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Evidence for Corneal Endothelial Cell Hypertrophy during Postnatal Growth of the Cat Cornea

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Endothelial cell counts made from specular micrographs of 1-month-old kitten and adult cat corneas demonstrate that a progressive increase in endothelial cell size and a reduced endothelial cell density occurs during the postnatal development of the cat cornea. In addition to confirming the difference in cell size, scanning electron micrographs show that kitten endothelial cells are much more pleomorphic than those of the adult. When the number of corneal endothelial cells/mm^2 and the size of the whole cornea are calculated for the kitten and adult, hypertrophy rather than mitosis appears to be the principal mechanism responsible for maintaining a confluent endothelial cell monolayer during the postnatal development of the feline cornea. Hypertrophy also appears to play a role in establishing the adult corneal endothelial cell population of the rabbit when the previously published data of others are treated in a similar manner to those of the kitten and adult cat. Thus, endothelial cell hypertrophy plays a role in establishing an "adult" endothelial cell monolayer in species that have a widely divergent corneal endothelial cell mitotic capacity. Invest Ophthalmol Vis Sci 24:247–250, 1983

The corneal endothelium of the adult cat, like that of the human, has little regenerative potential.[1,2] In addition, the cat appears to be a valuable model for corneal transplantation because surgically associated endothelial cell loss and the incidence of spontaneous graft rejection are similar to those observed in the human following penetrating keratoplasty.[3] The cat is also an excellent model in which to study induced homograft rejection.[4] In order to characterize the development of the corneal endothelium in this species, we have studied the changes in endothelial shape and size that occur concomitantly with corneal growth.

Materials and methods. A total of 16, 1-month-old kitten and ten adult cat corneas were studied. Corneas were removed and placed in buffered tissue culture medium that contained 5% dextran (mw 40,000). A laboratory specular microscope was used to photograph the central corneal endothelial surface.[5]

The number of endothelial cells/mm^2 was determined by counting a minimum of three separate frames per specimen. Cell counts were made by an experienced observer who did not know the source of the photographs. The variability in the cell count was found to be less than 3% when previously counted photomicrographs were included among those of the present animal series. The number of endothelial cells per cornea was estimated by calculating the surface area of the cornea using the formula, Area = $2\pi r$, h. (Calculations treating the adult cat cornea as a sphere

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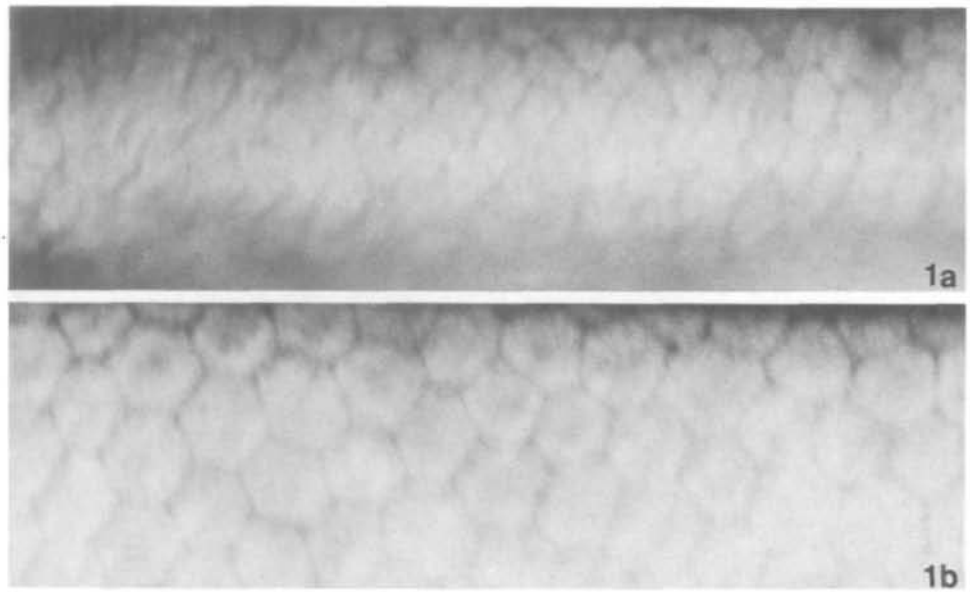

Fig. 1, A and B. A, Specular micrographs of kitten and **B,** adult cat (1b) corneal endothelium illustrating the difference in cell size (original micrographs ×200).

and then, more accurately, as an ellipse yielded values within 5% of one another. The simpler mode of calculation is used in this paper; a description of the elliptical surface formula and its application to a number of species is in preparation.) Radius of curvature measurements for cats of different ages have been reported by Freeman.[6] The actual measurements used in this study were: kitten, $r_1 = 5.4$, $r_2 = 4.5$, $h = 2.4$; cat, $r_1 = 8.5$, $r_2 = 7.5$, and $h = 4.5$ mm.

Corneas processed for scanning electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, that contained 4% (wt/vol) sucrose and 2 mM Ca^{++}, postfixed in buffered 1% OsO_4, dehydrated in ascending concentrations of ethanol, and critical point dried from CO_2. The corneas were sputter coated with gold-palladium prior to viewing.

Results. The mean endothelial cell count of 1-month-old kitten corneas was 6200/mm^2 (±230), while that of the outbred adult cat population was 2403/mm^2 (±177). Specular micrographs of kitten (Fig. 1A) and adult (Fig. 1B) corneal endothelia emphasized the distinctly smaller size of the kitten cells. Scanning electron micrographs of kitten (Fig. 2A) and adult cat (Fig. 2B) endothelia confirmed the size difference between the young and older animals. In addition, the arrangement of kitten endothelial cells was decidedly more pleomorphic than the ordered hexagonal surface appearance of the adult cells. The pleomorphism of the kitten endothelial cells was a consistent finding.

When both the number of endothelial cells/mm^2 and the extent to which the size of the cornea changes during maturation are known, it is possible to determine the relative contributions that cellular hypertrophy (enlargement) and mitosis make in establishing the adult endothelial cell population. In Figure 3 the number of endothelial cells per cat cornea are plotted in two hypothetical circumstances: one where mitosis is unrestricted and the final endothelial cell population/area is equal to that of the 1-month-old kitten; and the other where no further cell division takes place after the first month with the final endothelial cell population resulting exclusively from cellular hypertrophy (enlargement in the absence of cell divisions). When the observed final number of endothelial cells per adult cat cornea is compared against the two hypothetical models, it is evident that mitosis contributes very little to the postnatal development (beyond 1 month) of the feline corneal endothelial cell population. The imprecision of our measurements could result in the final endothelial cell population, 580,000 cells/cornea, being over- or under-estimated by as much as 15%. Even so, the overall contribution by mitosis to the corneal endothelial cell population during maturation appears to be relatively small.

Scanning electron microscopic studies of 1-month-old kitten corneas frequently demonstrated a number of cells that appeared to be dividing within the endothelial cell monolayer (Fig. 4). We have not observed similar mitotic cellular configurations in adult cat corneas. Serial specular microscopic examinations made on a separate group of three kittens over a 6-month period indicated that the number of cells/mm^2 dropped rapidly between 4 weeks (6000/mm^2) and 8 weeks (4100/mm^2), suggesting that cell division within the endothelial monolayer ceases at about 1 month. A progressive but less rapid decline in the

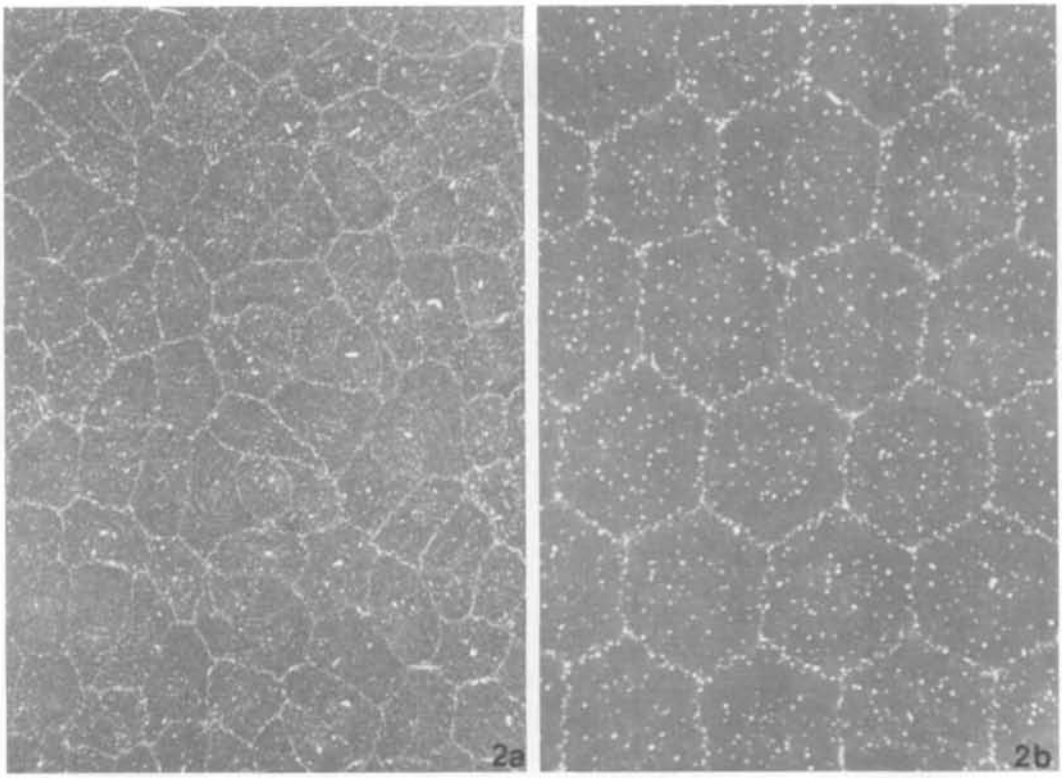

Fig. 2, A and B. A, Scanning electron micrographs of kitten and B, adult cat corneal endothelium. In addition to being much smaller, the kitten endothelium is considerably more pleomorphic than that of the adult cat (both ×1,125).

number of cells occurred during the ensuing 4 months (3 mos, 3700/mm^2; 4 mos, 3500/mm^2; 5 mos, 3100/mm^2; and 6 mos, 2800/mm^2).

Discussion. The number of endothelial cells/mm^2 and the number of endothelial cells/cornea decreases with age in man.[2] A recent study by Gwin et al[7] has demonstrated a progressive decrease in the number of endothelial cells/mm^2 with age in the dog. The inability of human endothelium to regenerate is well known, whereas the regenerative capacity of canine endothelium is thought to be limited.[8] Rabbit corneal endothelium, a cell type possessing a high mitotic potential,[1] also decreases in the number of cells/mm^2 with maturation.[9,10] However, because of postnatal corneal enlargement, the total number of endothelial cells/cornea increases between 130%[9] and 160%[10] over the number of endothelial cells present on the newborn (6–14 days) rabbit cornea.

When the postnatal growth of the cat cornea is considered together with the number of corneal endothelial cells/mm^2 in the kitten and cat, it is evident that mitosis contributes relatively little (~14%) to the final cell number present on the adult feline cornea, assuming that all cells resulting from division are retained within the cell monolayer. When a similar consideration is applied to the rabbit, it appears that cellular hypertrophy, even in this species that has a great mitotic potential, also accounts for a significant degree of coverage on the adult rabbit cornea. [Calculations of 6-day-old and 13-month-old rabbits made from the data by Von Sallman et al[10] indicate a sim-

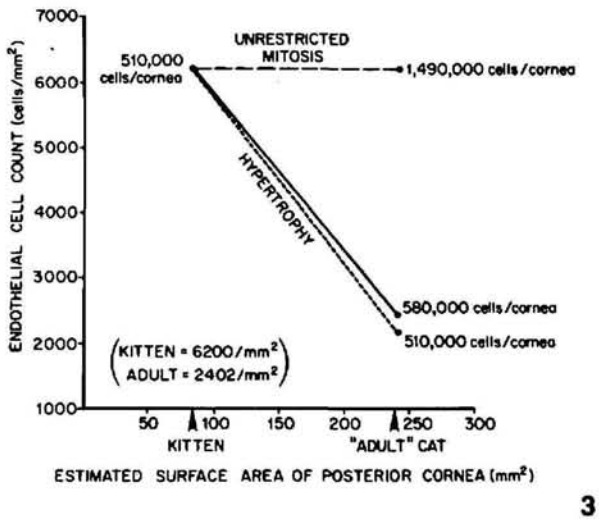

Fig. 3. The contributions made by mitosis and hypertrophy in establishing the endothelial cell density of the adult cornea are illustrated. When the actual number of cells on the adult cornea is plotted against two theoretical models, it is evident that cell division makes a minimal contribution to the number of cells present in the adult.

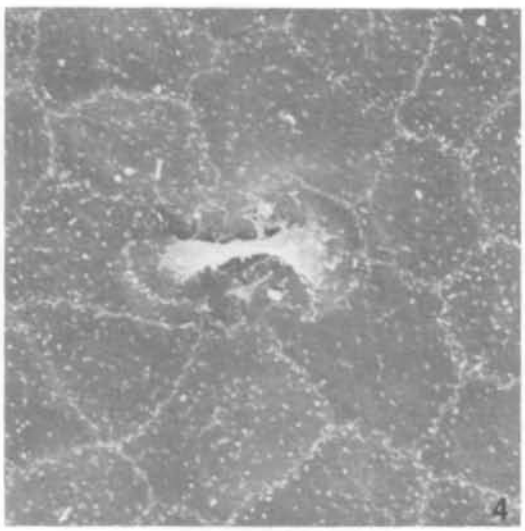

Fig. 4. Scanning electron micrograph of what appears to be a dividing cell in the corneal endothelium of the kitten. Such cellular conformations are relatively common in the kitten but have not been observed in the adult cat (×2,000).

ilar mitosis/hypertrophy relationship to that of the cat; in an unpublished series from our laboratory, four 1-week-old and 15 "adult" rabbits indicate an approximate 50% contribution by hypertrophy.] Therefore, the capacity of an endothelial cell population to divide does not necessarily mean that the process of cellular hypertrophy plays a relatively unimportant role in establishing the "adult" corneal endothelial cell density.

The pronounced pleomorphism observed in the kitten endothelium has also been observed in newborn (6-day-old) rabbit corneal endothelium.[9] The reason for this pleomorphism is unknown. A possible explanation is that mitotic activity[10] combined with the rapid enlargement of the cornea during the postnatal period imposes a continuous requirement for readjustment of cells within the monolayer. Such a continuous readjustment would result in a less ordered cellular arrangement.

Key words: corneal endothelium, cat.

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Vasoactive Intestinal Peptide and Cholinergic Neurotransmission in the Ciliary Muscle

Ryo Suzuki and Shunsaku Kobayashi

The effects of vasoactive intestinal peptide (VIP) on mechanical activity and electrical stimulation of the bovine ciliary muscle in vitro were compared. The generated contractions were enhanced by application of eserine and were abolished completely in the presence of atropine. VIP (10^{-8}–10^{-6} M) enhanced the response to electrical stimulation, while the contractile amplitude of the ciliary muscle to exogenous carbachol was not altered significantly by application of 10^{-7}–10^{-6} M VIP. The exogenous VIP had no direct effect on the muscle. As these data indicate that VIP