Cell size-shape relationships in corneal endothelium. GULLAPALLI N. RAO, LAWRENCE E. LOHMAN, AND JAMES V. AQUAVELLA.

The shape of corneal endothelial cells was studied from specular photomicrographs of 121 normal corneas. The predominant number of cells were hexagonal in shape (48% to 90%), with pentagonal (15% to 35%) and heptagonal (25% to 38%) cells constituting the greater portion of the remaining endothelium. Corneal endothelium with a greater number of hexagonal cells demonstrated minimal variation in cell size. (INVEST OPHTHALMOL VIS SCI 22:271-274, 1982.)

Normal human corneal endothelium is a monolayer of polygonal cells covering the posterior surface of the cornea. The integrity of this layer is vital for the maintenance of normal corneal transparency. Corneal endothelium demonstrates a decline in cell density with age and after exposure to different kinds of trauma. The effect of such morphologic alteration on corneal function, however, is not clear, since no direct correlation was observed between the degree of cell loss and corneal function as indicated by thickness. There is some evidence that endothelium with a greater degree of variation in cell size is more vulnerable to surgical trauma, probably because of low functional reserve.[1, 2] The degree of variation in cellular morphology is determined by cell shape among a number of morphologic parameters. In this study, we analyzed the normal corneal endothelium to investigate the relationship between cell shape and endothelial morphology, using cell size as a parameter.

Materials and methods. A total of 250 specular photomicrographs obtained from 127 eyes of 98 patients examined over a 3 year period formed the basis for this study. All cases were confirmed to be normal by biomicroscopic examination. Eyes with evidence of previous ocular disease were excluded. The age range was from 10 months to 82 years. The endothelium of the 10-month-old donor cor-

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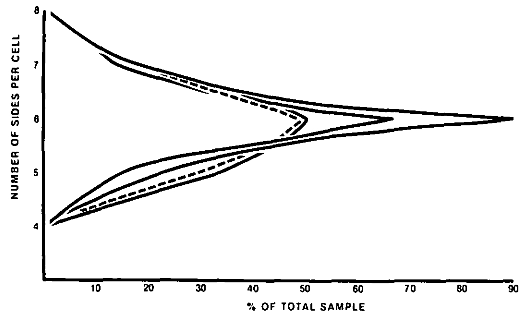

Fig. 1. Graph demonstrating the relative frequency of cells of different shapes in four different corneas represented by the four different lines. The shape of the cell is described as a function of the number of sides of each cell. The average number of sides per cell in a normal human corneal endothelium is about 5.5.

nea was photographed as soon as it was enucleated prior to corneal preservation.

The specular photomicrographs were enlarged, and overlays were made of the enlarged image for purposes of quantitative analysis with automated image analysis techniques.

These overlays were carefully analyzed for the shape of the cells. The cell shape was described as a function of the number of sides in each cell. This was done manually by counting the number of sides for each cell in each endothelial photomicrograph. The cells were described as pentagonal, hexagonal, or heptagonal. The relative frequency of these different shapes was then plotted. An attempt was then made to correlate the cell shape to the variation in cell size in each of the photographs. The mean cell size and the coefficient of variation were determined by using the automated large analysis system (FAS II; Bausch & Lomb, Inc.). The coefficient of variation is the ratio of standard deviation of the cell areas to the mean cell areas in a given sample of endothelium.

Results. Normal endothelium was seen to comprise mainly pentagonal, hexagonal, and heptagonal cells, with the presence of a minimal number of cells of other shapes. Fig. 1 demonstrates the relative frequency of cells of different shapes described as a function of the number of sides. We found that pentagonal cells constituted 15% to 35%, hexagonal cells from 48% to 90%, and heptagonal cells 25% to 38% of the cell population in any given sample. Cells of these three shapes were seen in all corneas. In a few corneas, however, cells of other shapes were also seen. Five of the 127 eyes had three-sided cells, with a range of 1.2% to 2.8%; 19 eyes had four-sided cells, with a range of 1.1% to 4.6%; and 16 eyes had eight-sided cells, with a range of 2.4% to 4.4%. As seen in Fig. 1, the curves for cell shape cross between five and six sides, indicating that on the average the number of sides per cell is 5.5.

We have also attempted to correlate the cell shape to the degree of variation in cell size. No statistically significant correlation was found between the pentagonal cells and the coefficient of variation in cell size ($r = +0.06$) (Fig. 2). In contrast, the number of heptagonal cells showed a strong positive correlation to the coefficient of variation in cell size ($r = +0.81$) (Fig. 3).

The number of hexagonal cells in the same sample was shown to be inversely related to the coefficient of variation in cell size ($r = -0.55$) (Fig. 4). No correlation was found between mean cell size and the cell shape ($r = +0.04$) in this group.

Discussion. In the present study, we observed that human corneal endothelium is made up of clusters of hexagonal cells, with a number of cells of other shapes interspersed among them. Of these, pentagonal and heptagonal cells constituted the greatest portion. The number of hexagonal cells was shown to determine the symmetry of endothelial cell structure. There was an inverse correlation between the number of hexagonal cells and the coefficient of variation in cell size of a given endothelium, suggesting that endothelium with a greater number of hexagonal cells demonstrates less variation in cell size. In contrast, if the percentage of heptagonal cells increases, the coefficient of variation increases in direct relationship.

Our observations in this study may be applied to determine the relationship between the endothelial cell structure and function. One can speculate that as the number of sides of a cell increases, it tends to become more rounded and this probably results in poorer interdigitation between adjacent cells, which in turn may result in excessive "leak" of fluid into the corneal stroma.[3] This may explain our earlier observations that corneas with greater variation in cell morphology demonstrated a greater increase in corneal thickness after surgery.

Further studies in the future correlating these observations on morphology to endothelial function by means of techniques such as fluorophotometry may help in further elucidating this complex relationship.

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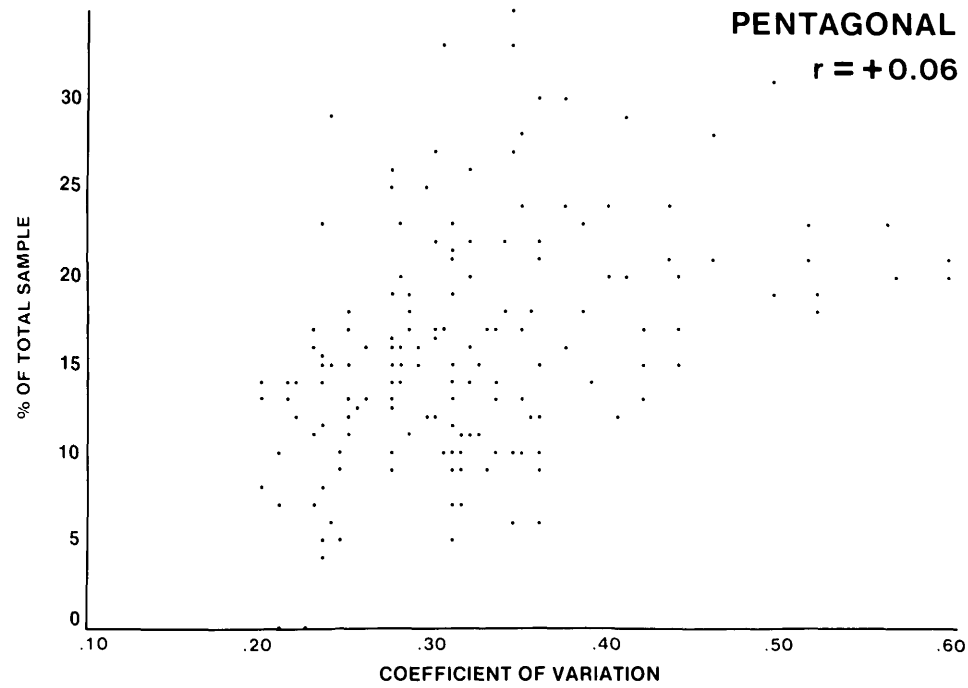

Fig. 2. Scatterplot showing the correlation between the degree of coefficient of variation in mean cell area to the number of pentagonal cells in a given sample of corneal endothelium. No correlation was found between the pentagonal cells and the coefficient of variation.

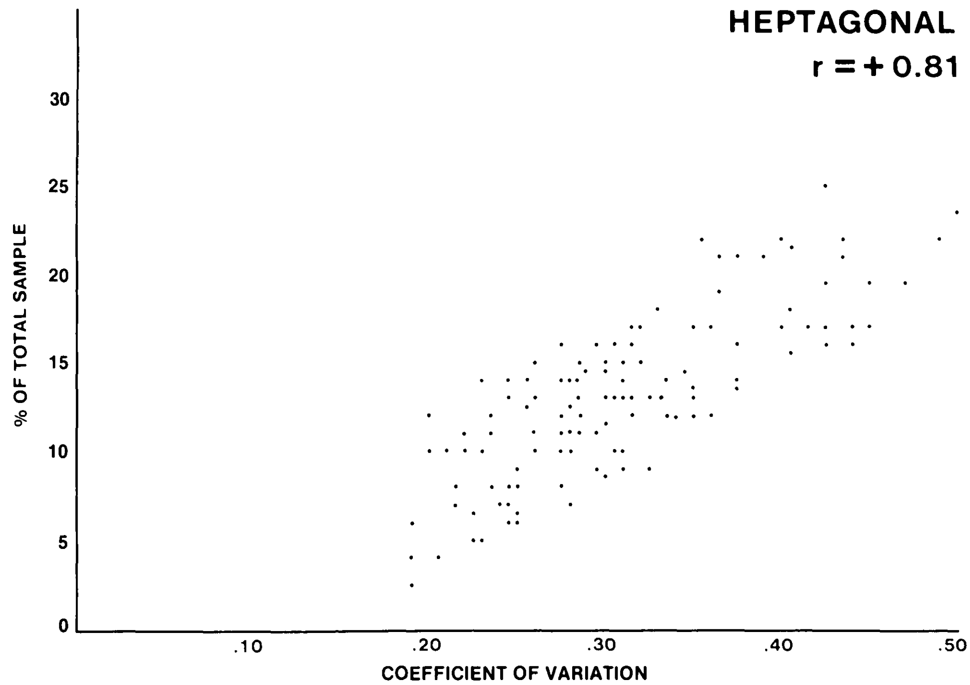

Fig. 3. Scatterplot demonstrating the correlation between number of heptagonal cells in a given sample of the coefficient of variation in mean cell area. There is a strong positive correlation between the factors.

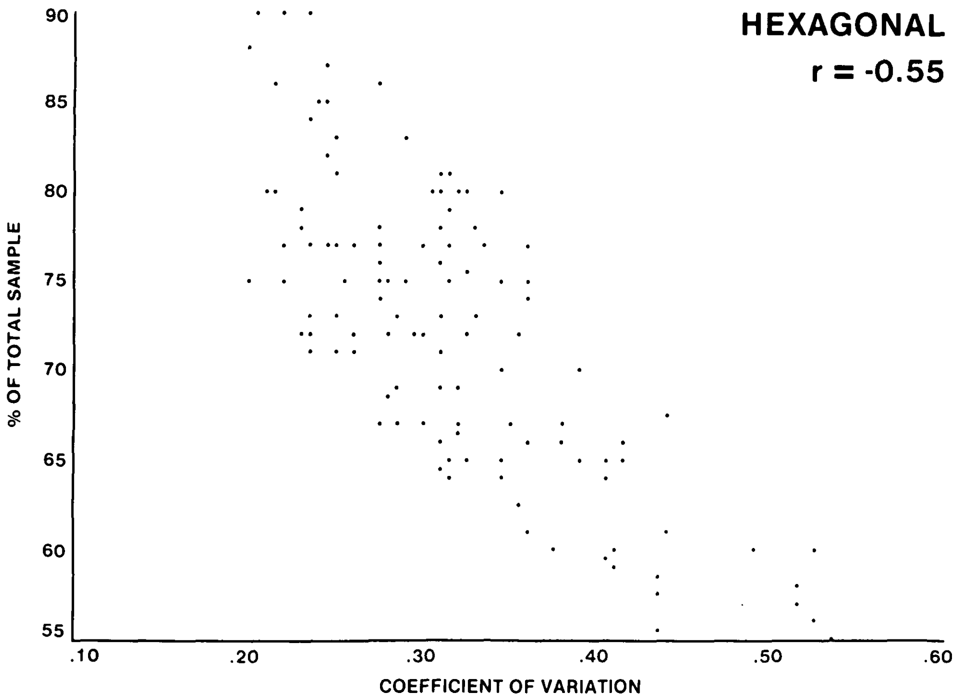

Fig. 4. Scatterplot demonstrating the correlation between the percentage of hexagonal cells in a given sample of corneal endothelium and the coefficient of variation in cell area. A significant negative correlation between the percentage of hexagonal cells in a given sample and the coefficient of variation is seen here.

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Elongation of cat eyes following neonatal lid suture.

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Development of the axial length of cat eyes was monitored through A-scan ultrasonography at various times after neonatal monocular lid suture. In every case the deprived eye was longer than its fellow eye. Upon reestablishment of the palpebral fissure, refractive state and corneal curvature were recorded. Although all the deprived eyes showed axial elongation, there was no consistent relationship between neonatal lid fusion and the degree of myopia. (INVEST OPHTHALMOL VIS SCI 22:274-277, 1982.)

Recent studies have shown that lid suture in visually immature macaques[1] and tree shrews[2] results in both an increase in axial length and myopia in the deprived eye. Myopia progresses with the duration of lid closure in macaques and is of the axial type, which is caused by elongation of the globe; no direct correlation has been shown between axial length and the degree of myopia in the tree shrew. Recently, an increase in axial length of the macaque eye after corneal opacification with multiple stromal injections of latex was shown,[3] a result similar, although somewhat less marked, to those of lid suture studies.

There are suggestions in the literature that after monocular deprivation, kitten eye development is similar to that of the primate. In single-unit studies of visual cortex of monocularly deprived cats, it was reported that the deprived eye was usually 1 to 2 diopters more myopic than the nondeprived eye.[4] The interocular refractive difference persisted after placement of equivalent contact lenses over both corneas and was therefore attributed to different axial lengths. However, axial dimensions of neonatally sutured cat eyes have been reported to show no consistent change[5]; a slight axial length increase was noted in some eyes and a slight decrease in others. In a recent study in which kittens were reared with optically induced anisometropia, five of eight kittens showed increased axial length in the defocused eye, whereas all eight kittens exhibited relative myopia in that eye.[6] The present study was undertaken to reinvestigate the axial development of the cat eye after monocular deprivation, and we report here a consistent increase in axial length of the deprived eye.

Materials and methods. One eye of each of 20 kittens was sutured closed between 13 and 80 days of age. Anesthesia was induced intramuscularly with 10 mg/kg ketamine hydrochloride (Vetelar; Parke-Davis) and supplemented with metofane gas administered through a small plastic mask. One to two millimeters were trimmed from the upper and lower lid margins, and the underlying conjunctiva was separated from the lid and joined together with interrupted sutures of 9-0 vicryl (Ethicon). The trimmed lid margins were then fused with interrupted 6-0 silk sutures, and antibiotic ophthalmic ointment was applied to the lid junction. If properly done, the lid margins fused reasonably well within 2 weeks, and although not totally deprived of light, the animals were certainly deprived of any pattern vision.

Measurement of axial length was made before and at various intervals after lid suture with either a Kretz Model 7100 MA ultrasonic ophthalmoscope or a Kretz Model 7200 MA echograph. Each cat served as its own control by comparison of the sutured and nonsutured eyes. It was generally sufficient to hand-hold neonatal animals, whereas adult animals were usually mildly sedated with a dose of intramuscular ketamine hydrochloride. In both cases the cornea was anesthetized with proparacaine HCl (Alcaine, 0.5%; Alcon Laboratories, Inc.) and a small plastic stand-off tube was placed on the cornea or the closed lid. The tube was filled with a hydroxypropyl methylcellulose solution (Goniosol; Smith, Miller & Patch) and the ultrasonic transducer was placed in contact with the methylcellulose rather than the cornea or lid. This helps in eliminating the measurement error arising when the transducer pushes against and depresses the cornea. Alignment with the geometric axis of the eye was made by positioning the transducer for maximum echoes from the cornea, anterior and posterior lens, and retina. If proper alignment is not made, echoes are reduced in size.

After the desired period of lid closure (257 to 635 days) each animal was reanesthetized as previously described and the palpebral fissure was reestablished. Any excessive bleeding was quickly stopped with a wet-field coagulator (Mentor) coupled to suitably small forceps. Tropicamide (Mydriacyl, 1.0%; Alcon) was instilled in each eye as a cycloplegic and mydriatic, and phenylephrine hydrochloride (Neo-Synephrine, 10%; Winthrop Laboratories) was instilled in each eye to retract the nictitating membrane. Refraction was done with a streak retinoscope and hand-held trial lenses, and the refractive power of the cornea was determined with a keratometer.

Results. Table I summarizes the results from the 20 cats that underwent monocular lid suture as kittens. Axial length was checked 12 or more times on the first six cats and the resulting axial length growth curves were found to be in close agreement with other published results.[7] Development of the axial dimensions of later cats was monitored less frequently. When the sutured eyes were finally opened (age at lid suture and opening listed in Table I), the deprived eye was longer than its fellow eye in every case. The difference in axial length ranged from 0.9 to 1.9 mm and the average elongation for all 20 cats was 1.37 mm. The average axial length of all deprived eyes was 20.17 mm compared with 18.80 mm for the controls. The difference in axial lengths was highly significant ($p < 0.001$, paired t test). Histologic examination was done on the eyes of two of the cats (CY-5, R-5); one was found dead in its cage and the other was used in another study. The measurements on the eyes were done by an ocular pathology lab with no previous knowledge of the experiment, and although the differences were somewhat less than those determined by ultrasound, they also showed axial elongation of the deprived eye (CY-5, 1.17 vs. 1.5 mm; R-5, 0.84 vs. 1.3 mm). Furthermore, they showed the elongation to occur primarily in the postequatorial segment of the globe with the anterior segment essentially unaffected.

Although all the deprived eyes showed axial elongation, only 10 of the 20 were myopic relative to their fellow eyes. The relative myopia ranged from 0.5 to 3.0 diopters. There was no correlation for the range of eyes covered in this study between the age of the animal at lid fusion or the duration of fusion and the refractive state of the adult. In nine of the 20 cats there was no refractive difference between the two eyes (no refractive examination was done on the cat found dead in its cage).

Keratometric measurements were made on 19

Table I. Intraocular differences after neonatal lid fusion

Animal No.	*Age at lid fusion (days)*	*Age at lid opening (days)*	*Difference in axial length (sutured − control) (mm)*	*Difference in K-readings (sutured − control) (diopters)*	*Difference in refraction (sutured − control) (diopters)*	
					Predicted	*Actual*
D-3	23	597	+1.0	−0.07	−2.93	−3.0
D-6	26	596	+1.9	*	*	−2.0
CY-5	18	289	+1.5	−3.00	−1.5	−1.5
CY-6	21	371	+1.5	−0.50	−4.0	0
CY-7	21	371	+1.5	−0.63	−3.87	0
CY-11	13	495	+1.3	*	*	0
CY-13	19	276	+1.7	−1.06	−4.04	0
CY-14	20	275	+1.9	−2.56	−3.14	−0.5
CY-15	36	655	+1.7	+0.06	−5.16	−1.0
CY-16	28	663	+0.9	−0.13	−2.57	−2.75
CY-17	28	663	+1.5	−1.50	−3.0	0
CY-18	36	655	+1.7	−2.12	−2.98	−0.5
R-5†	38	528	+1.3	—	—	—
R-6	38	620	+1.1	−1.37	−1.93	0
R-7	49	614	+1.1	−2.94	−0.36	0
R-11	19	495	+1.3	*	*	0
R-12	19	497	+1.3	−0.13	−3.77	−0.5
R-13	20	483	+1.0	+1.06	−4.06	−0.75
R-14	80	444	+1.1	−0.87	−2.43	0
R-16	68	444	+1.2	+0.12	−3.72	−1.5

*K-reading values too flat for measurement with keratometer.
†Cat was found dead in its cage.

of the cats after re-establishment of the palpebral fissure. In 10 of the 19, the cornea of the deprived eye seemed to compensate for the increase in axial length; that is, it was flatter than that of the fellow eye (negative values in K-reading column in Table I). In five of the remaining nine, the corneal curvature was essentially identical in the two eyes; in three of the cats the K-readings were flatter than the keratometer could measure; in one of the cats the control cornea was flatter than that of the deprived eye. The mean K-reading of the 19 control eyes in our study was 38.72 diopters and was significantly different than the mean K-reading of 37.91 diopters for the sutured eyes ($p < 0.01$, paired t test).

Knowing the axial length difference and the fact that 0.1 mm is equal to 0.3 diopters in the cat eye,[8] it is possible to predict the relative refractive error of the deprived eye. If the compensating refractive power of the cornea is then subtracted, the resulting value should be very close to the observed refractive power. Unfortunately, this did not work on a consistent basis (Table I). Of the 19 cats on which keratometry was done, the predicted and actual refractive values were essentially identical for four; for three cats the K-readings were flatter than the keratometer could measure; for 12 cats the predicted and actual refractive values differed by an average of 3.11 diopters. In all 12 cases there was relatively less severe myopia than would be predicted by consideration of axial length and keratometry measurements.

Discussion. These experiments have shown that the cat eye, like that of the monkey and tree shrew,[1, 2] elongates after neonatal lid closure. In each case the axial length of the deprived eye was significantly longer than that of its control. Previous investigators[5] concluded that lid suture results in no consistent change in axial dimensions of any ocular components of the cat's eye. They reported that the average difference between the eyes of six monocularly sutured cats was 3.1% and could be either an increase or decrease in axial length. We have recorded an average difference of 6.8% in axial length between the eyes of 20 monocularly sutured cats, and the difference always manifested itself as an axial length increase of the sutured eye. Why our results do not agree with those of the earlier deprivation study[5] is certainly not clear; however, they are entirely consistent with a report of noncorneal myopia in lid-sutured cats.[4]

Although all the deprived eyes in this study showed greater axial length than their controls, only half the eyes showed myopia relative to the fellow eye. In this respect, our results with cats differ from those of two studies with primates[1, 2] but agree with those of a third[9] in which the authors could not confirm a consistent relationship between neonatal lid fusion and the degree of myopia. It has been suggested that the refractive state of the eye is regulated postnatally by the level of accommodation necessary to maintain a clear image.[5, 10] In the absence of such accommodative signals during the course of lid suture, development of refractive state would be uncontrolled. Since we have seen only an increase in axial length and never a decrease compared with the control, our results suggest that all such unregulated development is in the myopic direction. Nevertheless, there was a tendency for the cornea of the deprived eye to flatten in compensation for the increased axial length. This was the case in 10 of 19 cats, with the deprived eye always having less corneal refractive power. However, even when corneal curvature and axial length differences between the control and deprived eyes were used to predict refractive state, the agreement between the predicted and actual refractive state was poor in nine of the 10 cats (see Table I). This is similar to the results of a previous report in which the magnitude of the difference in refractive error between two eyes on kittens reared with optically induced anisometropia could not be reliably predicted from axial length measurements.[6] Thus, although all cat eyes in this study elongated after monocular lid closure, we have no good explanation for the lack of consistency in refractive error.

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