tation and has been observed at birth. In PPMD, Descemet's membrane displays anterior banding, usually occurring during the fifth month of gestation, but lack of a uniform posterior granular layer suggests intrauterine damage to the corneal endothelium. If the abnormal corneal endothelial cells were displaced ectodermal cells, they would most likely have expressed their dysfunction at the same time as other derivatives of surface ectoderm, which are unaffected in this disease. All these factors are consistent with a most unusual transformation of corneal endothelial cells into keratin-containing epithelial cells. We have demonstrated that this change of cell type, an important feature of PPMD, distinguishes it from other disease of the corneal endothelium such as Fuchs' dystrophy.

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Cytochalasin B at concentrations of 1, 5, 10, 15, 20, or 40 µg/ml was continuously exchange-perfused into the eyes of nine living Macaca mulatta monkeys while intraocular pressure (IOP) was maintained at 25 mm Hg for 30 min. Pressures were then slowly reduced to 4 mm Hg to permit blood to reflux into Schlemm's canal as a tracer, and the eyes were fixed while maintained at 4 mm Hg IOP. Tissues were examined in all eyes by light and transmission electron microscopy. At concentrations of 1, 5, and 10 µg/ml cytochalasin B, the integrity of the endothelial lining of the trabecular wall of Schlemm's canal was maintained. At 15 µg/ml cytochalasin B, 6% of the length of the endothelial lining was disrupted; at 20 µg/ml, 54%; and at 40 µg/ml, 83%. There was a washout of extracellular material at the site of breaks and also a reflux of blood from Schlemm's canal into the trabecular meshwork in the same regions.

Intracameral infusion of cytochalasin B (CB) causes a large increase in gross outflow facility in the cynomolgus monkey. Since ruptures in the endothelial lining of the inner wall of Schlemm's...
Fig. 1. Macaca mulatta eye subjected to a continuous in vivo intracameral exchange perfusion of 1 $\mu$g/ml CB for 30 min while IOP was maintained at 25 mm Hg. In vivo fixation with glutaraldehyde following reduction of IOP to 4 mm Hg. Schlemm's canal (SC) is filled with plasma and red blood cells (rbc). The endothelial lining (el) of the inner wall of Schlemm's canal is intact. No red blood cells pass across the lining into the subendothelial space (SES). sec, Subendothelial cells. A, Light micrograph. B, Electron micrograph. (A $\times 800$, B $\times 5640$.)

canal also occur after exposure to CB, it has been suggested$^2$ that the ruptures may be the cause of the marked fall in outflow resistance. The CB concentration necessary to cause breakdown of the lining has not been established. The purpose of this study is to determine the minimum CB concentration necessary to cause extensive breakdown of the inner wall endothelium of Schlemm's canal.

**Materials and methods.** Macaca mulatta (rhesus macaque) monkeys weighing 4 to 5 kg were anesthetized with phencyclidine (0.3 mg/kg intramuscular), augmented with pentobarbital (intravenous). By means of a Sears$^3$ needle gun, three
Fig. 2. *Macaca mulatta* eye subjected to a continuous in vivo intracameral exchange perfusion of 40 μg/ml CB for 30 min while IOP was maintained at 25 mm Hg. In vivo fixation with glutaraldehyde following reduction of IOP to 4 mm Hg. Red blood cells (rbc) pass through disruptions in the endothelial lining (del) of the inner wall of Schlemm’s canal (sc) and into the subendothelial space (SES). Platelets (p) are present in regions of disruption of the endothelial lining (ed). Endothelial cell nuclei (ecn) are rounded, contain deep folds and notches, and bulge prominently into Schlemm’s canal. tl, Trabecular lamellae. A, Light micrograph. B, Electron micrograph. (A × 1200; B × 7500.)

21-gauge needles were introduced into the anterior chamber of each eye, with the infusion needle behind the iris. Exchange perfusion was achieved by connecting one needle to an infusion reservoir and another to a collecting reservoir. The third needle was connected to a transducer to monitor intraocular pressure (IOP) continuously throughout the experiment. Control of the exchange perfusion rate was achieved by adjusting the height of the infusing and collecting reservoirs relative to one another. Control of IOP was attained by adjusting the height of infusion and col-
Fig. 3. Percent disruption of endothelial lining of inner wall of Schlemm’s canal of *Macaca mulatta* monkeys subjected to a continuous in vivo intracameral exchange perfusion of CB for 30 min while IOP was maintained at 25 mm Hg. In vivo fixation with glutaraldehyde following reduction of IOP to 4 mm Hg. Control eyes (2) perfused with the drug carrier, DMSO, at 0.1% or 4% as well as the eye perfused with 1% CB had no lining breakdown. Asterisk, Standard error <1%.

In each experiment, the limbal area of the eye was divided radially into eight segments. Sections were examined from each of the segments except those in which randomly occurring fixation artifact in the segment or cutting artifact in the section precluded satisfactory measurements. Regional variability in the structural details along the canal was minimized by the technique of reducing IOP prior to fixation to flatten the endothelial lining into a relatively uniform sheet of tissue. The length of Schlemm’s canal from Schwalbe’s line to the scleral spur was measured at the level of the inner wall endothelium. The length of the inner wall ruptures in the same area were then measured and calculated as a percent of the length of the entire lining in that section.

In experiments preliminary to this study, heparinized red blood cells introduced into the anterior chamber passed through the trabecular lamellae into the subendothelial space in a variable, irregular, and unpredictable fashion, providing a poor tracer. In contrast, blood that was purposely refluxed into Schlemm’s canal at 4 mm Hg IOP uniformly was in apposition to the entire inner wall endothelium. Plasma, which stains an intense blue with toluidine blue, provided an excellent tracer to verify the presence of breaks. Red blood cells found at sites of discontinuity of the lining provided further confirmation of disruption. Additional verification of the presence and nature of breaks was obtained by examining sections by transmission electron microscopy.

Data at lower CB concentrations (≤15 μg/ml) were statistically analyzed by determining whether for equilibration while exchange perfusion with DMSO or CB at 0.25 ml/min was continued. While the same pressure and exchange rate were maintained, perfusion was switched to 4% phosphate-buffered glutaraldehyde for 30 min. The anterior segments were then removed and allowed to fix for 48 hr in 4% phosphate-buffered glutaraldehyde at 4° C. All eyes were postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Sections of 1 μm were stained with toluidine blue for light microscopy. For electron microscopy, sections were stained with Millonig’s uranyl acetate—lead citrate stain and then examined on an AEI Corinth 500 electron microscope.

A reticle micrometer on a Zeiss photomicroscope was used to determine the length of Schlemm’s canal and the length of disruption of the endothelial lining of Schlemm’s canal. All measurements were done at 250× magnification with a Zeiss Planapo lens. Sites of disruption of the endothelial lining were confirmed at 1000× magnification with a Zeiss Planapo oil lens.

In experiments preliminary to this study, heparinized red blood cells introduced into the anterior chamber passed through the trabecular lamellae into the subendothelial space in a variable, irregular, and unpredictable fashion, providing a poor tracer. In contrast, blood that was purposely refluxed into Schlemm’s canal at 4 mm Hg IOP uniformly was in apposition to the entire inner wall endothelium. Plasma, which stains an intense blue with toluidine blue, provided an excellent tracer to verify the presence of breaks. Red blood cells found at sites of discontinuity of the lining provided further confirmation of disruption. Additional verification of the presence and nature of breaks was obtained by examining sections by transmission electron microscopy.

Data at lower CB concentrations (≤15 μg/ml) were statistically analyzed by determining whether
or not disruption of the endothelial lining was observed, since we found such disruption to be rather uncommon. At higher concentrations (≥15 μg/ml) the actual disruption percentages, which were found to range from 0% to 100%, were used.

Results. In all sections, Schlemm's canal was filled with blood, and the trabecular meshwork was collapsed into a compact sheet of tissue rather than distending into the canal. The inner wall endothelium of Schlemm's canal was lying against the underlying subendothelial tissue and trabecular lamellae in a flat sheet as illustrated in Fig. 1. Plasma and red blood cells were in uniform position to the endothelial lining.

Disruption of the lining consisted of small breaks that occurred primarily at cell junctions as a result of exposure to lower concentrations of CB. Platelets were generally present at the site of breaks. At CB concentrations of 20 and 40 μg/ml, disruption, discontinuity, or loss of integrity of the lining was more extensive, with fragments of the inner wall endothelium lying in Schlemm's canal. In some areas the subendothelial cells detached from the underlying trabecular lamellae with their cytoplasmic processes still attached to the endothelial lining. In other areas the inner wall endothelium was detached both from adjacent endothelial lining and from subendothelial cell cytoplasmic processes.

Red blood cells were present at the site of discontinuity of the inner wall endothelium and within the subendothelial space adjacent to these ruptures. There was an absence of extracellular material in the subendothelial space adjacent to sites of disruption of the endothelial lining; there also was a disorganization of the architecture of the processes normally joining the endothelial lining to the subendothelial cells, as seen in the area of disruption in Fig. 2. Platelet aggregates were regularly present at the site of ruptures, and red blood cells were present in the ruptures and the subendothelial space adjacent to these ruptures.

There was no breakdown with 0.1% or 4% DMSO, the drug carrier. A total of 16 sections were examined, and since no breakdown was seen in any sample, there is no reason to assume DMSO causes any breakdown of the endothelial lining. As seen in Fig. 3, CB (1, 5, and 10 μg/ml) caused averages of 0.2%, and 0.8% breakdown, based on eight, 14, and 23 observations, respectively. A 15 μg/ml concentration of CB caused an average 6.6% breakdown (n = 6), whereas an increase to 20 or 40 μg/ml CB caused, respectively, averages of 54.8% (n = 13) and 83.3% (n = 4) breakdown or disruption of the endothelial lining. By Fisher's exact test, the proportion of cases with no breakdown was marginally significantly less at 15 μg/ml CB (3/6 cases) than at 10 μg/ml or less (43/46), with p = 0.016. By the two-sample randomization test, the average percent breakdown at 20 μg/ml was significantly greater than at 15 μg/ml with p = 0.0002, whereas the average percent breakdown at 40 μg/ml was marginally significantly greater than at 20 μg/ml, with p = 0.041.

Discussion. This report generally corroborates previous work,2 but it describes a substantially improved technique for evaluating the effects of cytochalasin. It combines an improved technique of constant exchange perfusion during exposure to the drug, with systematic reduction of pressure following exposure to flatten the endothelial lining, permitting blood to reflux into Schlemm's canal as a tracer. The technique permits a considerably more accurate control of drug concentration and pressure during drug exposure and during fixation than has previously been reported. The flattened endothelial lining and blood in Schlemm's canal consistently achieved with the technique also provide a more precise means of evaluating the extent of endothelial lining disruption than has previously been available.

The exchange perfusion technique permits one to introduce the drug while maintaining a stable, controlled IOP. The pressure is monitored and maintained at a known level throughout the experiments, so that both control and experimental eyes are subjected to identical treatment. The exchange perfusion also permits continuous exposure to a known concentration of drug and therefore provides more precise exposure than a single dose, which may result in less certain mixing of the drug. This procedure also eliminates the washout with aqueous turnover that would result in a gradually diminishing, unknown concentration of the drug during the experimental interval.

Disruption of the inner wall endothelium was not observed in previous experiments where exchange perfusion was maintained at 1 ml/min for 30 min with BSS.4 The exchange perfusion in the current study was done at 0.25 ml/min for 30 min, which should have even less effect on the integrity of the endothelial lining. Furthermore, all eyes were subjected to the same exchange perfusion rate, but the eyes with the DMSO drug carrier and the eyes exposed to low concentrations of CB did not show evidence of breakdown, indicating that it was the CB concentration rather than exchange perfusion which was responsible.

Control exchange perfusions with DMSO, the drug carrier, at 0.1% or 4% concentration did not cause breakdown of the endothelial lining. Four
percent DMSO was the concentration present in the perfusate with 40 μg/ml CB, whereas 20 μg/ml CB perfusate had 2% DMSO and lower concentrations of CB had correspondingly reduced concentrations of DMSO. The complete lack of inner wall endothelium breakdown with the use of the highest concentration of drug carrier containing 40 μg/ml CB caused 83% breakdown, reduced concentrations of DMSO. The complete lack of inner wall endothelium breakdown with the use of the highest concentration of drug carrier containing 40 μg/ml CB caused 83% breakdown, makes it highly unlikely that DMSO at the highest concentration (or the correspondingly reduced concentrations in the perfusate in the lower concentrations of CB) was a significant source of error in evaluating the degree of breakdown of the lining.

At positive IOP the inner wall endothelium of Schlemm’s canal is distended into a ballooning, highly irregular surface with many giant vacuole-like structures. Slow, systematic, in vivo reduction of pressure to 4 mm Hg in all cases caused the trabecular tissue adjacent to Schlemm’s canal to collapse into a relatively uniform, flat sheet of tissue. This configuration made evaluation of disruption of the endothelial lining much more satisfactory than was possible in preliminary experiments at higher IOP.

It has previously been observed that the endothelial lining of the inner wall of Schlemm’s canal may retain its integrity when IOP is reduced to levels below episcleral venous pressure. These morphologic findings have been used as evidence that a physiologic deformation of the lining may in fact act to maintain the blood-aqueous barrier in a one-way valve arrangement. Physiologic studies have confirmed this hypothesis by demonstrating that reverse facility in rhesus monkeys is 26 to 69 times smaller than the total facility in the normal eye.

The same studies have shown that sustained hypotony causes only a mild perturbation of the blood-aqueous barrier and concluded that significant breakdown of the blood-aqueous barrier is due to the trauma of sudden lowering of IOP and not due to a reflux response of the eye to an IOP lower than some critical level. Since a sustained low IOP itself does not represent a source of error of the endothelial lining when the IOP is reduced slowly, as was done in the current study, this should not be a source of error. The absence of breakdown or rupture of the inner wall endothelium of Schlemm’s canal in control DMSO eyes and at low concentrations of CB in this study provides further evidence to support the idea that slow lowering of IOP is not in itself a cause of disruption.

The degree of breakdown of the endothelial lining was clearly dependent on the CB concentration because there was a highly significant difference in breakdown or disruption occurring at a CB concentration above 15 μg/ml. Extracellular material was reduced or absent in the region of ruptures. Red blood cells passed freely through the endothelial lining at these sites, indicating that the degree of disruption should be sufficient to permit washout of extracellular material at normal perfusion pressures.

Bárány has shown in several animal species that outflow facility was greatly improved following introduction of hyaluronidase into the anterior chamber. Acid mucopolysaccharides have been demonstrated in the aqueous outflow channels, and Francois has suggested that they regulate the permeability of pathways for aqueous outflow. Svedbergh et al. observed washout of extracellular material in the meshwork in regions of breaks in the inner wall endothelium of Schlemm’s canal. They noted that this could account for the marked CB-induced fall in outflow resistance. They also noted repair of the endothelial lining within days. Their work raises the possibility that the reduced aqueous outflow facility in glaucoma could be at least partially reversed by the washout of extracellular material after exposure to CB. Following repair of the lining, there might be a persisting restoration of more normal aqueous outflow facility and intraocular pressure.

This report indicates that a continuous exposure to a CB concentration greater than 15 μg/ml for 30 min would be required to cause sufficient disruption of the endothelial lining to permit extensive washout of extracellular material in the meshwork. Questions remain concerning the CB concentration necessary to cause inner wall endothelium disruption with longer or shorter exposure times.

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Key words: cytochalasin B, aqueous outflow, Schlemm’s canal, trabecular meshwork, endothelium, blood-aqueous barrier

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The time course for the development of strabismic amblyopia in infant monkeys (Macaca nemestrina). LYNNE KIORPES AND RONALD G. BOOTH.

The time course for the development of acuity was followed in two experimentally esotropic infant monkeys (Macaca nemestrina). Surgical esotropia was produced at 6 days postnatal. Acuity values for both eyes of both infant monkeys were found to be normal through 4 weeks postoperatively. After this period of delay, amblyopia began to emerge. The deviated eyes showed poorer acuity than the nondeviated eyes at every subsequent age tested. These data support a developmental hypothesis for the emergence of strabismic amblyopia.

Previous behavioral research on strabismic amblyopia in animals has utilized an experimental paradigm which involves producing a strabismus at an early age, then testing for amblyopia a year or more later. Such studies have demonstrated that amblyopia can be induced by surgically creating esotropia in young kittens or monkeys during the first 3 months after birth. However an esotropia produced at older ages does not lead to amblyopia. It is also known that acuity is developing fairly rapidly during the time period in which both cats and monkeys are maximally susceptible to the deleterious effects of strabismus.

The fact that acuity is in an immature state during the time period when experimental esotropia leads to amblyopia suggests two types of hypotheses about the subsequent development of amblyopia. The first, which we will call a developmental hypothesis, is that the presence of esotropia disrupts the normal development of acuity. An example of a developmental disruption would be a simple arrest of acuity development at the onset of esotropia, as reported by Jacobson and Ikeda. However, clinical data suggest that in many cases amblyopia resulting from early esotropia is more than an arrest of development. A more complicated disruption of development would be necessary to explain the outcome of these cases. There are any number of more complicated disruptions of development which could lead to reduced acuity levels in the adult.

The second hypothesis, which we will call an adult-deterioration hypothesis, is that acuity develops normally, approaching adult levels, but then deteriorates some time later. For example, amblyopia could result from some kind of suppression which is not manifest until adult acuity levels would normally be reached, even though the esotropia had been present throughout.

In order to distinguish between these two hypotheses, it is necessary to look at the time course for the development of amblyopia following the onset of esotropia. In the present experiment we have followed the development of acuity for each eye of two monkeys made esotropic 1 week after birth. Our results are consistent with a developmental hypothesis but reflect a disruption of acuity development that is more complicated than a simple arrest. Some of these data have been reported previously.

Methods. Seven infant monkeys (Macaca nemestrina) were used in this study. All were reared according to normal protocol for this laboratory. Esotropia was produced surgically in the right eye of two infant monkeys on the sixth postnatal day. Refraction under cyclopia (1% Kupfers solution) prior to surgery revealed no anisometropia in either monkey. The surgical method was similar to that reported by von Noorden and Dowling. Briefly, the medial rectus was not resected but flows postnatal. Acuity values for both eyes of both infant monkeys were found to be normal through 4 weeks postoperatively. After this period of delay, amblyopia began to emerge. The deviated eyes showed poorer acuity than the nondeviated eyes at every subsequent age tested. These data support a developmental hypothesis for the emergence of strabismic amblyopia.

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