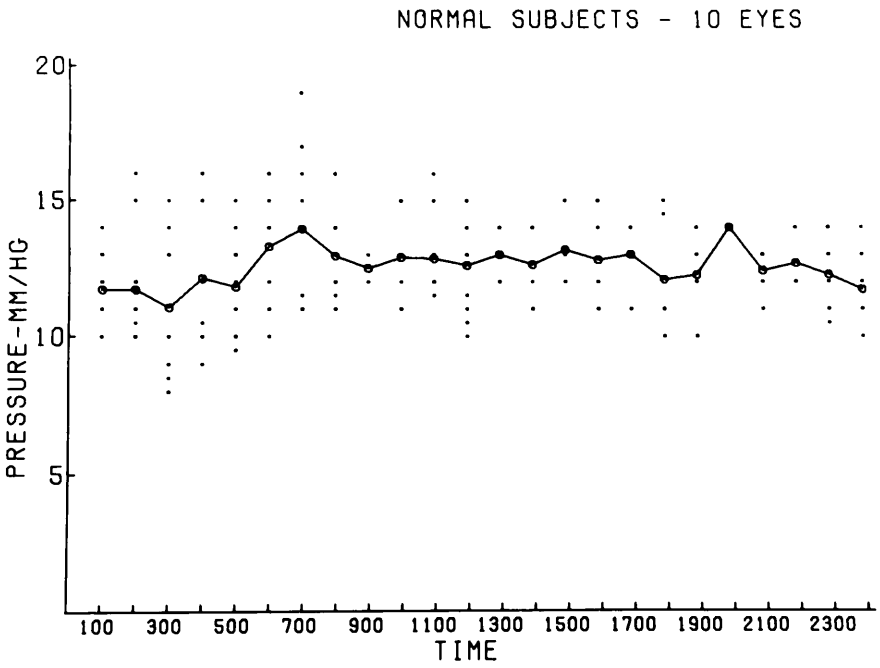

Fig. 4.

fluctuations in intraocular pressure, particularly in the early morning, have not received prior comment, and need to be evaluated more fully. If such fluctuations are common to all or most individuals, at all times of day, then the question of single ocular tension recordings and their significance must be reconsidered. The observation that intraocular tension drops to normal levels in the early morning even in severe glaucoma needs further confirmation in a larger group of patients. If this should prove the rule, then the question of appropriate nighttime medication for glaucoma subjects may need further study. The relationship between the diurnal fluctuations in intraocular tension and other physiologic parameters is presently under investigation as part of a larger study of the relation of sleep-waking cycles to clinical problems.[2]

Recording of hourly intraocular tension measurements may prove of value in the study of aqueous dynamics in normal and glaucomatous eyes. The present study shows that such repeated measurements are feasible without damage to the eye.

From the Departments of Ophthalmology* and Neurology,** Montefiore Hospital Medical Center and the Albert Einstein College of Medicine, 111 E. 210 St., Bronx, N. Y. 10467. Supported in part by funds from Public Health Service Grant OH00311, Clinical Research Center Grant RR-53, and an unrestricted grant from Research to Prevent Blindness. Manuscript submitted for publication Feb. 26, 1973; manuscript accepted for publication May 1, 1973.

REFERENCES

1. Langley, D., and Swanljung, H.: Ocular tension in glaucoma simplex, Br. J. Ophthalmol. 35: 445, 1951.
2. Weitzman, E. D., et al.: The twenty-four hour pattern of the episodic secretion of cortisol in normal subjects, J. Clin. Endocrinol. Metab. 33: 14, 1971.

Laser trabeculotomy in monkeys.* David M. Worthen and M. Gary Wickham.

The site of the greatest resistance to aqueous outflow in the monkey eye appears to be in the trabecular meshwork interior to Schlemm's canal.[1, 2] Perfused human eyebank eyes behave the same way.[3] The purpose of this study was to evaluate the effect of an argon laser on the trabecular meshwork in monkeys as seen at a microscopic level.

Four (4) *Cebus* and two (2) *Macaca* monkeys were tranquilized with Serynlan, then anesthetized with Nembutal. A Coherent Radiation Model 900 argon laser connected to a Zeiss photo slit lamp was used to deliver the argon laser beam. Treatments were done utilizing a 50 micron beam

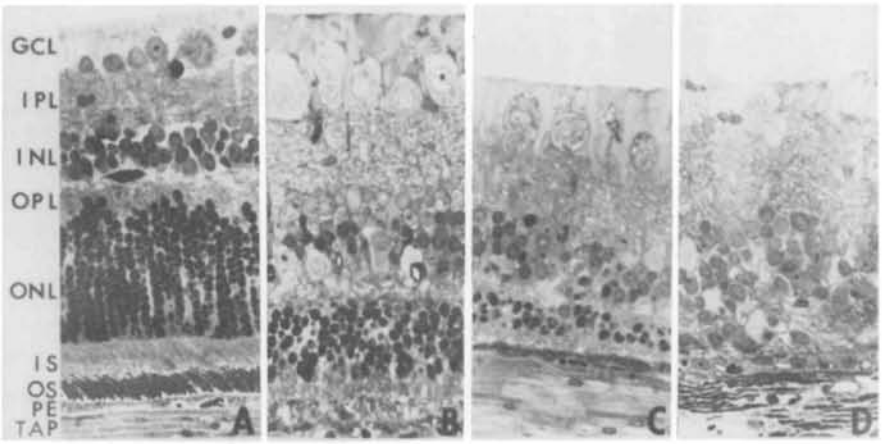

Fig. 7. Photomicrographs of retina from control cat fed commercial formula (A) and three cats fed the semipurified diet (B, C, and D). Anatomic layers are identified for the control only ganglion cell layer Gcl; inner plexiform layer, IPS; inner nuclear layer, INL; outer plexiform layer, OPL; outer nuclear layer, ONL; inner segment, IS; outer segment, OS; pigment epithelium, PE; and tapetum, TAP. Early (B), moderately advanced (C), and advanced stages (D) of the degeneration are represented (see text). Toluidine blue ×400.

spread involvement of the cone system even during the early stages of this degeneration could be seen in the cone ERG's which showed not only reductions in amplitudes but also delays in a- and b-wave implicit times.[16] The mechanism underlying delays in cone system implicit time in cats with retinal degeneration remains to be defined.

In contrast to the delays in cone system ERG b-wave implicit times, rod system ERG b-wave implicit times in the early stage were normal. Previous studies in man have shown that focal or patchy destruction of rods and cones is associated with reductions in ERG b-wave amplitudes with preservation of normal b-wave implicit times.[16] The present study has demonstrated that focal or patchy destruction of rods in the area centralis* is associated with reductions in rod ERG b-wave amplitude and normal rod b-wave implicit time. The idea that only a localized area of rods in the central retina is destroyed during the early stages is consistent with the normal histologic appearance of large areas of photoreceptors in the rod-dense peripheral retina. This is also consistent with the observation that the slope of the function describing the rod-dominated dark-adapted ERG amplitude vs. log-stimulus intensity was normal during the early stage. In moderately advanced stages large areas of rod photoreceptors were abnormal. The slope of the ERG amplitude vs. log-stimulus intensity function also became markedly abnormal, and the implicit times of the rod responses were delayed; both changes have been described in widespread degenerative retinopathies that occur in man.[9, 17-19]

Nutritionally induced retinal degeneration in the cat may prove to be an important model for the study of retinal degenerations in man. The delays in cone ERG b-wave implicit times seen in the early stages of practically all types of retinitis pigmentosa[17-19] have been stimulated for the first time in an animal model. When stimulus flash intensity was reduced so that cone system b-wave amplitudes from a normal subject could be matched with the reduced cone b-wave amplitudes seen in patients with retinitis pigmentosa, the implicit times of the normal subject were faster than the implicit times observed in these

*The ratio of rods to cones in the area of highest cone density (area centralis) of the cat retina has been shown to be approximately eleven rods to one cone.[15]

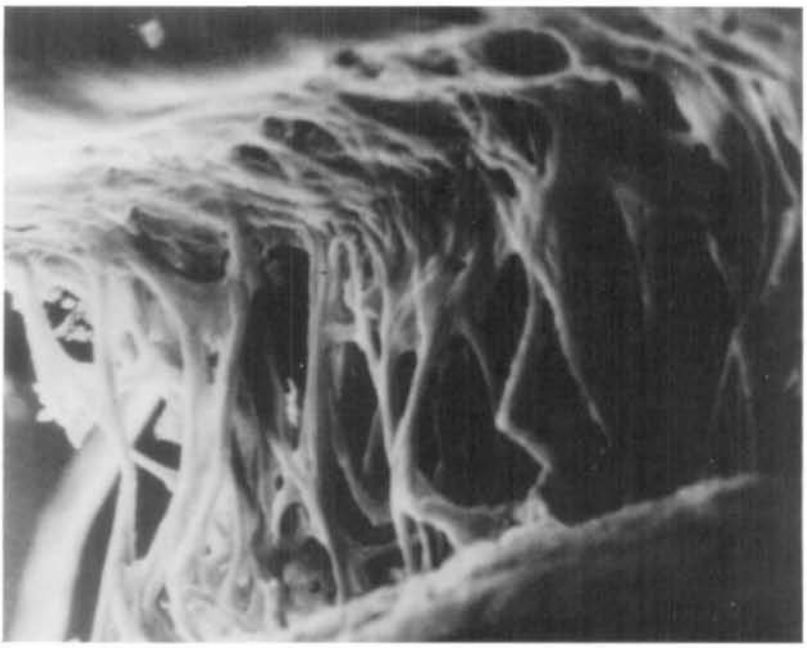

Fig. 1. Scanning electron photomicrograph illustrating the appearance of normal *Cebus* monkey angle. Note the smooth beams of the trabecular meshwork (below) and the even transition into the corneal endothelium (above). (×300.)

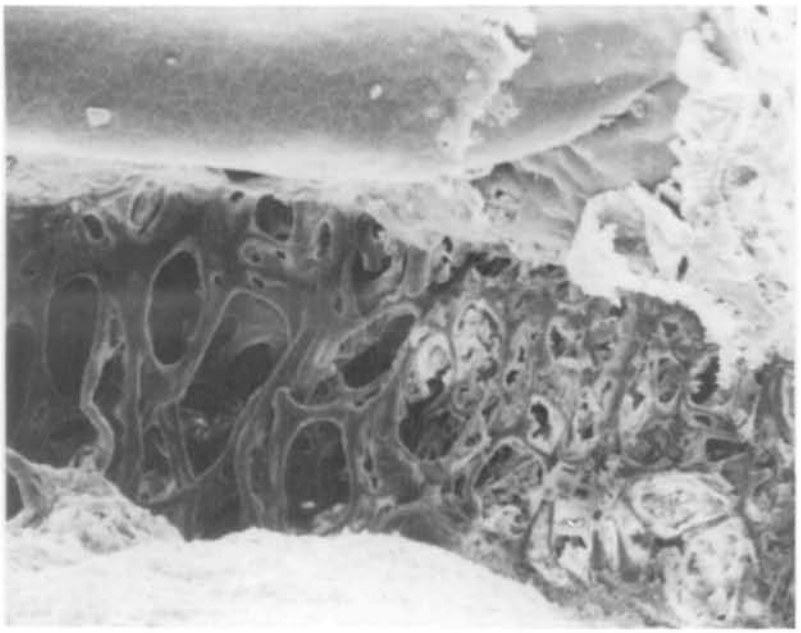

Fig. 2. *Cebus* monkey angle that received several bursts of 200 mW. laser energy for 0.2 second using a 50 μ beam. The differential effect seen between the denuded corneal endothelium above and the less affected trabeculum below is apparently due to the number of individual bursts delivered in each location. The openings in the treated trabeculum actually appear smaller than do those present in untreated area and appear white or "hot." (×500.)

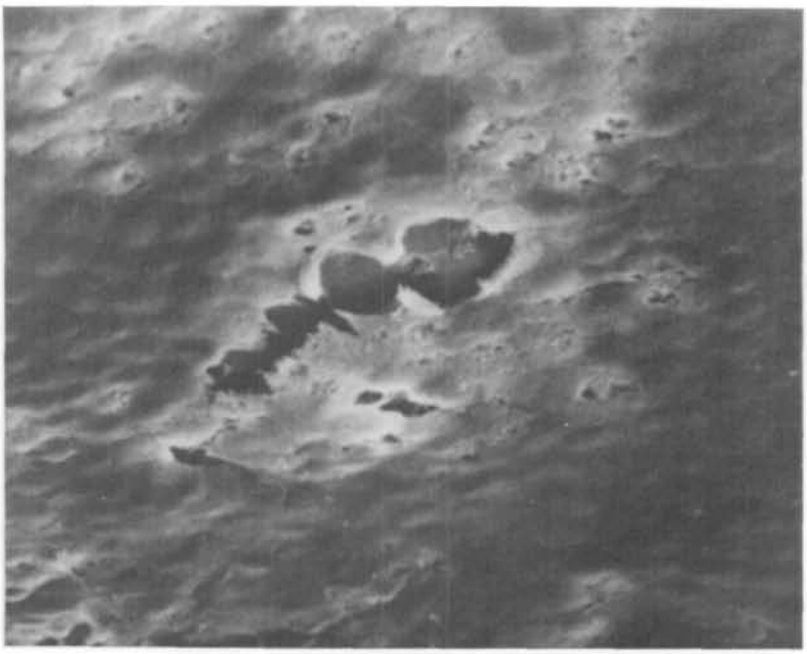

Fig. 3. Scanning electron photomicrograph of *Rhesus* monkey endothelium that received several 0.1 second bursts of 1,000 mW. laser energy at a 50 μ beam-spot size. Note the denuded endothelium which appears lifted off at the edges exposing Descemet's membrane below. The surrounding endothelial cells are thinned in areas where no direct burns were made. (×1,000.)

size delivered at 100 to 1,000 milliwatts with a shutter speed of from one-tenth of a second to continuous over two to three seconds. Coniolenses employed included the Zeiss four-mirror, the Lovac six-mirror, and the Goldmann three-mirror. It was necessary to perform a lateral canthotomy in order to use the latter two lenses. Of the 12 eyes involved, three served as control eyes and the other nine eyes received varying amounts of energy. The total energy delivered varied from 40 to 300 joules (joule = watt-second) per eye. During treatment the position of burns was recorded on a diagram and one animal of each species allowed to live at least three months after treatment. Two *Cebus* were killed at one month, and one of each species was killed the day after treatment. Both eyes of each monkey were processed for scanning electron microscopy.

Following a lethal injection of Nembutal, aqueous humor was removed from each eye and replaced with cold phosphate-buffered gluteraldehyde, the eye enucleated and immersed in the same fixative. The back of the eye and lens were removed and the anterior segment divided into wedges corresponding to the laser treatment areas. Each wedge was then processed for scanning electron microscopy.[4]

Clinical observations. Despite the high-energy levels used, none of the treated eyes showed continuous corneal change. All animals had some immediate posttreatment corneal edema corresponding to the treated areas. However, this edema seldom lasted for more than one day and even the animals receiving the highest energy levels had clear corneas at 24 hours. The creation of bubbles in the anterior chamber with laser burns was common. The aqueous would contain increasing amounts of cells and debris as the treatment progressed. There was a marked difference in the response to the laser beam in pigmented and unpigmented tissues. Oft-times after an unpigmented tissue area had been treated, a second application would cause a greater reaction, as if pretreatment had in some way altered the tissue and its response to the laser making it more like pigmented tissue, even though optically it appeared unchanged.

Scanning electron microscopy (SEM). Fig. 1 is a view of trabecular meshwork from a control eye.

Corneal endothelium. The corneal endothelium at the point of focus of the laser beam was subjected to two morphologically different types of damage. The most apparent was removal of discrete patches of cells leaving the underlying Descemet's membrane exposed (Fig. 2). The

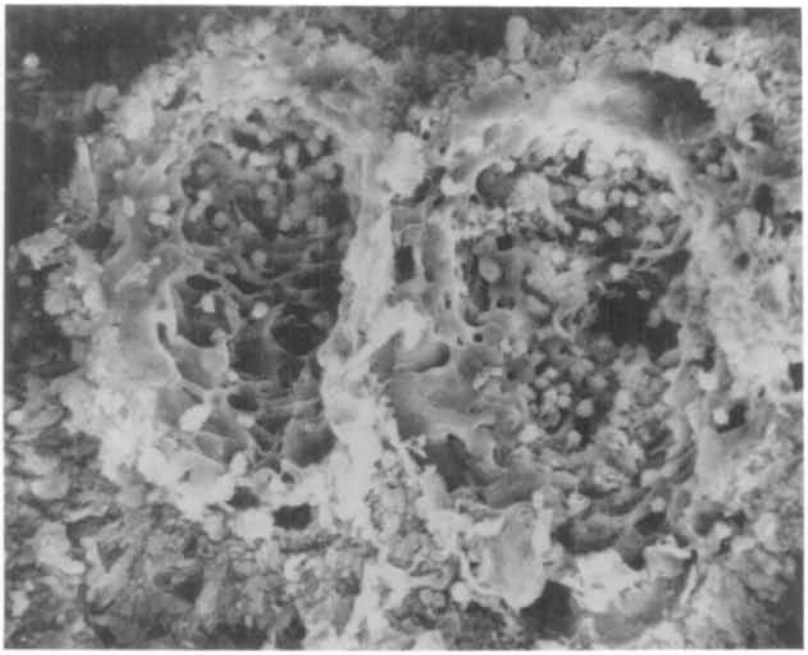

Fig. 4. Scanning electron photomicrograph of a *Rhesus* monkey iris that received several discrete burns of 100 mW. at 0.2 second. Note the two single burns in which the beam forced tissue in a line from the bottom to the top of the photomicrograph. "Melted" areas at the sides of the craters and the deposition of an amorphous cell mass at the end of the laser path (top) are typical results of the application of argon laser energy to the iris. (×400.)

denuded patches ranged from areas of approximately the same size as the beam to wholesale removal of the endothelium caused by the high-energy levels as seen in the right side of Fig. 2. The second type of damage, visible as thinned areas in the endothelium (Fig. 3), was much more subtle. These thinned regions did not always correspond with burns but occurred 100 to 200 microns from the burns.

Trabecular meshwork. Three types of change were noted and appeared to correlate with total energy density. The minimum change between treated and untreated trabeculums did not involve a physical rearrangement of tissue, but rather presented as an insulated area which appears more white or "hot" in the scanning photomicrograph. These were of a similar size to that of the beam diameter. Note the "hot" areas on the right side of Fig. 2. This change has not been documented in any manner other than scanning electron microscopy. On the basis of previous studies of insect material this change in appearance is like that associated with a change of dielectric constant.

The second type of trabecular damage consisted of compression of the beams and posterior push of the iris-trabecular junction. At higher energy density the laser beam appeared to act as a pressure source.

The third and most obvious type of meshwork damage occurred at high-energy densities. It consisted of a disruption of trabecular structure ranging from a moderate rearrangement of tissue to a more or less complete destruction of a section of meshwork that might also involve the adjacent cornea and iris.

Iris. The pigmented iris exhibited a dramatic demonstration of laser effect. Laser burns are clinically visible as whitish blemishes. Iris burns give a clear indication of the explosive effects in a pigmented tissue. The impact and angle of the laser beam can be assessed in each individual lesion by noting the pile of amorphous material left at the far site of the lesion (Fig. 4). In addition, on the sides of each burn crater isolated melanophores and bits of what appeared to be fused cytoplasm denoted a radial explosive effect.

The difference in the response of pigmented tissue vs. nonpigmented tissue to laser suggests the effect is largely due to thermal effect.[5] Two factors not realized before were the change in conductivity detected by SEM and the physical "pushing" of tissue by the laser beam. The altered response of nonpigmented tissue to a previous

laser burn may correlate with this changed conductivity. We are continuing to study the basic phenomenon occurring at the cellular level in hopes of gaining a better understanding of this response.

If laser energy were found to alter the resistance to outflow in human eyes, it might not be on the basis of excavation through the trabeculum but rather on the basis of cellular alteration. The severe destruction of corneal endothelium seen in these monkeys should be a warning of the potential damage that could be caused. The use of laser energy in the human angle should be tempered by the awareness of the severe damage that can be inflicted on iris and corneal endothelium.

The results of this study indicated the general energy level that might be applicable to a pigmented human eye. The data are not directly applicable to human glaucoma since these were normal monkeys and their trabecular meshwork is considerably different from that of the human eye. On the basis of the morphologic data it was concluded that an energy range level of 10 to 50 joules per eye should be reasonably safe. That could be achieved with an individual delivery of somewhere under 1,000 milliwatts at 50 microns for one second or less. On that basis, we feel that two-tenths of a second at 300 to 600 milliwatts with a 50 micron beam size would be a reasonable starting energy level for human subjects. Such a clinical study is now under way and will be the subject of a future communication.

From the Research Service, Veterans Administration Hospital, Gainesville, Fla. Supported by the Research Service, Veterans Administration.
Manuscript submitted for publication March 2, 1973; manuscript accepted for publication April 24, 1973.

Key words: laser, trabeculotomy, monkeys, glaucoma, scanning electron microscopy.

*Presented in part at the Association for Research in Vision and Ophthalmology Meeting, May, 1972, Sarasota, Fla.

REFERENCES

1. Peterson, W. S., Jocson, V. L., and Sears, M. L.: Resistance to aqueous outflow in the Rhesus monkey eye, Am. J. Ophthalmol. **72**: 445, 1971.
2. Ellingsen, B. A., and Grant, W. M.: Influence of intraocular pressure and trabeculotomy on aqueous outflow in enucleated monkey eyes, INVEST. OPHTHALMOL. **10**: 705, 1971.
3. Grant, W. M.: Experimental aqueous perfusion in enucleated human eyes, Arch. Ophthalmol. **69**: 783, 1963.
4. Wickham, M. G., and Worthen, D. M.: Scanning and transmission electron microscopy on the same tissue sample, Stain Technol. **48**: 63, 1973.
5. Watts, G. K.: Ruby laser damage and pigmentation of the iris, Exp. Eye Res. **8**: 470, 1969.

Density gradient of rabbit choroidal mast cells.* JAMES PRICE.

With the use of histologic sections or flat tissue preparations, tissue mast cells have usually been counted with the assumption that a Poisson distribution would fit their arrangement in tissue. And yet, these cells are almost invariably found in connective tissue concentrated at tissue interfaces of such structures as blood vessels and nerve fibers.[1, 2] Therefore, their density in any sample should be dependent on the local distribution of these structures.

After the work of Smelser and Silver[3] and that of Levene,[4] flat preparations were made from the albino rabbit uvea with the use of the retinal vessels for anatomic landmarks so that a reproducible preparation could be achieved. This paper will describe the density gradient of tissue mast cells that can be found in the albino rabbit's uvea.

Commercial house-bred albino New Zealand male rabbits were divided into groups based on weight. These are: Group A (N = 20), newborn, 0.10 kilograms (mean weight); Group B (N = 20), unweaned, 0.55 kilograms; Group C (N =

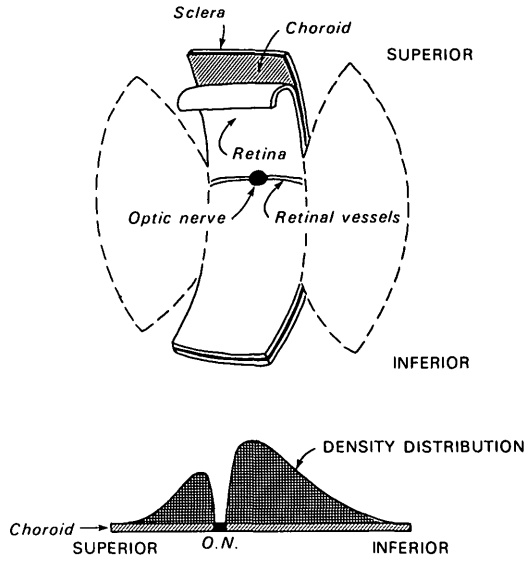

Fig. 1.