result of systemic and local changes in the chemical and ionic environment. Increased choroidal blood flow might facilitate the resorption of subretinal fluid by serving as a sink for fluid drawn off by ionic or osmotic gradients. A similar result would be achieved if the drug in some way increased the passive permeability of the RPE, although there is no evidence for such an effect.

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Key words: retinal detachment, retinal pigment epithelium, acetazolamide, rhegmatogenous, nonrhegmatogenous, central serous retinopathy

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The autoregulatory response of the retinal circulation to a short-term reduction in intraocular pressure (IOP) to hypotonic levels was studied in 15 normal subjects by means of the blue-field entoptic phenomenon. This phenomenon allows the perception of the leukocytes flowing in one's own retinal macular capillaries. This phenomenon allows the perception of the leukocytes flowing in one's own retinal macular capillaries. Subjects were asked to compare the leukocyte speed in one eye with that in the fellow eye while a scleral suction cup was used to raise the IOP in one eye to levels above 25 mm Hg for approximately 12 min. The release of the suction cup caused a drop in IOP to levels between 4 and 7 mm Hg, at which time all subjects reported a higher leukocyte speed (hyperemia) in this eye than in the fellow eye. After an average of 4 min the speed was observed to be equal in both eyes. The average IOP at which the equalization occurred was 6.8 ± 1.3 mm Hg. The retina can therefore normalize leukocyte capillary speed and presumably blood flow at IOPs at least as low as 6.8 mm Hg. The results of 16 experiments on the same eye of one subject suggest that under these experimental conditions, the lowest IOP for which the retina can fully autoregulate is around 6 to 7 mm Hg. (Invest Ophthalmol Vis Sci 23:124-127, 1982.)

Autoregulation, an intrinsic property of many organs, allows a tissue to maintain constant blood flow despite changes in perfusion pressure. A number of researchers have investigated the autoregulatory response of the retinal vasculature to decreased perfusion pressure induced by an elevation of intraocular pressure (IOP). Recent
work suggests that the human retina can normalize flow by autoregulation for a maximum increase of IOP to approximately 30 mm Hg. However, previous studies have not examined the retinal autoregulatory response after an increase in perfusion pressure produced by a reduction of IOP to hypotonic levels.

In this report, we demonstrate that the autoregulatory response of the normal human retina can normalize blood flow at IOPs at least as low as 6 to 7 mm Hg. This autoregulatory response has been investigated with the blue-field entoptic phenomenon, which allows the perception of leukocytes in one's own retinal macular capillaries.

**Subjects and methods.** Fifteen subjects (15 eyes) between 23 and 71 years of age (mean 37.6 ± 13) participated in this study; all had a normal eye examination and no history of systemic hypertension. On blue-field testing at normal IOP, all subjects observed the same number, speed, and spatial distribution of leukocytes in both eyes. The resting IOP, measured by pneumotonometry (Model 30R; Digilab), ranged from 9 to 16 mm Hg (mean = 13.6 ± 2 mm Hg). Brachial artery blood pressure, measured by sphygmomanometry, was between 90/55 and 110/70 mm Hg. The perfusion pressure (P), calculated from the relation $P = \frac{2}{3} P_m - IOP$, was 38.3 ± 5.9 mm Hg at normal IOP. $P_m$, the mean brachial artery pressure, was calculated according to the formula $P_m = 0.33 (P_s + 2P_d)$, where $P_s$ and $P_d$ are the systolic and diastolic brachial artery pressures. The factor $2/3$ accounts for the difference in pressure between the brachial artery and the ophthalmic artery.

The subjects were seated in front of two blue-field entoptoscopes (Model BFE-110; Medical Instrument Research Associates), each illuminating one eye. After the application of anesthetic drops, the IOP was measured in both eyes and a Digilab-Langham pressure cup was placed on the temporal sclera of one eye. Under pneumotonographic monitoring, the IOP was raised to a level between 35 and 40 mm Hg for approximately 4 min and then slowly decreased in small steps to a level between 25 and 30 mm Hg. After approximately 12 min of elevated IOP, the suction cup was removed quickly, allowing the IOP to fall to a hypotonic level. This IOP was measured and documented as IOP$_{off}$. The subjects were then asked to compare the leukocyte speeds between eyes until the speeds were perceived as equal. At that time the IOP was measured and documented as IOP$_{equal}$. The time between the removal of the suction cup and the equalization of leukocyte speed was documented as $T_{equal}$.

In one subject, 16 measurements were performed on the same eye on different days to evaluate the effect of IOP$_{off}$ on $T_{equal}$ and IOP$_{equal}$. These parameters were determined after the IOP had been maintained constant at pressures between 15 and 35 mm Hg for periods varying be-
between 5 and 25 min. The subject was not aware of the magnitude of the initial rise in pressure or of the IOPoff value.

**Results.** Immediately after the removal of the suction cup, all subjects reported a significantly faster leukocyte speed in the eye with lower IOP than that in the fellow eye. The distribution of leukocytes within the field of observation appeared unchanged. IOPoff ranged between 4 and 7 mm Hg. After a short duration the speed of leukocytes in the hypotonic eye appeared to decrease gradually and to equal that in the fellow eye after a Tequal of 4 ± 1.6 min. IOPequal ranged between 4 and 9 mm Hg (average = 6.8 ± 1.3 mm Hg). Values of IOPequal, which were obtained from three experiments in three subjects under the same experimental conditions, did not differ by more than 2 mm Hg for each subject.

Fig. 1 demonstrates the relationship between IOPequal and IOPoff and between Tequal and IOPoff obtained from 16 measurements performed on the same subject. IOPequal increased significantly with IOPoff when IOPoff was above 6 to 7 mm Hg, but appeared to be independent of IOPoff at values below this range. On the other hand, Tequal was strongly dependent on IOPoff below 6 to 7 mm Hg and less so above this pressure.

No significant correlation was found between the amount of IOP elevation above normal and Tequal or IOPequal, (2) the duration of elevated IOP and Tequal or IOPequal, and (3) the amount of IOP elevation times the duration of the elevation and Tequal or IOPequal.

**Discussion.** The increase in leukocyte speed observed immediately after the removal of the suction cup corresponds to a hyperemia produced by the sudden increase in perfusion pressure. A similar phenomenon was observed by Ffytche et al.3 in porcine eyes. The subsequent normalization of leukocyte speed at IOPs below resting value can be attributed to an autoregulatory response.

There is strong evidence that the changes in the observed leukocyte speed reflect proportional changes of macular retinal capillary blood flow.6 Our results therefore suggest that the retina can normalize blood flow by autoregulation for IOPs at least as low as 6.8 mm Hg on the average.

The series of experiments performed on one subject allows a more precise characterization of the factors affecting the autoregulatory response by eliminating intersubject variations. In this subject the lowest IOP for flow normalization was approximately 6 to 7 mm Hg. IOPequal was nearly constant for IOPoff values below this level and increased proportionally to IOPoff for values above this level. Tequal was, in most instances, between 1 and 2 min for values of IOPoff above 6 to 7 mm Hg. This range of Tequal is typical for the retinal autoregulatory response as previously reported4,6 and as indicated by recent laser Doppler measurements.8 The significant increase in Tequal at levels of IOPoff below 6 to 7 mm Hg is most probably due to retinal autoregulatory response that is insufficient to return leukocyte speed to normal. The delayed normalization of leukocyte speed occurs only through the slow rise in IOP and corresponding decrease in perfusion pressure produced by aqueous formation. Therefore 6 to 7 mm Hg appears to be the lowest IOP at which the retina can maintain normal macular blood flow in this subject under the described experimental conditions.

This study provides the first evidence for human macular retinal autoregulatory response to a decrease of IOP below normal. Knowledge of the extent of such response may have clinical relevance in the management of patients with ocular hypotony.

From the Department of Ophthalmology, University of Pennsylvania School of Medicine, and the Scheie Eye Institute, Philadelphia, Pa. This work was supported by NIH grant EY-01242 and Research Career Development Award EYO-0120 (C. E. R.) from the National Eye Institute, the Elaine O. Weiner Teaching and Researching Fund, and in part by a Fight for Sight Grant-in-Aid, Fight for Sight, Inc., New York. Submitted for publication Jan. 15, 1982. Reprint requests: Dr. Charles E. Riva, Scheie Eye Institute, 51 North 39th St., Philadelphia, Pa. 19104. Dr. Grunwald is on leave from the Eye Department, Beilinson Medical Center, University of Tel-Aviv, Israel.

**Key words:** autoregulation, blue-field entoptic phenomenon, leukocyte speed, ocular hypotonia, retinal blood flow, hyperemia, intraocular pressure

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The effects of fluorescein and Richardson's stain on corneal epithelial wound healing were compared in eyes of rabbits whose corneas had the epithelium removed by scraping or by n-heptanol. One eye of each rabbit was stained with fluorescein and the other eye was stained with Richardson's stain. JOHN L. UBELS, HENRY F. EDELHAUSER, AND KRISTINA H. AUSTIN.

The clinical problems of corneal epithelial erosion and persistent epithelial defects have led to several studies of the mechanisms and biochemistry of corneal wound healing. Evaluation of a corneal wound or infection requires the use of a topical staining technique. The most common method used clinically has been fluorescein staining. Fluorescein has also had wide usage in ophthalmic research and has known toxic effects on the corneas of humans or rabbits.

A second stain, known as Richardson's stain, has recently been used in laboratory studies of corneal epithelial wound healing. This is a histologic stain containing methylene blue, azure II, and borax; when applied to the cornea, this stain yields a dark blue, well-delineated stain at the location of an epithelial defect. It is visible under white light and can be conveniently photographed without special filters.

Several characteristics of Richardson's stain, however, raise questions about its suitability as a stain for corneal wounds. The pH is basic, 8.6, the osmolality is 15 mOsm and, most significantly, methylene blue is reported to be toxic to human and rabbit corneas. Comparison of two reports in the literature indicates that healing of chemically wounded corneas may occur more rapidly in fluorescein-stained corneas than in corneas stained with Richardson's stain. A controlled study was therefore undertaken to compare corneal healing rates with the use of fluorescein and Richardson's stain. Rates of wound closure were determined by measurement of the area of the wound over time. Corneal thickness was also measured as an index of the functional integrity of the regenerating epithelium.

Materials and methods
Experimental procedures. Albino rabbits (2 to 2.5 kg) were sedated with ketamine HCl (30 mg/kg) and the corneas of the animals were anesthetized with a drop of proparacaine HCl (0.5%). A central epithelial wound was made on both corneas of each animal by means of either the n-heptanol method of Cintron et al. or the scraping method described by Ho et al. A filter paper disc 6.5 mm in diameter was used for heptanol wounds, and scraped corneas were debrided within a 6.5 mm trephine mark. The right eye of each animal was stained by applying a moistened fluorescein sodium ophthalmic strip to the bulbar conjunctiva and rinsing the eye with an ophthalmic irrigating solution (Dacriose; CooperVision, San German, Puerto Rico). The left eye was stained with one or two drops of Richardson's stain—1% methylene blue and 1% azure II in 1% borax 1:1 (this stain should actually be called Mallory's methylene blue—azure II stain)—and rinsed with irrigating solution. The corneal wounds were photographed with 35 mm Ektachrome 160 film and a Kowa fundus camera equipped with a cobalt filter for fluorescein photography. Slides were projected onto paper and the corneal wound perimeters were traced. The areas of these tracings were determined by computerized planimetry (Apple II computer and graphics tablet) calibrated for magnification. After the initial photographs, the wounds were stained and photographed at 4, 12, 24, 30, 48, 54, 72, and 78 hr.

Healing was also monitored by measurement of corneal thickness with a Haag-Streit slit lamp and pachometer modified according to the method.