*Discussion.* A significant increase in facility of outflow did not occur to account for the decrease in intraocular pressure. We conclude from this experiment that the mechanism of action for the ocular hypotensive effect of timolol is not by increasing the outflow of aqueous humor from the eye. We believe that it is most likely that timolol decreases the secretion of aqueous humor, and experiments directly testing this hypothesis are underway.

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### Physiological effects of laser trabeculotomy in rhesus monkey eyes.

M. GARY WICKHAM, DAVID M. WORTHEN, AND PERRY S. BINDER.

*In a comparison of laser energy level and infusion response we found that high-level laser trabeculotomies caused a significant decrease in measured inflow with increasing infusion pressure. We feel that such treatments are ill-advised even though they apparently bring about a lower resting IOP and cause a visible effect at the time of laser delivery. Low-level laser trabeculotomy has the potential to effect a significant increase in measured inflow in normal monkey eyes at lower infusion pressures and may therefore be effective in increasing aqueous outflow in glaucomatous human eyes that do not have intraocular pressures in excess of 30 to 40 mm. Hg. From previous monkey and clinical studies we know that improper application of laser energy in the angle region will cause synechiae and hyperpigmentation regardless of energy level used, but in this study the treatment of the anterior meshwork and posterior corneal endothelium did not cause deleterious effects. Peripheral iridectomies have an effect similar to, but not as marked as, that of low-level laser trabeculotomy. No lens depression effect was seen in the comparison between untreated eyes and eyes with peripheral iridectomies.*

The treatment of glaucoma with laser energy in an attempt to reduce intraocular pressures in human eyes has been proposed in recent years,[1] and a substantial body of information on the clinically perceived effects of such treatments has accumulated.[2-4] However, there are no data describing quantitative changes in aqueous dynamics and the relationship of measured flow to the level of laser energy used. There is experimental evidence from studies on monkey eyes that excessive laser treatment can caused damage to the angle[5] as well as decreasing measured inflow and increasing intraocular pressure,[6] but there are no comparable observations on monkey eyes describing the effects of the minimum energy levels used to treat humans.[2] Most clinical lasers used in ophthalmology are argon lasers, and the literature on the microirradiation of the cell[7,8] indicates that the green wavelengths emitted by the argon laser interact with many cell constituents including chromosomes and mitochondria and could cause submicroscopic alteration in the outflow pathway cells.

The purpose of the present study was to obtain preliminary information on the physiological changes in the angle tissues of normal rhesus monkey eyes irradiated at specific laser energy levels. Iridectomies were performed to test the comparative influence of surgical alteration and test the need for intracameral communication to prevent posterior lens displacement during constant-pressure infusion.[9]

*Methods and materials.* Eighteen adult male rhesus monkeys weighing 4.5 to 13 kg. were utilized in a 3-year study of the effects of laser trabeculotomy on measured inflow as estimated by a technique of randomized constant-pressure infusion.[10] A mean value for each left eye was obtained under the following conditions: (1) untreated, (2) immediately pretreatment (the eye
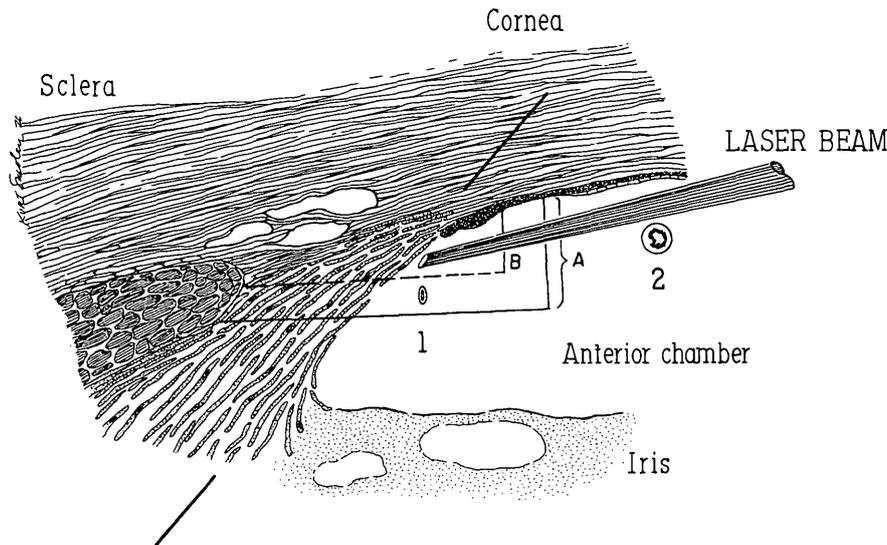
Fig. 1. Semidiagrammatic view of the anterior chamber angle of the rhesus monkey eye illustrating the spatial characteristics of the transchamber-directed laser beam. The beam cross-section at focus on the meshwork is oval (1) because of the angle of incidence to the meshwork. At either overfocus or underfocus (2) the beam diameter is much larger than at focus. In addition, as diagrammatically illustrated in 1 and 2, the power is not evenly distributed in the cross-section of the beam. The angle view provides two desirable windows. Window A demonstrates the *maximum* posterior extent of a valid treatment, and B represents the *optimum* area for laser placement. Drawing done to scale from one of the experimental eyes.

having been cannulated once previously), (3) immediately posttreatment, and (4) a second posttreatment examination at up to 9 months. Cannulations were done at a minimum interval of 2 weeks and a maximum interval of 1 year. Seven left eyes received a set of argon laser lesions with a total energy of 1.5 J. (0.2 sec./0.05 W. average power/150 exposures) over 2 clock hours of the inferior trabecular meshwork (low treatment). Seven left eyes received a total of 41 J. (0.2 sec./1.2 W. average power/170 exposures) equally spread over 2 clock hours of the superior and inferior meshwork (high treatment). Four left eyes served as untreated controls. The right eyes of each animal received no laser treatment and were evaluated each time with the contralateral eye. Each right eye received a peripheral iridectomy following the first constant-pressure infusion run to prevent lens depression[9] and to provide a comparative estimate of the effect of ocular surgical intervention.

Prior to laser treatment each animal was anesthetized with 1 mg./kg. phencyclidine hydrochloride (Sernylan; Bio-Ceutic Laboratories, St. Joseph, Mo.) and 5 to 10 mg./kg. sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.). The monkey was then strapped into a papoose board (Olympic Surgical Co., Seattle, Wash.) and held vertically with its chin on the chin-rest of the laser slit lamp. A

pulsed laser (Britt Corp., Los Angeles, Calif.) with a peak power of 20 W., a pulse width of 130 $\mu$sec, and a continuously variable firing rate of 5 to 500 bursts/sec. was used. The laser had a theoretical minimum spot diameter of 40 $\mu$, but we estimate that the spot size at the meshwork was increased to 50 to 60 $\mu$ because of scatter along the optical path. The laser beam was directed through a Goldmann one-mirror gonioscopic lens applied to the cornea with an intervening layer of Gonio-Gel (Muro Pharmaceutical Laboratory, Inc., Quincy, Mass.). The physical relationship of the beam to the meshwork (Fig. 1) was determined by the incident angle of view through the one-mirror lens. We estimate that the maximum angle obtained was 30° from the vertical axis of the meshwork but that most of the time we were working between 20° and 25° from vertical. The minimum spot size is obtainable only in a limited plane of focus. The high angle at which the beam strikes the meshwork will provide an oval cross-section (Fig. 1, 1) and less energy per unit area. If the meshwork is struck by a cross-section of the beam away from focus (Fig. 1, 2), the energy level per unit area will rapidly decrease as a result of the increasing area of the beam cross-section. Another important factor relative to the incident angle of the laser-meshwork interaction is the position of the canal of Schlemm relative to the beam (Fig. 1). An
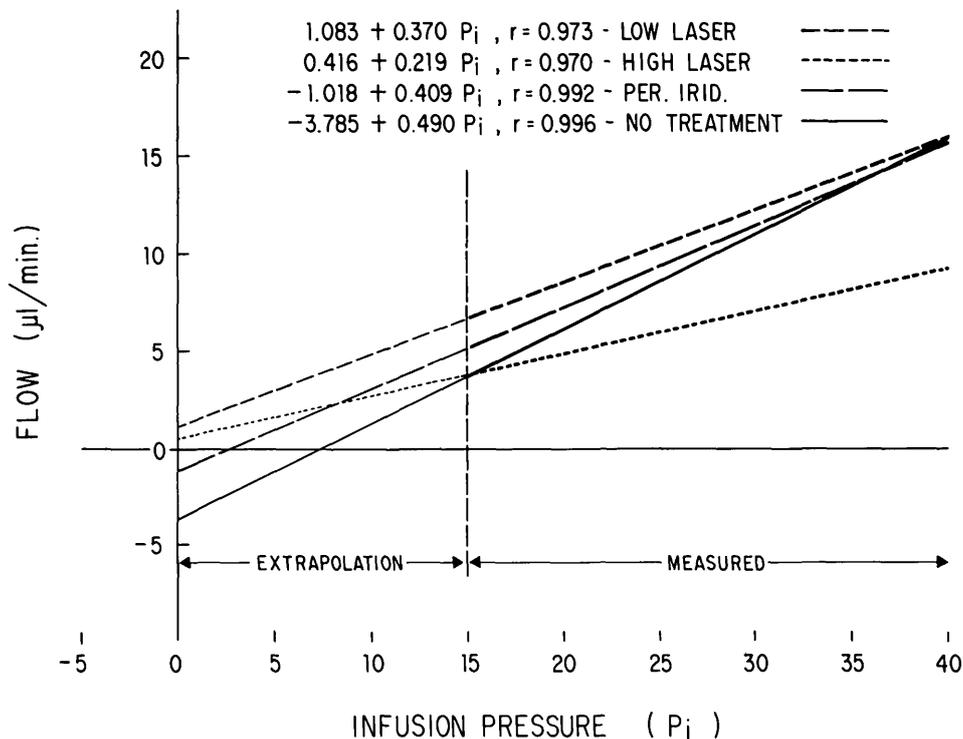
Fig. 2. Regression curves of the pressure-flow means for eyes receiving low-laser treatment, high-laser treatment, peripheral iridectomy, and no treatment. The high-laser curve is substantially different from the other three. At 15 mm. Hg infusion pressure, both the low-laser and peripheral iridectomy pressure-flow means differ significantly from the untreated one. Both laser curves have positive y intercept values, whereas the peripheral iridectomy and untreated curves have negative y intercepts.

Table I. Inflow values ($\mu$l/min.)

| Group | N* | 15 mm. Hg | 20 mm. Hg | 25 mm. Hg | 30 mm. Hg | 35 mm. Hg | 40 mm. Hg |
|---|---|---|---|---|---|---|---|
| Untreated | 900 | 3.04 ± 2.31 | 6.17 ± 3.00 | 8.86 ± 3.10 | 11.35 ± 4.37 | 13.32 ± 4.63 | 15.41 ± 5.44 |
| Peripheral iridectomy | 648 | 4.49 ± 2.28 | 7.46 ± 2.76 | 9.85 ± 2.96 | 11.30 ± 2.99 | 13.22 ± 3.41 | 15.05 ± 4.33 |
| Low-laser trabeculotomy | 162 | 5.59 ± 2.31 | 9.11 ± 3.79 | 10.65 ± 3.36 | 12.67 ± 3.84 | 14.82 ± 4.80 | 14.71 ± 3.71 |
| High-laser trabeculotomy | 162 | 3.51 ± 1.23 | 4.39 ± 1.30 | 6.29 ± 1.34 | 7.63 ± 1.92 | 8.26 ± 1.90 | 8.59 ± 1.82 |

*N = number of single observations of pressure-flow measurements.

imaginary line from the anterior extension of the canal to the point of entry of the laser beam through the cornea would pass through the posterior aspect of the corneal endothelium up to 50 $\mu$ anterior to Schwalbe's line. In the present study, the 1.5 J. treatments were confined to the optimum window (Fig. 1, B), but the effect of the 40 J. treatments could not be predictably limited although they, too, were placed at the anterior border of the meshwork.

Flow values obtained from the constant-pressure infusion procedure were analyzed with one-way and two-way ANOVA, paired and Students t test, and regression using the independent variables of pressure, run number, eye, weight category, anesthesia type and amount, date category, order done,

cannulation series, and surgical treatment. Pressure is especially important because of its previously described significant association with the flow means.[10] Significance levels of p > 0.001 for ANOVA and regression statistics and p > 0.005 for t statistics were established prior to data analysis.

Clinical examination of the monkey eyes, including biomicroscopy, retina examination, and photography, were made in a single-blind fashion by one of the authors (P. S. B.) to assess damage from cannulation and laser treatment.

*Results.* Table I lists the mean measured inflow values at each of the six infusion pressures for the untreated eyes, peripheral iridectomized right eyes, and laser trabeculotomy at low level and at

high level in left eyes. In each case the mean flow plus or minus the standard deviation is presented. Pressure-flow observations (n = 900) done on untreated eyes and those on the eyes after peripheral iridectomy (n = 648) showed a progressive increase in flow as the infusion pressure was increased. In contrast, pressure flow observations (n = 162) in left eyes with low-energy-level laser trabeculotomy showed a higher flow at the lower pressures than the previous two groups, with equivalence at the highest pressure. All three categories sharply contrast with the pressure-flow observations (n = 162) in left eyes with high-energy laser trabeculotomy treatments. These showed diminished flow at all pressures, with a less obvious increase of flow rates with increasing pressure. Both the eyes receiving peripheral iridectomy and low-energy-level laser trabeculotomy treatments showed a significant difference from the untreated controls at 15 mm. Hg (p <0.001). In contrast, the high-energy-level laser trabeculotomy treatment had significantly different results from those of the other three at 30 through 40 mm. Hg (p <0.001). At physiological pressure levels the flow rates for eyes receiving either a peripheral iridectomy or a low-energy-level laser trabeculotomy were much higher than those of both the untreated eyes and those receiving high-energy-level laser.

Fig. 2 presents the regression curves calculated from the pressure-flow means. The curves showed a clear-cut difference between eyes. The high-laser-treated eyes showed a lower slope and a positive y intercept. The low-laser-treated eyes had a steeper slope but also a positive y intercept. The peripheral iridectomy and control eyes had respectively steeper slopes, and both had negative y intercepts. The power of the regression analysis and the high correlation values renders these data very valuable. The difference between the low laser and the other three groups was highly significant (p <0.001) at 15 mm. Hg, significant (p <0.005) at 20, and marginally significant at 25. The high laser was statistically (p <0.001) different from the remaining three at pressures from 30 to 40 mm. Hg. The marked contrast between the two laser treatments can be seen in those curves where the low laser had higher flow at low pressures and the high laser had low flows at high pressures.

Table II presents the change in flow divided by the change in pressure in each category between 15-25 and 25-40 mm. Hg. These figures emphasize that in all cases this quasi-"facility" value arithmetically decreases, but the drop is most marked in the laser treated eyes.

Clinical examination of laser-treated eyes failed to show any obvious defects. In repeated examinations laser-treated eyes were not detected. In addition there was no evidence that the laser

**Table II.** Calculated* C values

|  | *15-25 mm. Hg* | *25-40 mm. Hg* |
|---|---|---|
| Untreated | 0.59 | 0.44 |
| Peripheral iridectomy | 0.53 | 0.35 |
| Low-laser trabeculotomy | 0.51 | 0.28 |
| High-laser trabeculotomy | 0.28 | 0.15 |

*Values were obtained by subtracting grand flow means at indicated infusion pressures and then dividing by the pressure differential. The same data base as that in Table I was used.

treatments caused any obvious clinical iris, lens, or corneal damage. Under routine circumstances the only clinical signs of ocular manipulation were the cannulation needle tracks in the corneas and the iridectomies in the right eyes. The resting intraocular pressure was lowest in the high-level-laser treated eyes.

*Discussion.* The analysis of pressure-flow data as a function of treatment points out several important facts. (1) High-level laser treatments cause a reduced inflow at the three highest infusion pressures even when the energy is applied over narrow, localized areas. (2) Low-level laser treatments effect a significant increase in inflow at 15 mm. Hg and a marginally significant increase at 20 and 25 mm. Hg as well. (3) Thus low-level laser and high-level laser treatments modify inflow in significantly different ways. (4) In in vivo eyes, peripheral iridectomies cause changes similar to, but less marked than, those caused by low-laser treatments. This would suggest that the high-energy-level laser trabeculotomy had rendered the outflow pathway inelastic or scarred and that flow would not increase as the pressure was increased.

An extrapolation of the data on the laser treatment of normal monkey eyes to the treatment of glaucomatous human eyes is difficult because of the lower normal outflow rate of human eyes and the abnormally high intraocular pressures associated with primary open-angle glaucoma. However, the use of single or double treatments to the anterior meshwork of normal monkey eyes with argon laser energy causes neither clinically visible changes in angle structure nor secondary pathology when delivered at specific energy levels and to a specific site. Involvement of the iris base in a treatment with even a small amount of energy will cause clinically visible synechiae and/or hyperpigmentation. To avoid this complication, the laser spot must be placed at the far anterior boundary of the meshwork. A minimum portion of the angle should be involved in a treatment. High amounts of energy (+10 J.) should not be used. The 40 J. treatments described in this paper were easy to perform, and it is easier to overtreat in an attempt to gain a visible effect[2] than it is to treat at the 1.5 J. level. A summation of our experi-

ence with the argon laser trabeculotomy technique in both normal monkey eyes and glaucomatous human eyes[2] indicates that the use of the technique is most appropriate in those glaucomatous human eyes that have relatively low intraocular pressures (<30 mm. Hg). The intraocular pressure data obtained from our experience with the laser technique in humans implies the same feature quantitatively shown in the monkey experiment: low level laser treatments do not significantly change the slope of the pressure-flow curve. However, the shift of the y intercept to a positive value will cause a significant upward increase in "outflow" ($\cong$ measured inflow) at near-physiological infusion pressures. Clinically this means an effect will be maximum in cases of mild, not severe, intraocular pressure elevations. Although the pressure-flow data best fit a linear regression curve, the calculated facility value in each group decreased as the pressure was increased. This may suggest that laser treatment has the effect of increasing flow at low pressures and reducing flow as the pressure is increased. However, the fact that infusion rates were higher with low laser is lost when a ratio is used. This use of ratio is a common error in analysis of statistical results, pointing to the greater value of raw data rather than ratios such as a percentage or facility value.

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### Effects of catecholamines on the intraocular pressure of conscious owl monkeys. J. W. LAMBLE AND A. P. LAMBLE.

*Conscious unsedated owl monkeys responded to locally applied epinephrine (0.125 to 2 percent) with concentration-related reductions of intraocular pressure lasting over 5 hours. Some animals reacted initially to the higher concentrations with an increase in pressure. Isoproterenol (0.2 percent) caused a reduction, but norepinephrine (1 percent) gave a significant increase in intraocular pressure. Sotalol hydrochloride (2 percent), which was free of intrinsic effects, caused reversal of the pressure reduction seen with a submaximal (0.25 percent) epinephrine concentration. These results are related to published data on healthy humans.*

A previous report[1] described effects of miotics on pressure in the eyes of unsedated owl monkeys, and the present study extends use of this model to an investigation of locally applied sympathomimetic amines. The relatively greater potency of norepinephrine at $\alpha$-adrenoceptors and isoproterenol at $\beta$-adrenoceptors, plus the fact that epinephrine is effective at both, have been used to analyze the roles of the receptor types in mediating intraocular pressure (IOP) effects.

*Methods.* The methods have been outlined previously.[1] Thoroughly trained conscious owl monkeys *(Aotus trivirgatus)*, 0.8 to 1.5 kg., were restrained during measurements in loosely fitting cloth bags. Tonometry, with a pneumatic instrument based on the Digilab (Cambridge, Mass.) floating probe, was performed under 0.5 percent oxybuprocaine hydrochloride–induced local anesthesia. (-)-Epinephrine bitartrate* (E), (-)-norepinephrine bitartrate† (N), (-)-isoproterenol bitartrate* (I), and sotalol hydrochloride were each freshly prepared as solutions in sterile physiological saline. Dose volume was 20 $\mu$l for all solutions except those containing I, of which 10 $\mu$l were applied. In the text, all concentrations

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*Supplier, Sigma Chemical Co., London, U.K.
†Supplier, Koch-Light, Colnbrook, U.K.