

Multiple Dosing of Prostaglandin F_{2α} or Epinephrine on Cynomolgus Monkey Eyes

III. Histopathology

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Prostaglandins (PGs), or their derivatives, are potent ocular hypotensive agents which may prove useful in glaucoma therapy. PGF_{2α} (250 μg in 50 μl saline) or epinephrine 2% solution (50 μl) was topically applied twice daily for 2 weeks to one eye of six cynomolgus monkeys for each agent. Contralateral control eyes received their respective vehicles. By light microscopy, there was no evidence of inflammation, corneal changes, retinal pathology (including cystoid macular edema), or other adverse effects. Likewise, by electron microscopy of the peripheral cornea, anterior chamber angle, iris base and ciliary body, no differences were noted between treated and control eyes. Therefore, multiple dosing with PGF_{2α} in subhuman primate eyes did not result in notable histopathological changes that would contraindicate a clinical trial in glaucoma patients. Invest Ophthalmol Vis Sci 29:1428-1436, 1988

Topical application of prostaglandins (PGs), or their analogs, are effective ocular hypotensive agents in normotensive and/or glaucomatous rabbit, cat, monkey and human eyes.¹⁻¹² The hypotensive effect after multiple dosing in cats^{4,13} or monkeys^{9,11,14} is achieved without clinically significant adverse effects that would contraindicate a multiple-dose clinical trial.

However, the only previous histopathological studies were done following exogenous administration of high doses of PGE₁ or E₂, PG types known to produce a considerably greater irritative response when compared with PGF_{2α}.^{1,15} These studies demonstrate disruption of the tight junctions of the nonpigmented

layer of the ciliary epithelium in rabbits¹⁶ or prominent structural changes in ciliary epithelium at the anterior portion of the pars plana in cynomolgus monkeys.¹⁷ Prior to performing a multiple-dose study in human subjects, careful histopathological studies are required in subhuman primates after multiple dosing with PGF_{2α} in presumed therapeutic doses.

The present study examines the histopathological effects of PGF_{2α} applied topically in multiple doses to cynomolgus monkey eyes, and compares them with those produced by similarly applying epinephrine, which clinically produces an ocular hypotensive effect mediated, at least in part, by PGs.^{9,14,18}

Materials and Methods

Twelve adult female cynomolgus monkeys, 3-5 kg, were used in this study. They were secured in primate chairs for all experimental measurements and treated twice daily, 5 days each week for 2 weeks with PGF_{2α}, 250 μg or epinephrine 2% to one eye, and the respective vehicle to the contralateral control eyes, as previously described.⁹ All experimental manipulations previously performed on these eyes during the 2 weeks of treatment have been carefully described in companion studies.^{9,14} Most noteworthy is that each eye underwent an anterior chamber paracentesis with a 28-gauge needle under microscopic control 1 week prior to enucleation.¹⁴

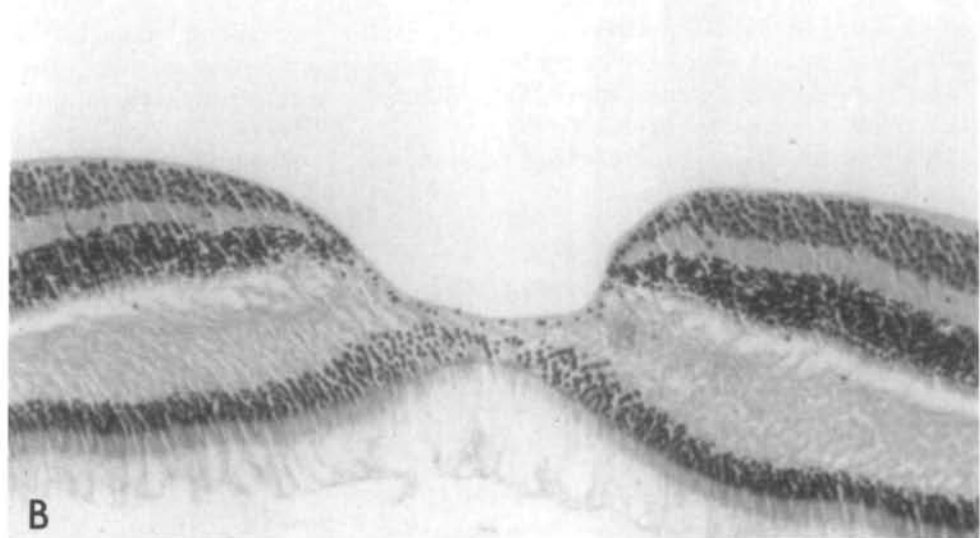
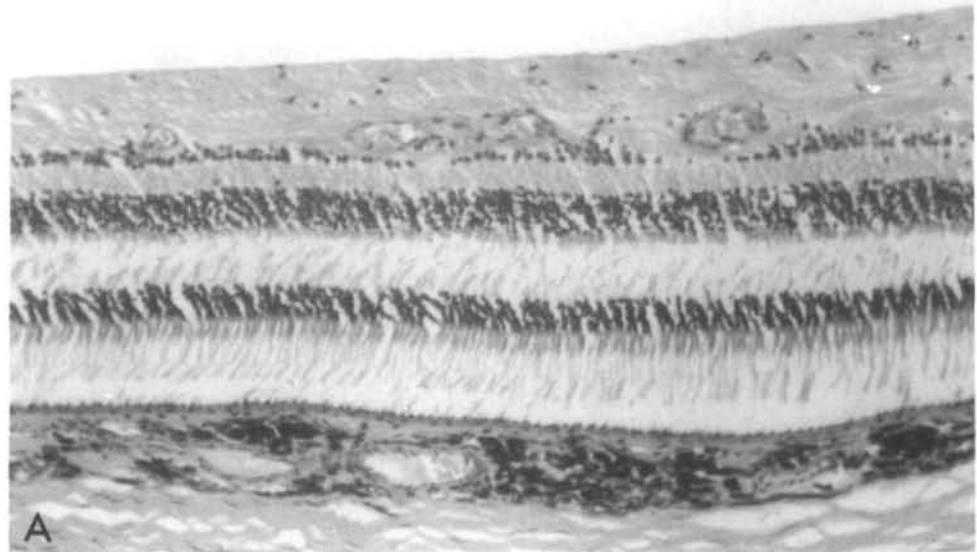
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Fig. 1. (A) Photomicrograph of retina of an eye treated with $\text{PGF}_2\alpha$ vehicle shows no abnormalities in retina, retinal pigment epithelium or choroid. (B) Light microscopy of foveal zone in an eye treated with epinephrine is within normal limits (both hematoxylin and eosin, original magnifications $\times 350$).



Four hours after the last topical application of the active drug or vehicle, bilateral enucleations were carried out under phenobarbital (25–35 mg/kg, intramuscularly) sedation. After making a 3–4 mm puncture wound at the pars plana with a #11 Beaver blade, all eyes were immediately immersed in triple aldehyde fixative, which consisted of formaldehyde 1%, acrolein 1% and glutaraldehyde 2% in 0.1 M cacodylate at pH 7.4 with 5 M CaCl_2 . Several weeks later, the eyes were rinsed with ethanol in a standard fashion, sectioned and paraffin-embedded in a standard fashion.

Sections with hematoxylin eosin stains were prepared with careful attention to include a section through the fovea in almost all eyes.

Electron micrographs were prepared of the peripheral corneal endothelium, anterior chamber angle structures, iris root and anterior ciliary body. The freshly enucleated eyes, which had been placed immediately in a freshly prepared mixture of triple aldehyde fixative for a minimum of 48 hr, were postfixed in buffered osmium tetroxide 1%. Epoxy-embedded tissue was sectioned 1 μm thick and stained with tolu-

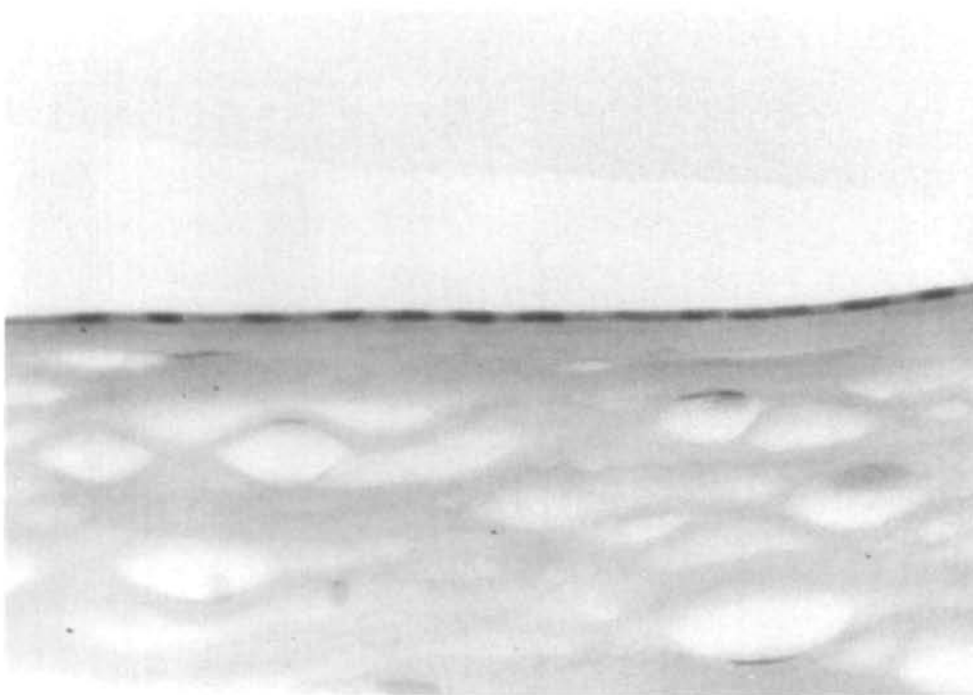


Fig. 2. Peripheral cornea in an eye treated with $\text{PGF}_2\alpha$ is normal (hematoxylin and eosin, original magnification $\times 1400$).

idine blue. Thin sections, cut 400 angstroms thick with a diamond knife, were mounted on copper grids after being stained with uranyl acetate and lead citrate. Sections were examined with a JEOL transmission electron microscope operated at 60 kV.

All light microscopy and electron photomicrographs were evaluated by four expert ophthalmic pathologists in a masked fashion.

This investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Results

Careful examination of at least three hematoxylin and eosin stained sections from each eye revealed no significant difference between any eyes treated with $\text{PGF}_2\alpha$, epinephrine or their respective vehicles (Figs. 1-3). Specifically, there was no evidence of any inflammatory, degenerative or morphological changes in any tissues. Cystoid macular edema was not observed in any eyes (Fig. 1B). Occasional mild iridial stromal hemorrhages were noted in some experimental and control eyes, which apparently was a result of the paracentesis performed 1 week earlier.

Electron microscopic studies of the peripheral cornea, anterior chamber angle structures, iris root and the anterior ciliary processes in each eye also failed to demonstrate any significant differences between experimental and control eyes (Figs. 4-9). Occasional trabecular cells displayed prominent cytoplasmic organelles (Figs. 4A, 7), including rough endoplasmic

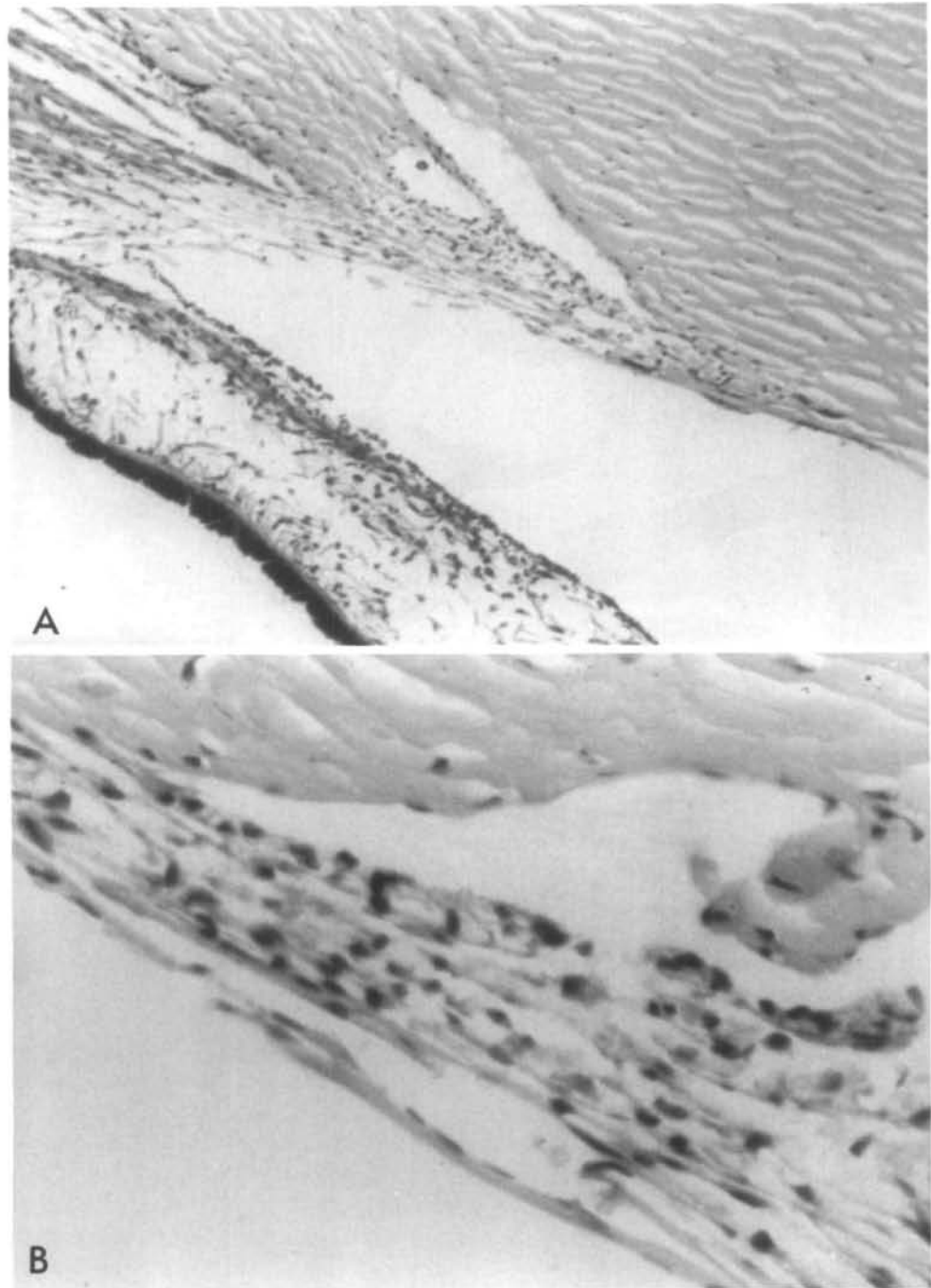
reticulum. Other cells showed 6 nm microfilaments consistent with actin (Figs. 5, 7). Intracellular vacuoles were observed in some endothelial (Fig. 6) and trabecular cells, with some disarray of the extracellular matrix. There were occasional dilated intertrabecular spaces. Focal cytoplasmic degeneration was observed in endothelium of the anterior trabecular meshwork and cornea (Fig. 7). Some trabecular and corneal endothelial cells showed myelin figures and mitochondrial swelling (Fig. 4B). Other trabecular cells displayed loss or "indistinctness" of cytoplasmic detail (Fig. 8). Pigmented ciliary epithelium showed normal basement membranes and cytoplasmic organelles (Fig. 9). All of these changes occurred with equal frequency in experimental and control eyes.

Overall, when examined in a masked fashion, the experimental eyes could not be distinguished from their contralateral vehicle-treated eyes.

Discussion

These results demonstrate that multiple doses of $\text{PGF}_2\alpha$ in subhuman primate eyes do not produce any adverse histopathological effects when compared with contralateral vehicle-treated eyes. Previous histopathological studies after exogenous PG administration that demonstrated adverse effects on the ciliary epithelium in rabbit¹⁶ or cynomolgus monkeys¹⁷ used high doses of PGE_1 or PGE_2 , types of PGs known to cause a greater rise in intraocular pressure and breakdown of the blood-aqueous barrier when compared with $\text{PGF}_2\alpha$.^{1,15} Of note is that PGs have

Fig. 3. (A) Low power photomicrograph of an eye treated with $\text{PGF}_2\alpha$ shows no abnormalities in the anterior chamber angle structures (hematoxylin and eosin, original magnification $\times 350$). (B) Higher power view of trabecular meshwork in an eye treated with epinephrine is unremarkable (hematoxylin and eosin, original magnification $\times 1400$).



been found to have a beneficial effect on corneal endothelial cell morphology in tissue culture.¹⁹

Several clinically used ocular hypotensive agents in glaucoma therapy, such as phospholine iodine or pilocarpine, seem to produce various adverse morphological effects, such as significant tissue damage to trabecular meshwork, iris sphincter, ciliary body and lens.²⁰⁻²² In primary culture of human trabecular endothelium, epinephrine was found to have a reversible direct effect on cell morphology, phagocytosis

and mitotic activity at concentrations of 10^{-5} or 10^{-6} M, but not at 10^{-7} M.²³ In the current study, the concentration of epinephrine reaching the trabecular meshwork may have been too low to produce the alterations when the 2% solution was applied topically twice daily in vivo.

Recent studies have suggested that $\text{PGF}_2\alpha$ reduces IOP primarily by increasing unconventional outflow through uveoscleral drainage.^{5,9,11,24-27} This enhanced uveoscleral drainage may result from PG-induced al-

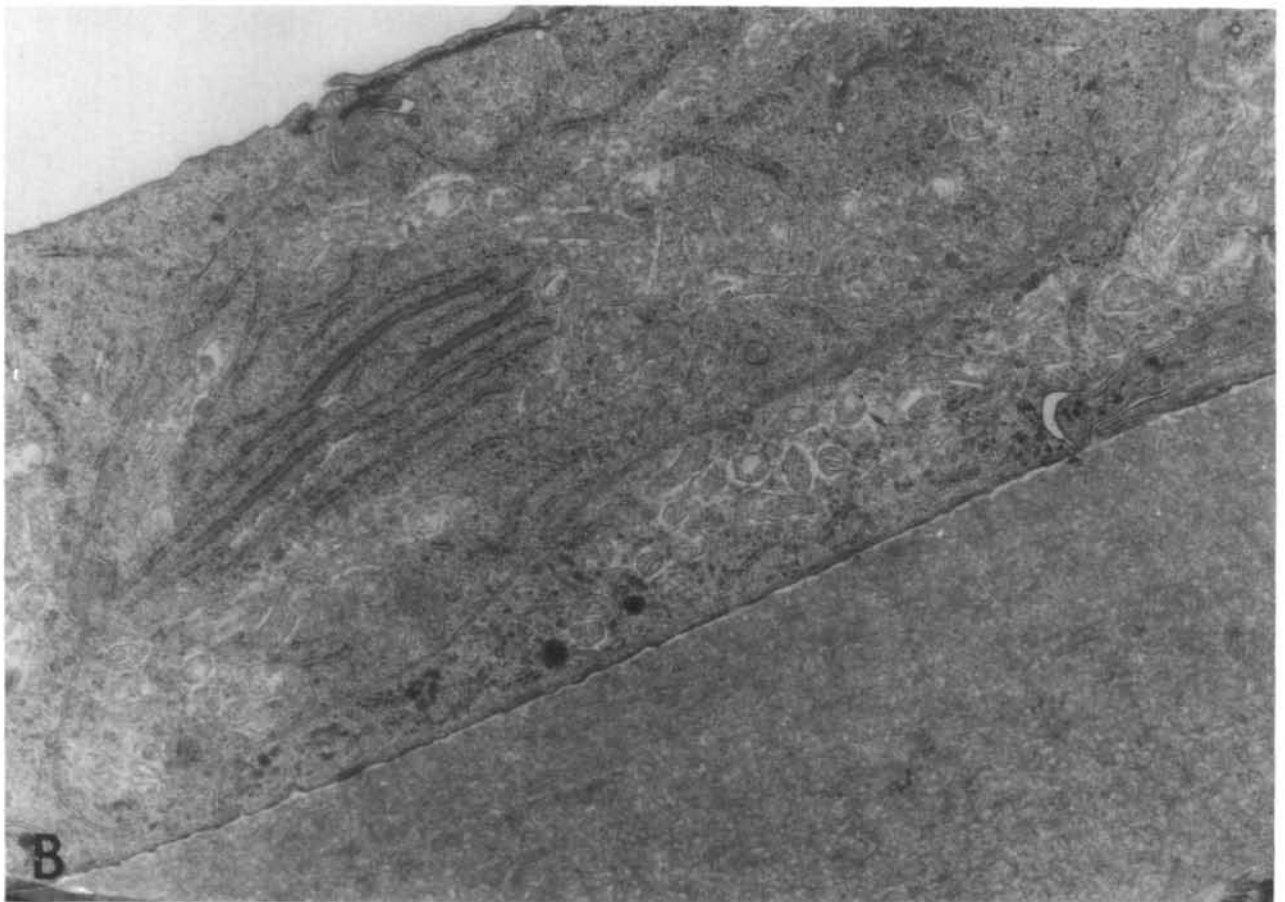
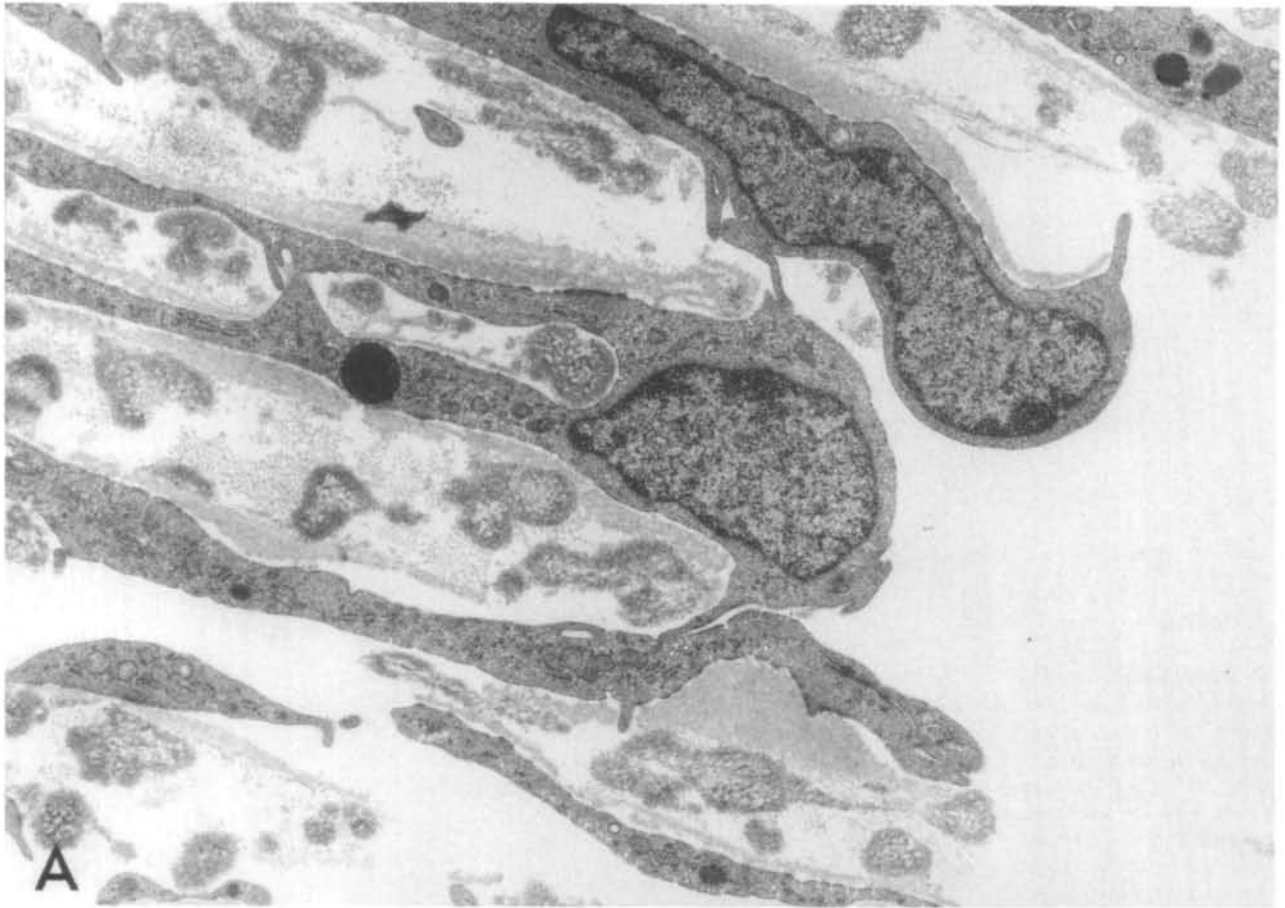


Fig. 4. (A) Electron micrograph of trabecular cells in a $\text{PGF}_2\alpha$ treated eye shows normal nuclei and cytoplasmic organelles. Basement membranes and collagen beams are unremarkable. (B) Corneal endothelium in a $\text{PGF}_2\alpha$ -treated eye is normal. Cellular borders and organelles are clearly visible and normal (original magnifications $\times 16,666$).

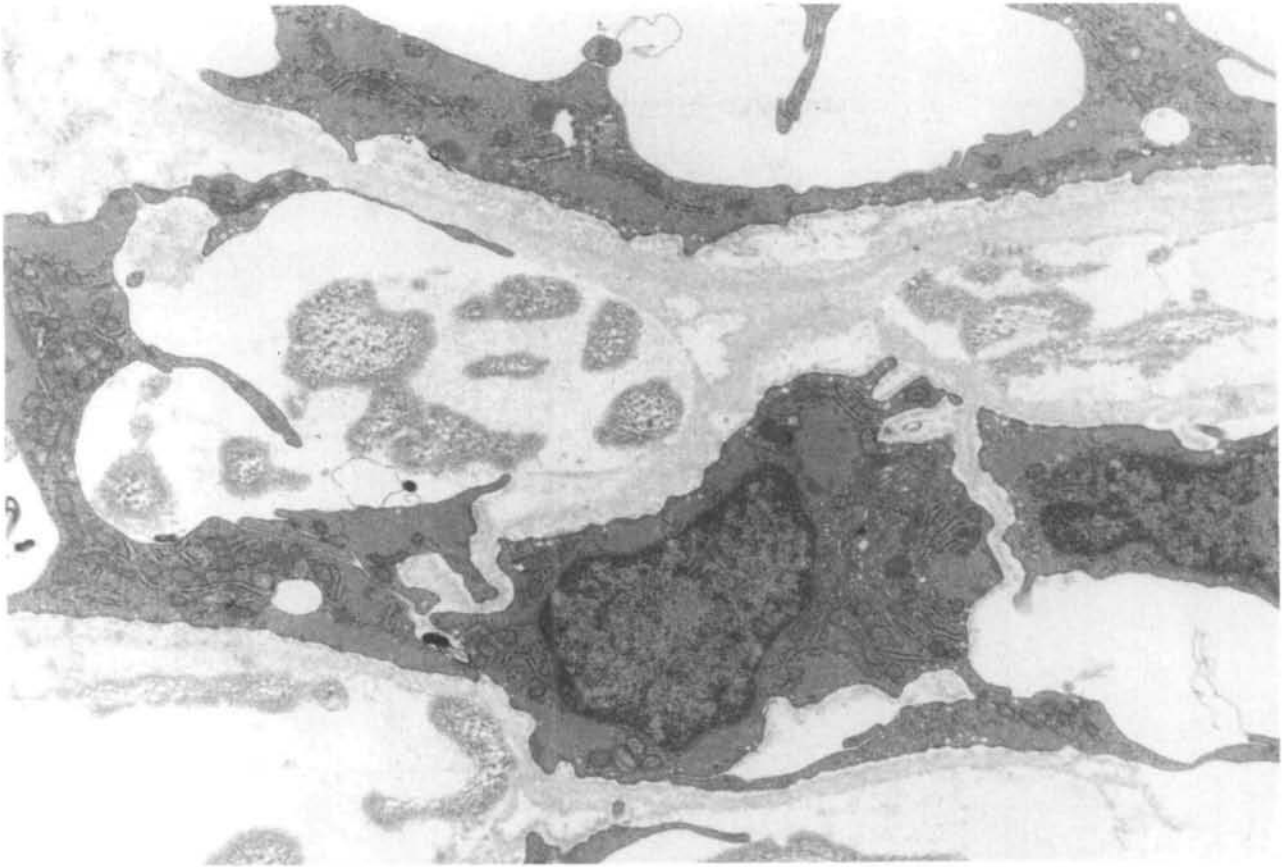


Fig. 5. Electron micrograph of trabecular meshwork of an eye treated with $\text{PGF}_2\alpha$ vehicle shows cells are intact and contain prominent organelles, rough surfaced endoplasmic reticulum, and actin filaments. (original magnification $\times 16,666$).

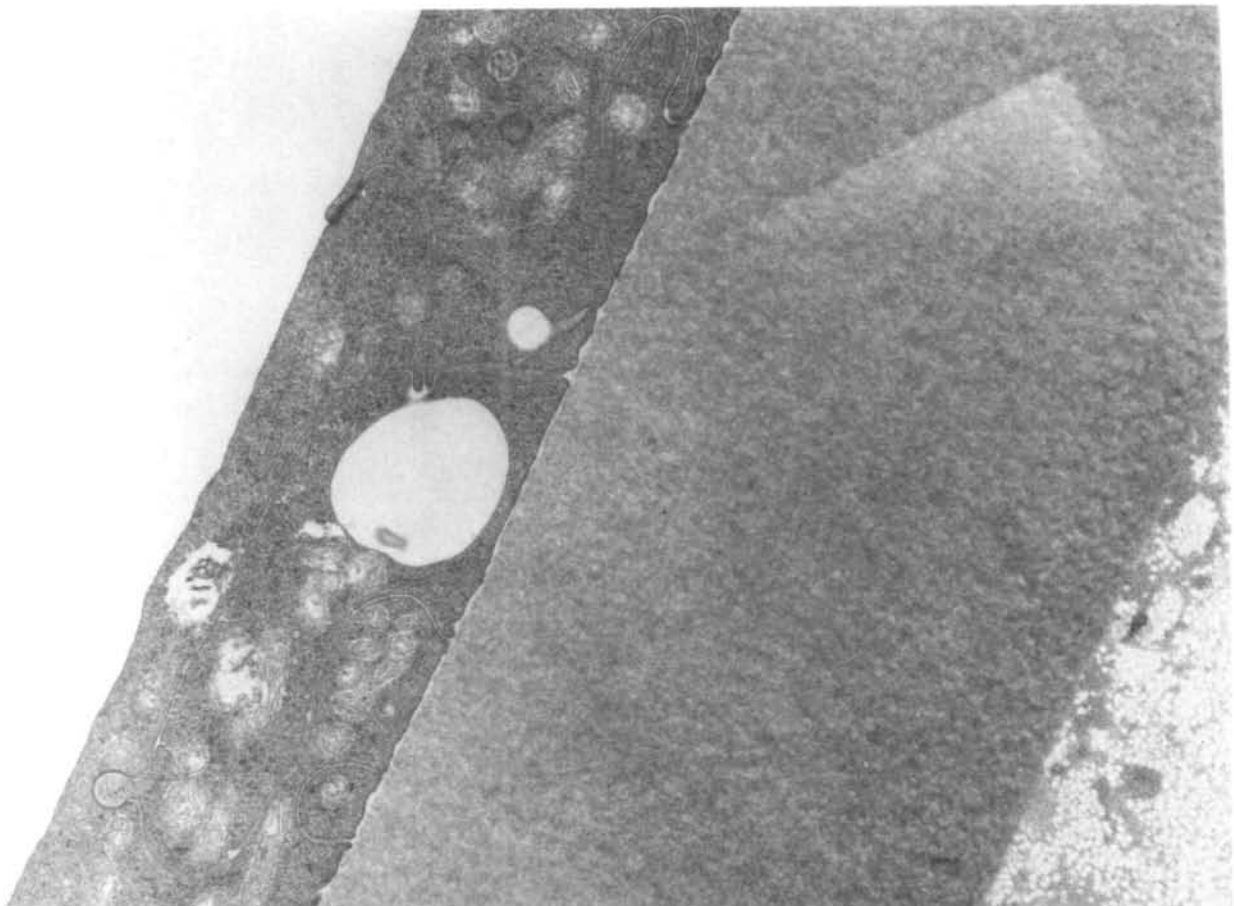


Fig. 6. Endothelial cells of an eye treated with epinephrine show normal organelles with an occasional intracytoplasmic vacuole. (original magnification $\times 16,666$).



Fig. 7. Trabecular meshwork of an eye treated with epinephrine vehicle shows normal-appearing cells with prominent organelles and cytoplasmic actin filaments. Collagen beams are unremarkable. (original magnification $\times 16,666$).

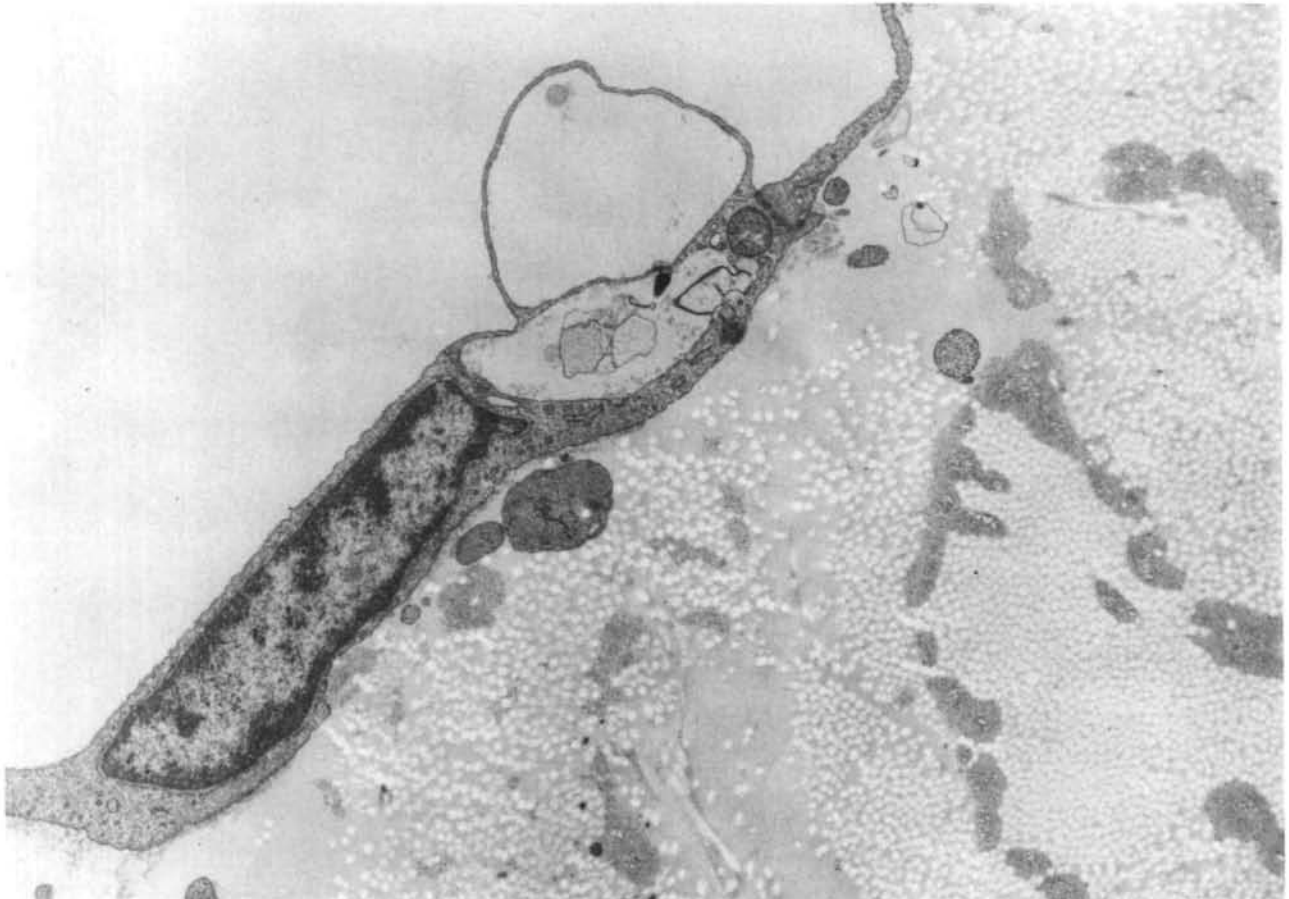


Fig. 8. Trabecular meshwork of an epinephrine vehicle-treated eye shows some trabecular cells with a mild loss of cellular detail and an endothelial cytoplasmic vacuole. (original magnification $\times 16,666$).

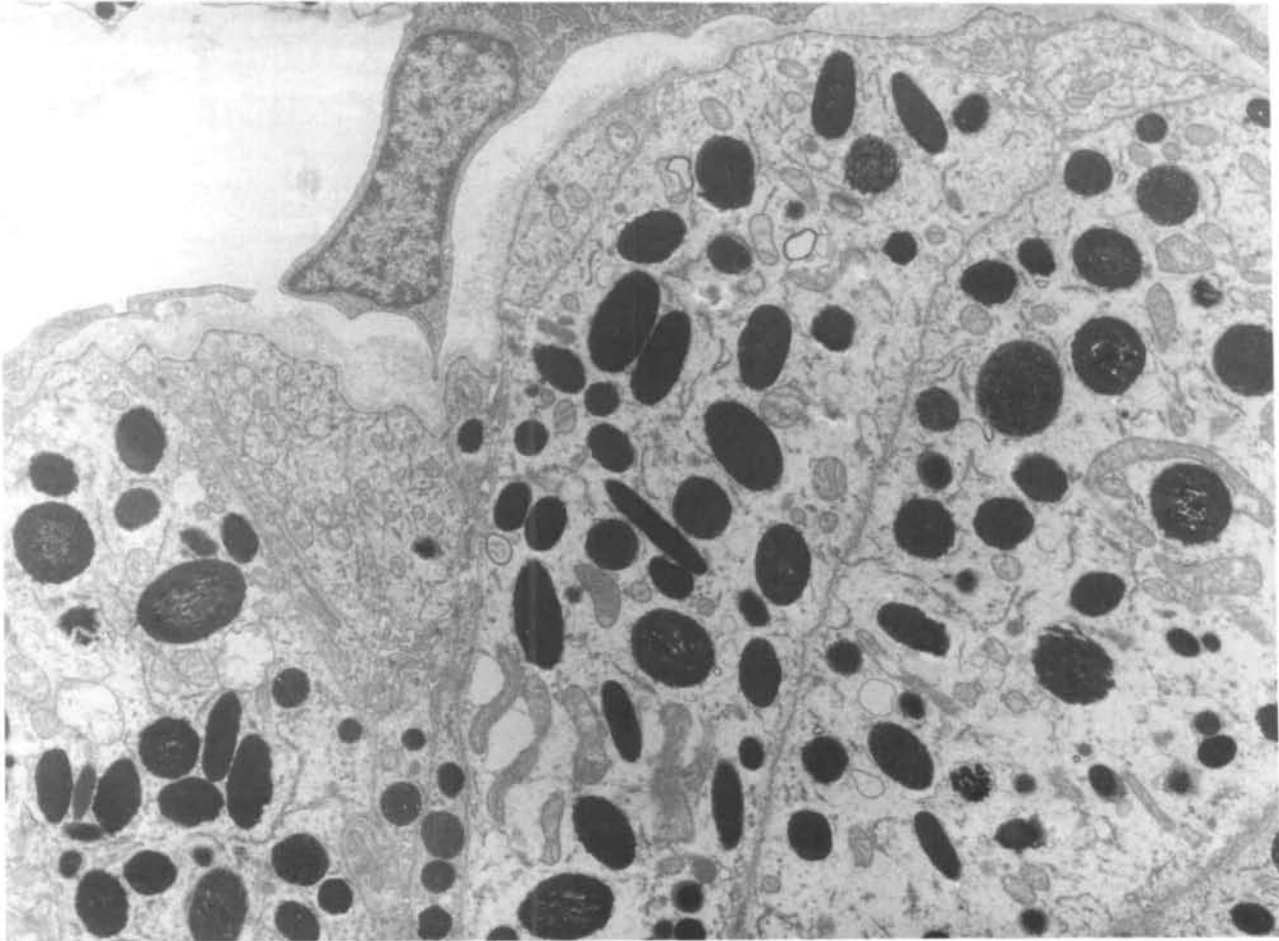


Fig. 9. Pigmented ciliary epithelium of a PGF₂α-treated eye. A normal basement membrane and basal cell membrane infoldings are present. The cytoplasmic pigment granules are completely melanized. A normal complement of cytoplasmic organelles is present. (original magnification ×12,500).

teration of extracellular material between ciliary muscle bundles (Lutjen-Drecoll, unpublished observations). This interesting possibility should be further pursued by in-depth ultrastructural evaluation of the ciliary muscle.

In conclusion, the failure to demonstrate any adverse histopathological effects after multiple dosing with PGF₂α in subhuman primate eyes is encouraging. Based on the results of this study, there does not appear to be any contraindication to its use in a multiple-dose clinical trial.

Key words: prostaglandins, epinephrine, monkeys, histopathology, eyes

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