were administered topically immediately before microwave exposure. For each subject, a nonexposure period of at least two weeks was allowed between groups of three exposure sessions. Before each exposure protocol, diagnostic procedures (e.g., wide-field corneal specular microscopy, fluorescein iris angiography) were performed to document the absence of pre-existing or residual (from previous microwave expo-

ures) corneal and iris abnormalities. In addition, slit-lamp biomicroscopy, a routine clinical diagnostic procedure, was performed to examine ocular structures for other abnormalities, including cataract formation.

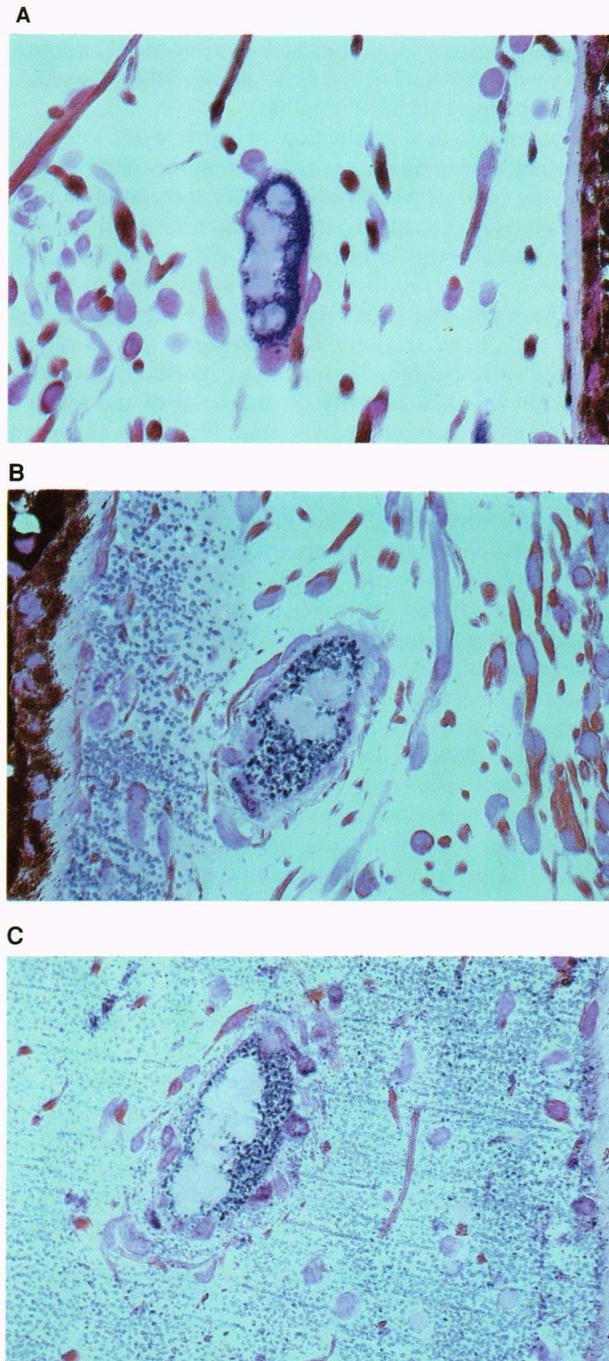
Histopathological evaluation of some eyes was also performed to determine iris vascular permeability to HRP following microwave exposure. After the completion of the diagnostic procedures, HRP was administered intravenously and allowed to circulate for fifteen minutes. The primate was then sacrificed with an intravenous overdose of sodium pentobarbital. The eyes were enucleated and fixed, and 2- $\mu$ m sections were prepared for histology. Sections were examined using light microscopy and evaluated for vascular integrity on the basis of the relative amount of extravascular HRP present. Figure 7A shows an HRP-processed section of iris from a control/sham primate subject. In Figure 7B, a similar section of iris tissue taken from a “microwave-only” (10 mW/cm<sup>2</sup>) exposed primate subject demonstrates extravascular HRP near the iris pigment epithelium. Figure 7C shows a timolol-pretreated, microwave-exposed (10 mW/cm<sup>2</sup>) primate subject; HRP has diffused from the vessels into the surrounding stroma throughout the field of view.

Results of diagnostic examination following drug pretreatment and exposure to various power densities of microwave radiation are presented in Tables 2 and 3. As in the previously described studies, sham exposure (0 mW/cm<sup>2</sup>) and exposure to levels less than 10 mW/cm<sup>2</sup> for three days without drug pretreatment produced no increase in iris vascular permeability to fluorescein and no observable change in the corneal endothelium. Similar results were also observed in primates that received either timolol or pilocarpine pretreatment and were sham-exposed.

Pulsed microwave exposure (2.45 GHz) at an average incident power density of 10 mW/cm<sup>2</sup> (SAR, 2.6 W/kg) without drug pretreatment produced minor leakage of fluorescein into the anterior chamber of the eye (a score of 2). Corneal endothelial lesions were also observed. At a higher exposure level (15 mW/cm<sup>2</sup>), iris vascular leakage and the number of endothelial lesions increased.



**Figure 6.** Fluorescein angiograms of the primate iris. **A.** Primate fluorescein iris angiogram three minutes postinjection (no vascular leakage). **B.** Primate fluorescein iris angiogram demonstrating a significant increase in vascular permeability after pulsed microwave exposure (15 mW/cm<sup>2</sup>).



**Figure 7.** Primate iris sections prepared for histopathological evaluation. **A.** Section of normal (sham-exposed) primate iris demonstrating horseradish peroxidase (HRP) confined to vascular lumen. **B.** Section of microwave-exposed ( $10 \text{ mW/cm}^2$ ) only primate iris demonstrating moderate leakage of HRP. **C.** Section of iris from a primate pretreated with timolol maleate and exposed to pulsed microwaves ( $10 \text{ mW/cm}^2$ ).

Exposure at average power densities of 5, 10, and  $15 \text{ mW/cm}^2$  with timolol pretreatment increased the amount of fluorescein leakage from the iris vasculature (Fig. 8) and increased the number of endothelial lesions (Fig. 9) when compared with effects observed with microwave-only exposure at these power densities. Irises of primate subjects pretreated topically with one drop of

pilocarpine and exposed at these power densities also demonstrated an increase in fluorescein leakage and a concomitant increase in the number of endothelial lesions when compared with microwave-only exposed primates. At an exposure level of  $10 \text{ mW/cm}^2$ , pretreatment with either timolol or pilocarpine increased effects from minor to moderate (Tables 2 and 3). Moderate changes were observed after exposure to  $5 \text{ mW/cm}^2$  (a subthreshold microwave-only exposure) with drug pretreatment.

Timolol or pilocarpine pretreatment combined with microwave exposure at  $15 \text{ mW/cm}^2$  produced major changes in the degree of vascular leakage and the number of corneal endothelial lesions when compared with sham-exposed primate subjects. Because of the degree and consistency of ocular effects produced, few primates were exposed to the higher power level ( $15 \text{ mW/cm}^2$ ) with drug pretreatment.

Timolol reacted synergistically with microwaves to lower the threshold, which was an unexpected outcome since, again, timolol had been reported to protect the iris vasculature from thermal insult.<sup>11</sup> To determine a microwave threshold value following timolol pretreatment, we expanded the scope of this research. A number of primates were pretreated with timolol and exposed at power densities of 0.2 or  $1 \text{ mW/cm}^2$ . Results are summarized in Table 4. In primates pretreated with timolol and exposed to  $0.2 \text{ mW/cm}^2$ , iris angiography and corneal specular microscopy failed to demonstrate observable changes in the eye when compared with normal nonexposed eyes and sham-exposed controls. Primates pretreated with timolol and exposed to increased power density ( $1 \text{ mW/cm}^2$ ) demonstrated moderate iris vascular leakage, as shown in Figure 8B. A moderate number of corneal endothelial lesions (eleven to fifty lesions/field, as shown in Fig. 9B) were also observed under these exposure conditions. The data therefore suggest that, with timolol pretreatment, a microwave ocular damage threshold lies between 0.2 and  $1 \text{ mW/cm}^2$  (SAR, 0.05 to  $0.26 \text{ W/kg}$ ).

To date, similar experiments with pilocarpine pretreatment before microwave exposure have not been conducted. The study of primates exposed to pulsed microwaves without drug pretreatment indicates the threshold of ocular effect to be about  $10 \text{ mW/cm}^2$  (SAR,  $2.6 \text{ W/kg}$ ).

## RETINAL CHANGES

The retina (Fig. 10A), a thin, transparent tissue that lines the interior of the eye, is subdivided into well-defined layers: the retinal pigment epithelium, rod and cone layer (photoreceptors), external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, capillary layer, and nerve fiber layer. The posterior focal point of the ocular optical system in the retina is the macula. (The retina and the optic nerve are derivatives of the forebrain; consequently, their morphology and physiology are similar to those of the brain.)

The photoreceptors (Fig. 10B) serve as the sensory receptors of the visual pathway. The outer segment of the photoreceptors is composed of stacked discs that undergo constant renewal. This outer segment contains photopigments that initiate the visual response to light. When light

**Table 2.** Changes in iris vascular permeability.

Power density avg. (mW/cm <sup>2</sup> )	Specific absorption rate (W/kg)	Mean $\pm$ standard error of the mean		
		No drug <sup>a</sup>	Timolol <sup>a</sup> (0.5%)	Pilocarpine <sup>a</sup> (2.0%)
0	0	1.0 $\pm$ 0.00 (2)	1.0 $\pm$ 0.00 (9)	1.0 $\pm$ 0.00 (2)
5	1.3	1.0 $\pm$ 0.04 (8)	2.7 $\pm$ 0.31 (5) <sup>b</sup>	2.4 $\pm$ 0.55 (4)
10	2.6	2.1 $\pm$ 0.28 (6) <sup>b</sup>	2.7 $\pm$ 0.44 (3) <sup>b</sup>	2.8 $\pm$ 0.51 (5) <sup>c</sup>
15	3.9	2.8 $\pm$ 0.26 (6) <sup>b</sup>	3.5 $\pm$ 0.50 (2) <sup>b</sup>	4.0 $\pm$ 0.00 (2) <sup>c</sup>

<sup>a</sup>Number of eyes examined appears in parentheses.<sup>b</sup>Probability <0.001 when compared with sham-exposed subjects.<sup>c</sup>Probability <0.01 when compared with sham-exposed subjects.**Table 3.** Changes in the corneal endothelium.

Power density avg. (mW/cm <sup>2</sup> )	Specific absorption rate (W/kg)	Mean $\pm$ standard error of the mean		
		No drug <sup>a</sup>	Timolol <sup>a</sup> (0.5%)	Pilocarpine <sup>a</sup> (2.0%)
0	0	1.0 $\pm$ 0.00 (3)	1.0 $\pm$ 0.00 (9)	1.0 $\pm$ 0.00 (4)
5	1.3	1.2 $\pm$ 0.13 (8)	2.8 $\pm$ 0.33 (4) <sup>b</sup>	2.9 $\pm$ 0.66 (4) <sup>b</sup>
10	2.6	1.9 $\pm$ 0.31 (8) <sup>b</sup>	4.0 $\pm$ 0.00 (3) <sup>b</sup>	3.2 $\pm$ 0.31 (6) <sup>b</sup>
15	3.9	2.8 $\pm$ 0.15 (6) <sup>b</sup>	4.0 $\pm$ 0.00 (2) <sup>b</sup>	3.0 $\pm$ 0.00 (2) <sup>b</sup>

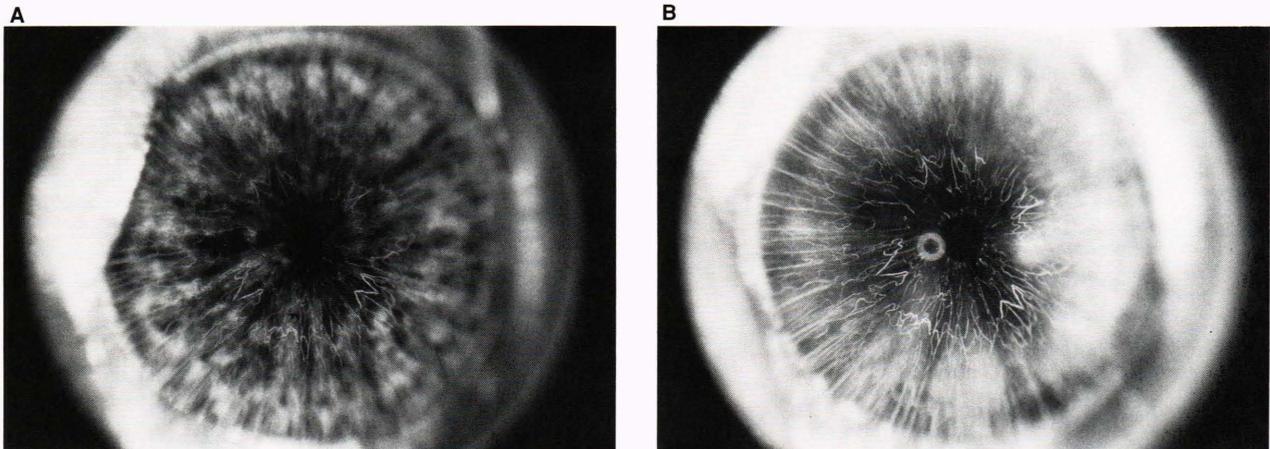
<sup>a</sup>Number of eyes examined appears in parentheses.<sup>b</sup>Probability <0.001 when compared with sham-exposed subjects.

is absorbed by these pigments, it is converted to chemical energy, which is then converted to an electrical signal that is transmitted via the retinal neurons and the optic nerve to the brain.

The outer nuclear layer of the retina contains the nuclei of the photoreceptors. From the outer nuclear layer, the photoreceptors send nerve fibers, or axons, which synapse with the neurons of the inner nuclear layer. This

transition zone is called the outer plexiform layer. We know little about the reversibility of photoreceptor changes, although some experts speculate that all but the loss of the photoreceptors' nuclei are probably reversible.

During early investigations into the effects of microwave radiation on the corneal endothelium, several primate eyes were removed at sacrifice for histopathological analysis. Preliminary histological analysis of the retina,



**Figure 8.** Fluorescein angiograms of the primate iris. **A.** Primate fluorescein iris angiogram ten minutes postinjection demonstrating minor degree of vascular permeability after microwave exposure (10 mW/cm<sup>2</sup>) only. **B.** Primate fluorescein iris angiogram ten minutes postinjection demonstrating moderate vascular permeability following pretreatment with timolol maleate and exposure to pulsed microwaves (10 mW/cm<sup>2</sup>).

**Table 4.** Ocular changes with timolol pretreatment.

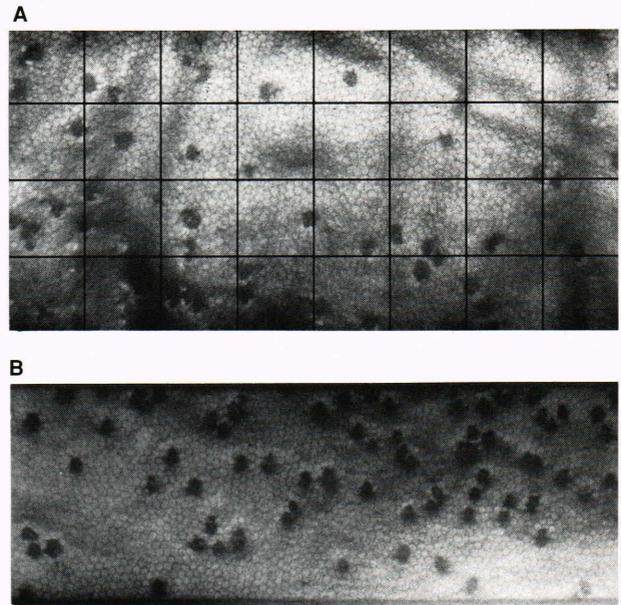
Power density avg. (mW/cm <sup>2</sup> )	Specific absorption rate (W/kg)	Mean ± standard error of the mean	
		Endothelial damage <sup>a</sup>	Vascular permeability <sup>a</sup>
0	0	1.0 ± 0.00 (9)	1.0 ± 0.00 (9)
0.2	0.05	1.2 ± 0.17 (5)	1.0 ± 0.00 (5)
1.0	0.26	3.3 ± 0.25 (7) <sup>b</sup>	2.9 ± 0.31 (7) <sup>b</sup>

<sup>a</sup>Number of eyes examined appears in parentheses.

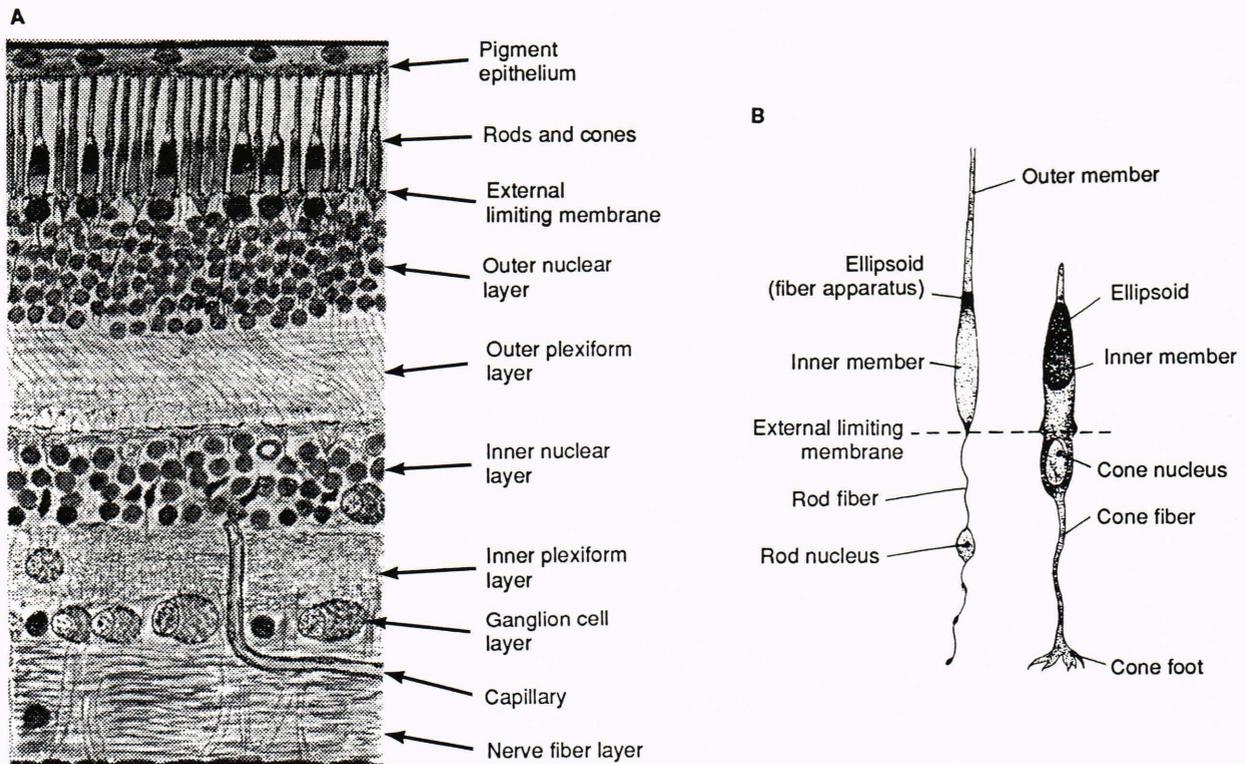
<sup>b</sup>Probability <0.001 when compared with sham-exposed subjects.

performed by Gerard Luty of the Wilmer Institute and D. Scott McLeod of APL, demonstrated significant photoreceptor damage and retinal detachment. A clear cause-effect relationship explaining this damage could not be established, because of the mixed exposure protocol of the primate subjects and the use of halothane gas anesthesia during microwave exposure. Researchers also questioned the possibility of pre-existing retinal pathology contributing to the effects observed. A new protocol was therefore established to address these concerns.

To eliminate possible pre-existing retinal pathology, ocular electrophysiologic testing was performed before exposing the primates to microwaves. All measurements and analyses were under the direction of Dr. Mary John-



**Figure 9.** Specular micrographs (×120) of the primate corneal endothelium. **A.** Primate corneal endothelium showing a minor number of lesions twenty-four hours postexposure to pulsed microwaves (10 mW/cm<sup>2</sup>) without drug pretreatment (×120). **B.** Primate corneal endothelium showing a moderate number of lesions twenty-four hours postexposure to pulsed microwaves (10 mW/cm<sup>2</sup>) after pretreatment with timolol maleate (×120).



**Figure 10.** Illustration of the retina of the eye. **A.** Layers of the retina. **B.** The retinal photoreceptors. (Figures reprinted, with permission, from Vaughn, D., and Asbury, T., *General Ophthalmology*, 8th Ed., © 1977 by Lange Medical Publications, Los Altos, Calif., p. 116 and 117, respectively.)

son of the Wilmer Institute. Each primate was again tested after microwave exposure to measure possible changes in retinal function. Electroretinography (ERG) was used to measure the transient action potentials initiated by a change in the light energy falling on the photoreceptors of the retina. It yields information regarding the earliest stages of the visual process. The basic clinical ERG protocol entails a scotopic test (rod photoreceptor response) and a 30-Hz flicker test (cone photoreceptor response).

The exposure protocol called for a four-hour exposure on three consecutive days for three consecutive weeks. Microwave exposure parameters were 1.25 GHz, pulsed ( $0.5 \mu\text{s}$ , 16 pps), 1-MW peak, producing an SAR of 4.0 W/kg. The primates were restrained but not anesthetized during microwave exposure (Fig. 11). Just before the first exposure and twenty-four hours after the ninth and last exposure, ERG was performed. Figure 12 shows "before and after" ERG's; a 60% reduction in the scotopic or rod response and a 90% reduction in the 30-Hz flicker cone response are seen.

Following the three-week exposure protocol and ERG testing, the primates were euthanized and the eyes prepared for light microscopy. Figure 13 shows the histopathology observed in an exposed primate. The effects include photoreceptors with degenerative changes both in the nuclei and in the outer and inner segments. These changes correlate with the ERG findings demonstrated in Figure 12.

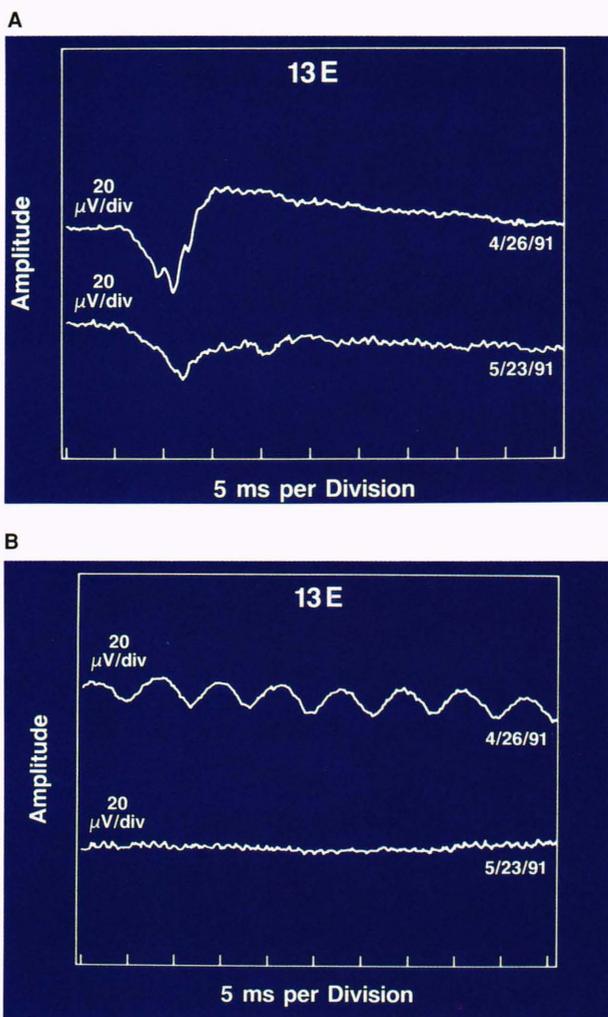
## SUMMARY

The experiments reported herein demonstrate that exposure to relatively low levels of microwave radiation can result in significant ocular changes in the nonhuman primate. These changes range from cellular disruption to altered visual function. At  $10 \text{ mW/cm}^2$ , we have observed corneal endothelial lesions, increased iris vascular permeability, and degenerative changes in the cells of the iris and the retina. When the primates were pretreated with timolol maleate, a ten-fold reduction in the threshold microwave power density that produces these effects was observed. At an exposure level of  $10 \text{ mW/cm}^2$ , ERG has demonstrated a significant decrease in visual function. Measurements of the SAR of these low-level exposures to microwaves indicate strongly that significant tissue heating is not the mechanism responsible for the exhibited changes.

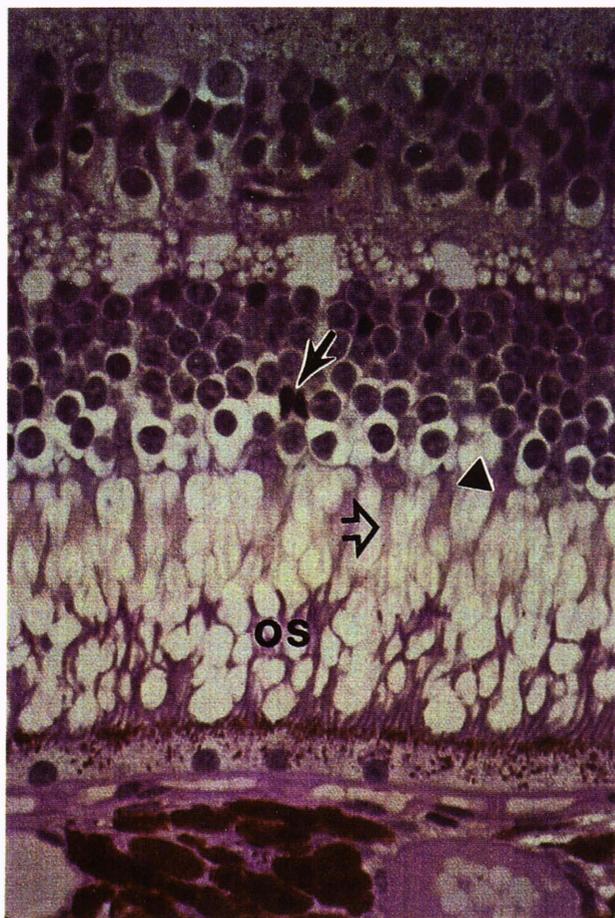
In the United States, several permissible exposure standards for RF radiation have been promulgated and revised over the years. The current frequency-dependent American National Standards Institute guideline<sup>13</sup> for occupational and general population exposure permits unlimited exposure to  $1 \text{ mW/cm}^2$  at frequencies from 30 to 1500 MHz, and exposure to  $5 \text{ mW/cm}^2$  at frequencies above 1500 MHz. The most recent standard, Institute of Electrical and Electronic Engineers C95.1,<sup>14</sup> permits unlimited exposure to  $10 \text{ mW/cm}^2$  in controlled environments for frequencies above 1.5 GHz. The American Conference of Governmental and Industrial Hygienists<sup>15</sup> recommendation permits occupational exposures of  $10 \text{ mW/cm}^2$ , whereas the National Council on Radiation Protection and Measurements<sup>16</sup> recommends that occu-



**Figure 11.** Restrained, nonanesthetized primate positioned for ocular irradiation.



**Figure 12.** Electrophysiological measurement of the primate retina (from primate subject 13E) before and after pulsed microwave exposure. **A.** Scotopic electroretinogram demonstrating rod response before and after microwave exposure (60% reduction). **B.** 30-Hz flicker electroretinogram demonstrating cone response before and after microwave exposure (90% reduction). Signal amplitude is the signal produced by the retina before (upper curve) and after (lower curve) microwave exposure.



**Figure 13.** Macular region of the retina from a microwave-exposed primate. Degeneration of the photoreceptor nuclei (arrow) and disappearance of nuclei (arrowhead); degeneration of outer segments (OS) and inner segments (open arrow) is evident.

pational exposure not exceed  $1 \text{ mW/cm}^2$ . The data presented in our study suggest that ocular effects may actually occur at exposure levels close to or below those recommended by these safety guidelines, particularly when medications such as timolol are being used. At the very least, our observations indicate that the current safety guidelines lack an inherent safety factor, which heretofore has been considered to be an important aspect of any safety guideline.

On the basis of some of our early histological findings, it seemed likely that cellular change could occur at exposure levels below  $10 \text{ mW/cm}^2$  in either sensitive individuals or under prolonged exposure conditions. In recognition of this possibility, and to provide a sufficient safety factor between the level where biological effects occur and levels recommended for human exposure, APL adopted its own safety standard<sup>17</sup> for microwave exposure—an exposure limit of  $0.1 \text{ mW/cm}^2$  for all employees, regardless of whether they work with microwave-generating equipment or not.

The results observed in our studies were obtained under specific laboratory conditions, including prolonged

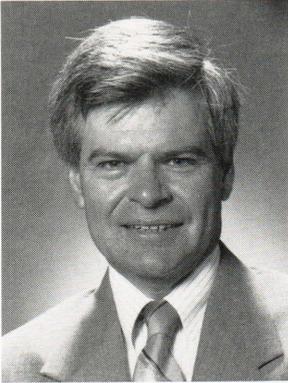
microwave exposure and the use of anesthesia. These exposure sessions do not necessarily duplicate conditions found in the environment. The ocular effects obtained, however, were demonstrated in a nonhuman primate model, which is anatomically and physiologically similar to the human eye. The data suggest strongly that individuals could be subject to ocular effects from previously unsuspected low-level microwave exposure with low average absorbed dose rates. Attempts to extrapolate the data beyond the actual conditions of the experimental paradigm should be pursued with appropriate caution.

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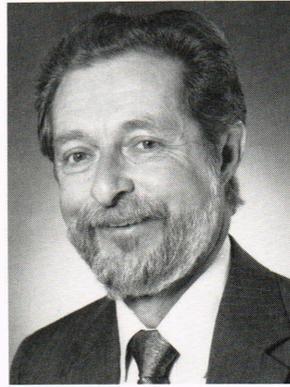
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