Reports

Endothelial cell density in relation to morphology.

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Corneal endothelium of 121 normal corneas was studied with the clinical specular microscope, and the relationship between cell density, cell morphology, and age was examined. Our observations indicate a decrease in cell density with age in homomegethous endothelium but no such correlation in a polymegethous endothelium.

A number of investigators have used cell density as a quantitative description of the corneal endothelium. Different studies on normal corneal endothelium[1, 2] have shown a decrease in endothelial cell density and an increase in pleomorphism with age.

In earlier reports,[3, 4] we described two distinctly different types of endothelial cell morphology, which we designated as "homomegethism" and "polymegethism."

The categorization of these two types was based on the degree of density in cell size in the endothelium of a given cornea. A cornea with an endothelium having cells of obviously different sizes is considered to have polymegethous endothelium. The largest cell in this endothelium is several times bigger than the smallest cell. In contrast, a homomegethous endothelium demonstrates cells which are of relatively uniform sizes.

In one of the earlier studies[5] designed to examine the relationships between endothelial cell morphology and corneal deturgescence, we also noted an apparent relationship among the three factors: endothelial cell morphology, cell density, and age. Although the expected decrease in endothelial cell density with age was seen in homomegethism, no correlation between cell density and age was found in the polymegethous group. We undertook further investigation of this hypothesis, and the results are presented in the present communication.

Materials and methods. A total of 121 corneas were included in this study. These subjects were selected from either candidates for cataract extraction or volunteers from the hospital staff. None of the volunteers had a history of any ocular pathology. In the surgical patients, the unaffected eye was selected for inclusion in the study.

All subjects were given a complete eye examination, and any eye showing evidence of anterior segment pathology was excluded. Pachometry was obtained on all eyes included in the study group.

The corneal endothelium was then examined and photographed with a Syber clinical specular microscope. At least 10 photographs were obtained of the endothelium from the central cornea in each eye. Eyes showing evidence of cornea guttata were excluded from this study. The endothelial cell densities were determined from at least five different specular photomicrographs of the central cornea, and the mean values were obtained for each cornea. The methodology used in determining the cell density was the same as described earlier.[2]

On the basis of the subjective evaluation of the endothelial cell morphology which was described in earlier studies, all the corneas were classified under two different categories. Of these 121 corneas, 60 were classified as homomegethous (Fig. 1) and 61 as polymegethous (Fig. 2). In seven of the volunteers, the endothelium of two corneas was found to belong to two different morphological categories.

All these data were subjected to statistical analysis, the results of which are included in the Results section.

Results. The mean endothelial cell densities for each subject were calculated. These values were then plotted as a function of the subject's age. The relationship between the cell density and the age for the 60 subjects in the homomegethous group were then plotted as shown in Fig. 3. Linear regression equation describing the curve which best fits these data is shown in the upper right hand corner of the figure. The standard error of estimate for this equation was ±306. The limit lines on either side of the curve represent 1 standard error of the estimate.

The data obtained from the 61 polymegethous corneas were plotted in a similar fashion, and the linear regression equation was calculated (Fig. 4). As shown, the slope of the curve was much less steep than in the homomegethous groups, and the standard error of the estimate was larger, 427 vs. 306 in homomegethous corneas.

The correlation coefficients between age and cell density were determined for each of the two groups. In the homomegethous group, the r value was -0.615 and is highly significant ($p < 0.001$). In the polymegethous group, on the other hand, the r value was not significant at -0.211 ($p < 0.1$). As can be seen in the homomegethous group, the numerical value of the correlation was highly significant. The fact that it has a negative sign indi-

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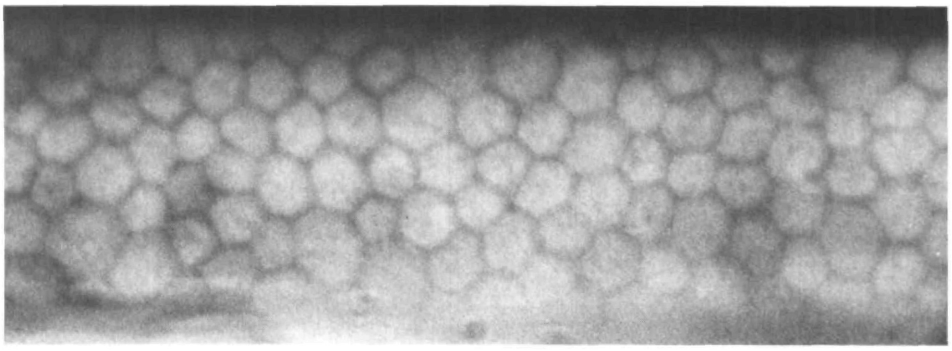

Fig. 1. Specular photomicrograph of corneal endothelium demonstrating homomegethism in a 62-year-old white man. Cell density = 2867/mm^2.

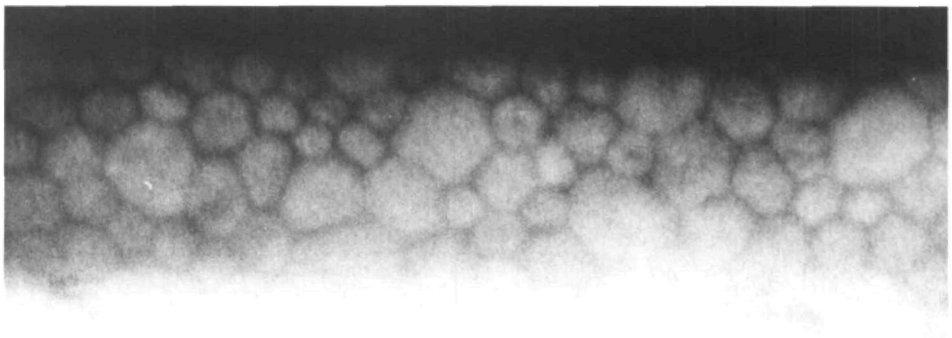

Fig. 2. Specular photograph of corneal endothelium demonstrating polymegethism in a 64-year-old white man. Cell density = 2700/mm^2.

cates that the correlation was inverse, which showed a decrease in cell density with increasing age. According to the sign of the correlation coefficient calculated for the polymegethous group, cell density tended to decrease with age. However, since the numerical value of the coefficient was well below the level required for statistical significance, this tendency was not particularly reliable. That is, the probability of finding a low cell density in a young person having a polymegethous endothelium is not much different from the probability of finding high cell density in an older subject having this type of endothelial cell morphology.

We determined the group mean standard error for each of the two morphological categories by averaging the standard errors of each subject's mean cell density. Since the group mean standard error included all individuals in the category, age was not an interacting factor. Sample size was also not a factor; therefore the magnitude of the standard error depended on the existence of real variation in cell density. The results are given in Table I. The range in size of the individual standard errors and the mean standard error for the entire group was notably smaller in the homomegethous groups. The difference between the values in the two morphological categories was highly significant, according to the t test.

Table I. Mean standard errors of individual endothelial cell densities

Homomegethism	*Polymegethism*
± 63.2	± 79.9
(28-107)	(26-294)
$p < 0.01$ by t test	

Discussion. Endothelial cell density forms only one of the many components in the endothelial cell morphology. The relationship between cell density and cell morphology is not clearly understood. Previous studies indicated that cell density decreases with age, with an increase in the degree of pleomorphism.[1, 2] From our studies[3, 4] of normal corneal endothelium, we observed that there

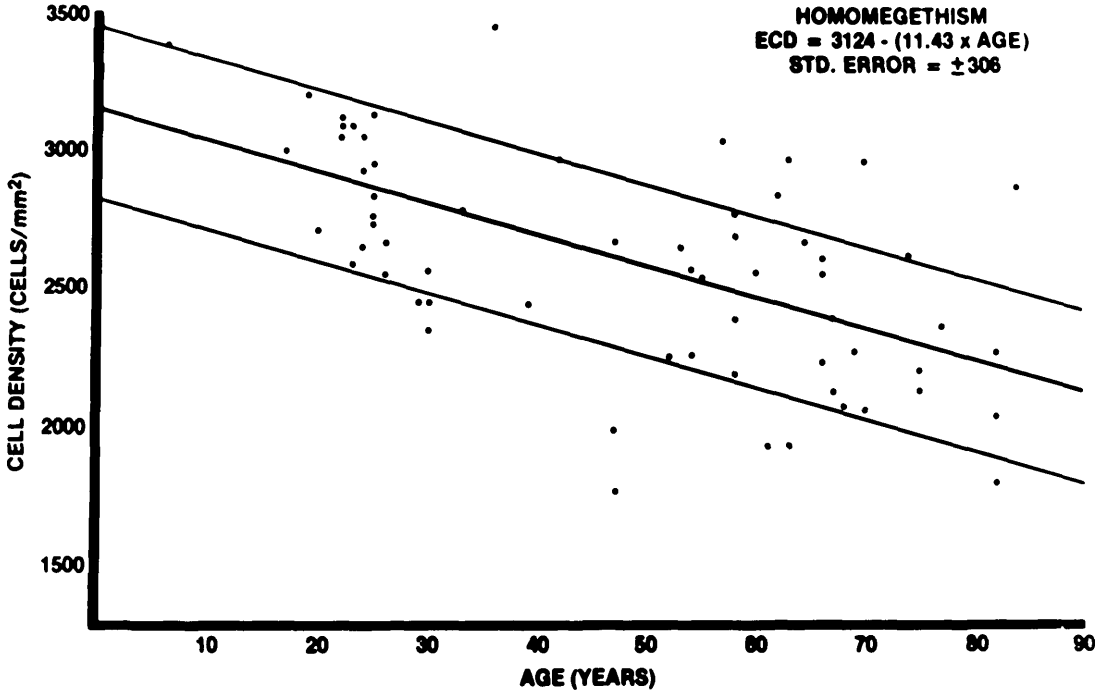

Fig. 3. Relationship between cell density and age in homomegethous group. *ECD*, Endothelial cell density.

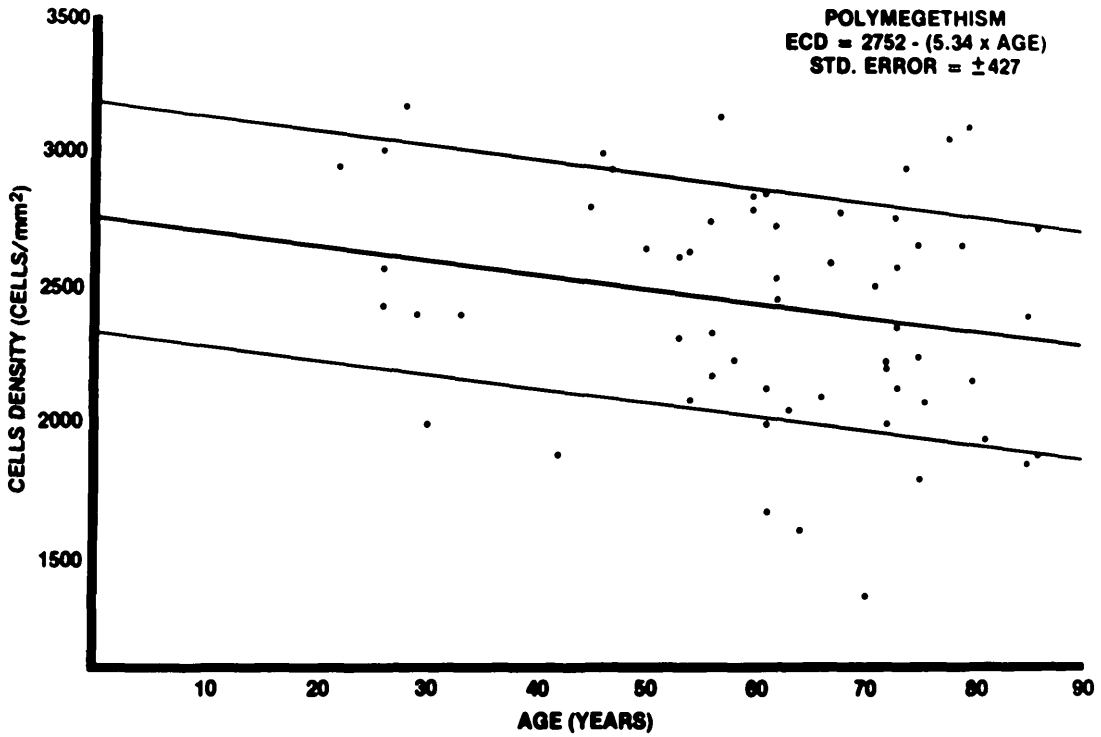

Fig. 4. Relationship between cell density and age in polymegethous group. *ECD*, Endothelial cell density.

is a great variation in the endothelial cell morphology in the normal corneas, and we divided the endothelium into two morphological categories, homomegethous and polymegethous. Upon further analyzing our data, we[5] observed that those corneas with homomegethous endothelium tend to show a decrease in cell density with age but that corneas with polymegethism do not essentially follow this rule. From our present study, it can be clearly seen that cell density certainly decreases significantly with age when the endothelial cells are regular in size and arrangement. However, if the endothelial cells are irregular in size and arrangement, the endothelial cell densities did not correlate with age. This study also demonstrated that a polymegethous endothelium shows a significantly greater variation in cell density within the central cornea regardless of age. These findings give a more quantitative basis to our categorization of endothelial cell morphology. Relative uniformity in cell size and arrangement seen in homomegethous corneas is a reflection of uniform cell density throughout the endothelium. The morphological irregularities seen in polymegethism are associated with some degree of regional differences in cell density and cannot be considered a simple effect of age.

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Permeability of retinal capillaries in rats with inherited retinal degeneration.

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The permeability of retinal capillaries in RCS rats with inherited retinal degeneration was investigated with horseradish peroxidase used as a tracer. Five-week-old rats showed typical degeneration of photoreceptor cells and accumulation of outer segment debris, but retinal capillaries were not permeable to peroxidase. At 10 weeks of age, capillaries in the inner retina appeared normal, but many in the outer retina were leaky. Peroxidase activity in these latter vessels was demonstrable in the basal laminae of endothelial cells and pericytes and in vesicles on the luminal and abluminal sides of the endothelium. Tracer also permeated the intercellular spaces in the surrounding area. The number of leaky capillaries in the outer retina increased during the course of the dystrophy. The site of the leak in permeable capillaries has not yet been established; it may be due to an alteration of the endothelial cell junctions or of transcellular vesicular transport. In the choriocapillaris, peroxidase permeated Bruch's membrane and the basal infoldings between adjacent pigment epithelial cells; tracer progression along the intercellular spaces was blocked at the zonulae occludens at the apicolateral border. The RCS rat may be a useful model for studying the morphological basis of changes in capillary permeability associated with retinal degeneration.

Retinal dystrophy in the Royal College of Surgeons (RCS)[1] rat is a recessively inherited disorder characterized by progressive degeneration of the photoreceptor cells, beginning by about the third postnatal week.[2] This process continues with a progressive thinning of the outer nuclear layer until almost all the photoreceptor cells have degenerated and have been replaced by a layer of debris. Gerstein and Dantzker,[3] using the trypsin-digest method, found that photoreceptor degeneration in RCS (tan-hooded) rats was accompanied by degenerative changes in the retinal capillaries. In this report we demonstrate that the retinal capillaries of RCS rats become leaky to intravenously injected horseradish peroxidase during progression of the dystrophy.

Materials and methods. RCS-*p*+ (pigmented) rats[4] and normal, Long-Evans Blue rats of both sexes were used in this study. They were reared in cyclic light (12 hr light–12 hr dark) at a room illumination of approximately 10 to 15 ft-cd from overhead fluorescent lamps. Room temperature was controlled at 21° C, and the rats were fed Purina Chow. Animals were sacrificed at 5, 10, 15.5, and 28 weeks of age. For enzyme tracer stud-

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