# Scanning Slit Confocal Microscopic Observation of Cell Morphology and Movement within the Normal Human Anterior Cornea

*James D. Auran, MD, Charles J. Koester, PhD, Norman J. Kleiman, PhD, Rachel Rapaport, J. Scott Bomann, Barbara M. Wirotsko, MD, George J. Florakis, MD, Jan P. Koniarek, PhD*

***Purpose:*** Noninvasive in vivo observations of the anterior human cornea were performed to study cell structure and dynamics. Cellular elements were identified by their location, morphology, and pattern of movement. The hypothesis that cells in the epithelial layer of the normal cornea migrate centripetally was tested.

***Methods:*** Using a scanning slit confocal microscope with a new 0.75-numeric aperture contact objective, individual cells of normal human corneas were observed over time, quantifying the velocity and direction of cellular movement within the basal epithelial layer.

***Results:*** Basal epithelial cells, wing cells, the basal epithelial nerve plexus, and the subepithelial nerve plexus were identified readily. Centripetal motion was observed for three corneal cell types: basal epithelial cells, basal epithelial nerves, and unidentified cellular elements (possibly Langerhans cells). The unidentified cellular elements moved along the length of the basal epithelial nerves. The basal epithelial nerve plexus maintained a roughly stable topology as it slid centripetally. New nerve material appeared at the site of entry of the nerve into the epithelium. No growth cones were present at the distal termini of the growing epithelial nerves.

***Conclusion:*** In the midperiphery of the normal human cornea, basal epithelial cells and nerves slide centripetally, probably in concert. Unidentified cellular elements used the basal epithelial nerve plexus as a pathway for intraepithelial movement. Observations in this study suggest that neurite growth occurred by the addition of new membrane material along the length of the axon rather than at a distal growth cone.

*Ophthalmology 1995;102:33–41*

In human adult corneal epithelium, the continual cell renewal process—mitosis, maturation, and sloughing—is thought to consist not only of anteriorly directed cell movement (from the basal layer to the corneal surface)

Originally received: May 2, 1994.
Revision accepted: August 22, 1994.

From the Department of Ophthalmology, Edward S. Harkness Eye Institute, Columbia University, New York.

Supported by a grant from the David Warfield Fellowship Program in Ophthalmology of the New York Community Trust and the New York Academy of Medicine (Dr. Auran), a grant from the Edward S. Harkness Clinical Eye Society, and an unrestricted departmental grant from Research to Prevent Blindness, Inc, New York, New York.

Presented as a poster at the American Academy of Ophthalmology, Annual Meeting, Chicago, November 1993.

Charles J. Koester has a proprietary interest in the scanning slit confocal microscope. None of the other authors has a financial or proprietary interest in the instrumentation used in this study.

Reprint requests to James D. Auran, MD, the Edward S. Harkness Eye Institute, 635 West 165th St, Box 11, New York, NY 10032.

but also of centripetal tangential sliding (migration of cells toward the inferocentral cornea): the "X-Y-Z" hypothesis.[1–3] Such centripetal migration of all epithelial cell layers, including the basal epithelial cell layer, has been observed in India ink-labeled epithelial cells in the normal mouse cornea.[4] Before the current investigation, human corneal epithelial cell migration had not been directly observed in vivo.

The human corneal epithelium is richly innervated. Stromal nerve bundles enter the anterior cornea at the limbus, coursing centrally and anteriorly. Stromal nerves are relatively thick and straight, with widely spaced branches that diverge at acute angles. As these nerves reach the anterior limits of the stroma and the interface between the stroma and Bowman's layer, they form a poorly characterized network[5–7] (the subepithelial nerve plexus). Anteriorly directed nerves, emerging from the anterior stromal nerves, perforate Bowman's layer at an estimated 400 peripheral sites[8] to enter the basal epithelial cell layer. Other nerves enter the corneal basal epithelium at the limbus. Together, these nerves form the basal epithelial nerve plexus. Nerves of this plexus have been identified histopathologically in three locations: (1) between basal epithelial cells and the underlying basement membrane, (2) between adjacent basal epithelial cells (several micrometers anterior to the basal lamina), and (3) completely enveloped by individual basal epithelial cells.[5,8–10] Each nerve contains 1 to 40 axons.[8,10] Adjacent nerves intermingle via numerous connecting elements. The basal epithelial nerves have varicosities (beads),[8,10,11] which have been identified as axonal efferent and sensory terminals.[8,10]

Until the current investigation, normal human corneal epithelial nerve dynamics have not been investigated in vivo. There is some evidence, however, that corneal epithelial nerves participate in centripetal migration in the normal cornea. Light microscopy of fluorescent dye-labeled rat corneas demonstrated that epithelial superficial nerve endings undergo topologic changes over time.[12] Human histopathologic studies show that anterior epithelial nerve branches follow an essentially vertical path (i.e., perpendicular to the anterior corneal surface) as they run anteriorly from the basal epithelial nerve plexus.[5,11] This finding supports the premise that, if centripetal epithelial slide is occurring, nerves in the epithelial layer and epithelial (basal and wing) cells are moving laterally at approximately the same velocity. If the basal epithelial nerve plexus were fixed in position, the flow of epithelial cells toward the inferocentral cornea would exert lateral mechanical pressure on the anterior epithelial branches, inducing them to follow an oblique course toward the surface (angled toward the inferotemporal cornea).

To investigate these processes further, a newly modified scanning slit confocal microscope[13,14] was used to perform noninvasive in vivo observations of the architecture and dynamics of the human anterior cornea, especially the basal epithelial layer.

## Subjects and Methods

The wide-field scanning slit confocal microscope used in this study was coupled with a 0.75-numeric aperture contact objective,[14] rather than the previously described 0.35-numeric aperture contact objective.[13] The microscope has a theoretical resolution of less than 1 $\mu$m and an optical section thickness (for an infinitely bright object) of as small as 40 $\mu$m.[14] This facilitated observation of very dim objects.

After approval by the Columbia—Presbyterian Medical Center Institutional Review Board, the corneas of four of us were examined. To establish that topical anesthesia had no effect on the appearance of the basal epithelial layer during confocal observation, examinations were performed on one subject with and without prior instillation of topical anesthetic (0.5% proparacaine drops). Thereafter, all measurements were performed after instillation of anesthetic drops. No vital dyes were used at any time.

Multiple corneal landmarks were examined serially over time, in all subjects, to determine stability of their position. These landmarks consisted of the corneoscleral limbus, anterior stromal nerves (including the subepithelial nerve plexus), entry points where nerves emerged from Bowman's layer into the epithelium, stromal banding and basal lamina ridges induced by pressure from the flat contact element (anterior corneal mosaic),[15] and the nuclei of nonneural stromal cellular elements.

The left cornea of a 23-year-old woman who did not wear contact lenses was observed on three occasions (days 0, 43, and 72) over a 72-day period. At each observation session, a distinctive portion of the subepithelial nerve plexus approximately 2 mm nasal to the superotemporal (1:30 clock hour) limbus was photographed at a depth of 59 to 70 $\mu$m posterior to the anterior corneal surface (Fig 1).

A left cornea stromal nerve of 35-year-old man who wore disposable soft contact lenses was mapped from its point of entry into the cornea, which was at the superotemporal limbus (1:30 clock hour), 170 $\mu$m posterior to the anterior surface. The nerve extended nasally through the stroma, gradually sloping anteriorly. The nerve broke up into a branching network (the subepithelial nerve plexus) in the superotemporal cornea midperiphery 2.5 mm nasal to its limbal origin and 50 $\mu$m posterior to the anterior corneal surface. A point where a branch of this nerve entered the epithelium was readily recognizable, due in particular to a bright object contiguous to the nerve entry site. This nerve entry point into the epithelium (Fig 2) and underlying stromal nerves (including the subepithelial nerve plexus) in the area were observed serially over 438 days. The basal epithelial nerve plexus was examined on three occasions over a 3-week period (Fig 3). At each session, multiple photographs of overlapping areas were obtained with the plane of focus at the level of the basal epithelial nerve plexus, allowing two-dimensional reconstruction of plexus topology in photographic print montages.

In the left cornea of a 63-year-old man who did not wear contact lenses, we identified a bright object contiguous to a site of nerve entry into the superior midperipheral epithelium approximately 1.5 mm from the 12-o'clock limbus. The basal epithelial nerve plexus in this

area was examined four times over a 20-day period (Fig 4).

The left cornea of a pregnant 27-year-old woman who wore disposable soft contact lenses was observed over a 61-day period. An area 2.5 mm nasal to the temporal limbus (3-o'clock limbal meridian) was observed serially on five occasions (days 0, 33, 34, 47, and 61). At no time were signs or symptoms of epithelial trauma present in this or the other contact lens-wearing subject.

Best images of the basal epithelial cell outlines were obtained when the plane of focus was 10 μm anterior to the basal epithelial nerve plexus (Fig 5). The cell membranes of the basal epithelial cells are so dim that good confocal images are obtained only when the plane of focus is at mid-cell height. At a plane of focus 5 μm anterior to the basal lamina, the outlines of the basal epithelial cells were faintly visible (at the limit of detectability) and photographic prints are of marginal quality. At this focal plane, the basal epithelial nerve plexus is at the outside limit of the optical section and is poorly imaged. In the cornea of the 35-year-old man, a bright object (a presumed cellular aggregate) at a site of entry of the nerve into the basal epithelium was imaged at this plane of focus. This object remained stable in position (Fig 2) and was used as a reference point for serial observations of the basal epithelial cells over time (Figs 6 and 7).

Photographs were taken with TMAX 400 ASA black and white print film (Kodak, Rochester, NY) push-processed to 1600 ASA with high-contrast printing.

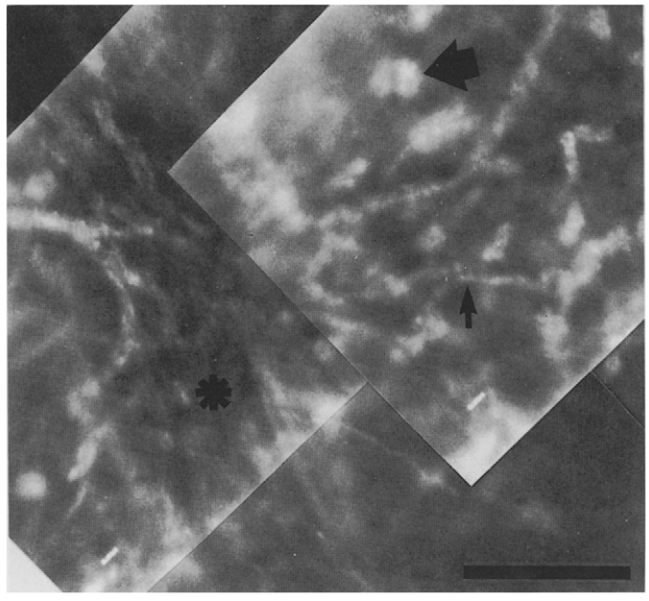

Figure 1. A photographic montage of an anterior stromal nerve entering from the left and branching out to form the subepithelial nerve plexus (thin arrow). Nuclei (thick arrow) of nonneural stromal cellular elements and a dark band of the anterior corneal mosaic (*) are readily identified. Bar = 100 μm.

## Results

No qualitative difference in anterior stromal and basal epithelial (including nerve and basal epithelial cell) confocal microscopic appearance was noted with and without the use of topical anesthetic.

Irregular-shaped bright objects, distinct from the stromal nerves, were abundant in the anterior stroma of all subjects (Fig 1). Over the course of a single observation session, the objects were stable in position and appearance. However, during serial observation sessions, the objects often moved slightly; changed in size, shape, and brightness; and, in some cases, disappeared.

The corneoscleral limbus, stromal nerves (including the subepithelial nerve plexus), entry points of nerves into the epithelium, and the anterior corneal mosaic remained fixed in position relative to each other over observation periods of 20, 60, 72, and 438 days in the four subjects. The stability of the positions of these landmarks, coupled with the 40- to 80-μm optical section thickness of images obtained with the scanning slit system, enable us to return to and photograph precise locations in the epithelium and anterior stroma repeatedly over time.

On confocal microscopy, normal anterior stromal nerves were relatively thick, somewhat radially oriented, and gently curved over long distances. Branches usually were separated by hundreds of microns, and the branches diverged at acute angles. The nerves had smooth edges and a bright uniform texture, which showed little internal detail. As these nerves reached the anterior limits of the stroma (nearing the interface between the stroma and Bowman's layer), they either sent branches directly into the epithelium through Bowman's layer or broke up into a network of profusely branched nerves—the subepithelial nerve plexus (Fig 1). Nerves of this plexus had narrowly separated branches diverging and converging at both acute and right angles. These nerves were of very low contrast, with a granular texture and irregular edges. The plexus was sparse and patchy in distribution, with the patches limited to the midperipheral cornea. Smaller nerves of the subepithelial nerve plexus were occasionally beaded.

The basal epithelial nerve plexus appeared as roughly parallel beaded nerves, which formed a vortex pattern as they arched toward the area just inferior to the corneal apex. In addition, the nerves appeared to branch at acute angles with numerous connecting fibers (Fig 3). This plexus appeared approximately 48, 44, and 47 μm posterior to the anterior corneal surface in the three subjects in whom it was examined. Entry sites of nerves into the basal epithelium frequently were found at the apices of the applanation pressure-induced epithelial basal lamina ridges of the anterior corneal mosaic (Figs 2–4).

Nerves of the superotemporal basal epithelial nerve plexus of the 35-year-old man shifted centripetally toward an area just inferior to the corneal apex. The velocity of movement averaged 17 μm daily. Different plexus topologic features (i.e., branch points) shifted at different velocities (range, 14.9–18.1 μm daily). There were variations in the velocity of individual reference points over time (Fig 3). The nerve movement velocity of a superotemporal basal epithelial nerve of the 63-year-old man averaged

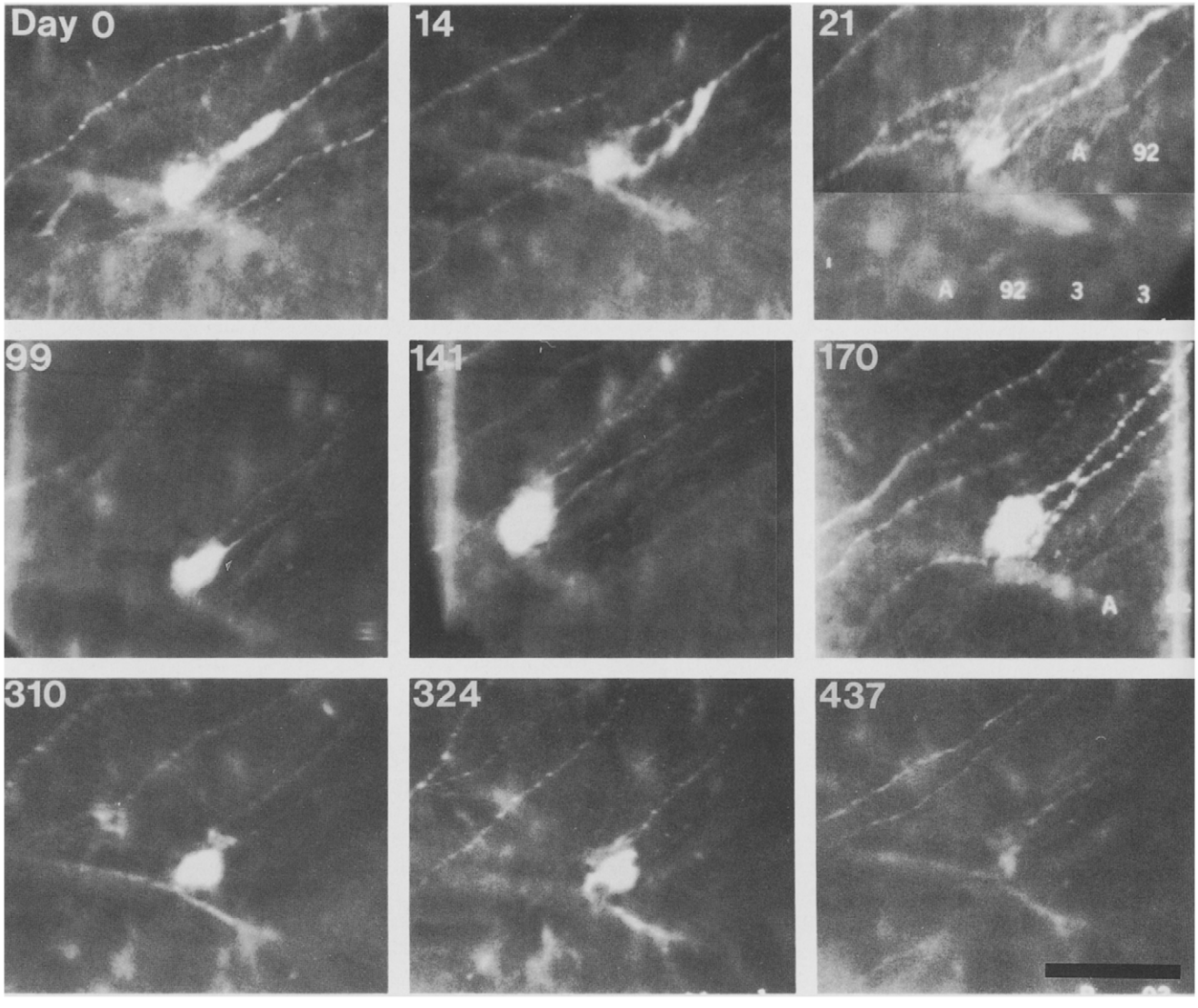


Figure 2. An entry point of a nerve into the corneal epithelium followed for 438 days in a 35-year-old man. In these photographs, taken at the plane of the basal epithelial nerve plexus, beaded epithelial nerves run from lower left to upper right. A blurred stromal nerve runs from mid-left to lower right. Bar = 100 μm.

13.3 μm daily (Fig 4). Again, velocity varied at different times and branch points, ranging from 11.0 to 16.2 μm daily. In the 27-year-old woman, the velocity of movement of a temporal basal epithelial nerve was measured at 5.5 μm daily over an interval of 14 days. Reference points (beads) on another nerve (without branch points) in the area were observed to move 6.8 μm daily over two examinations scheduled 3 days apart.

The topology of the basal epithelial nerve plexus varied slightly as the plexus slid centripetally. Nerve segments between clearly identifiable bifurcation points and distinctive bends could be tracked over time moving away from the point where they entered the epithelium. The shape and length of these nerve segments varied slightly as the axons slid centripetally (Figs 3 and 4). No distal growth cones were observed.

In all subjects, at a few sites of nerve entry into the epithelium, large irregular-shaped bright objects were observed contiguous with the nerve. The bright objects were continually changing in size (range, 11 × 27 to 42 × 46 μm) and shape over time, but they always remained in contact with the nerve (Figs 2–4, 6, and 7).

In all subjects, small bright elements were observed contiguous with basal epithelial nerves at various points distal to the nerve's point of entry into the epithelium (Figs 3 and 4). The objects varied in size (range, 11 × 13 to 13 × 31 μm) and shape over time. Such an object was followed over time in the cornea of the 35-year-old man. The object moved centripetally along the length of the nerve with a velocity of up to 4 μm daily relative to the nerve (18.2–22.7 μm daily relative to the stroma) (Fig 3). In the cornea of the 27-year-old woman, a similar object moved along the nerve at approximately 10 μm daily relative to the nerve (16.8 μm daily relative to the stroma).

Basal epithelial cells were best observed when the microscope was focused 10 μm anterior to the basal epithelial

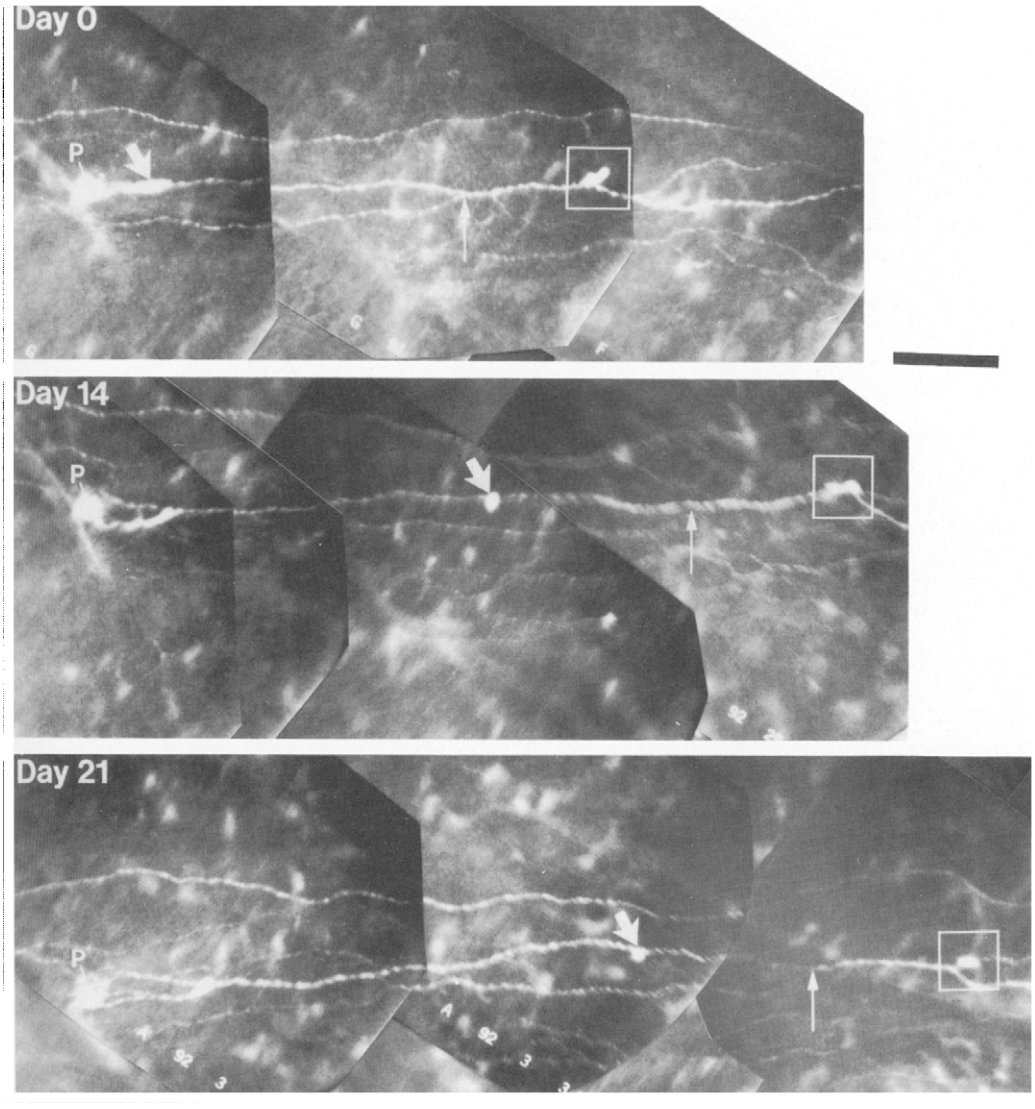


Figure 3. Photographic montages of the basal epithelial nerve plexus obtained at days 0, 14, and 21 in a 35-year-old man. Notice centripetal movement of nerve topologic features (e.g., the branch point marked by the long, thin arrow). The blurred, dim oblique linear object adjacent to the nerve entry point is the stromal nerve from which the epithelial nerve has branched. A motile cellular element, probably a Langerhans cell (thick, short arrow), appears to bud off from the main element and move centripetally along the nerve. A similar element is observed passing a branch point of the nerve (square). The corneal apex is to the right. P = the entry point of the nerve into the epithelium with an associated irregular-shaped bright object. Bar = 100 $\mu$m.

nerve plexus and basal lamina (30–35 $\mu$m posterior to the anterior corneal surface) (Fig 5). Cell outlines were bright and cell contents were dark, unlike the confocal microscopic appearance of corneal endothelial cells, in which cell outlines were dark and contents were light. Occasionally, a white object was visible in the cell center, suggestive of a cell nucleus. The basal epithelial cells were 10 to 20 $\mu$m in diameter.

In the 35-year-old man, over a 24-hour period, the basal epithelial cells slid 23 $\mu$m centripetally relative to the nerve entry site (Figs 6 and 7). Repeat measurements showed 29 and 32 $\mu$m of basal epithelial cell centripetal slide over 24-hour periods.

## Discussion

The objects observed in the anterior stroma had the appearance of cells or portions of cells. The anterior stroma has a mixed population of nonneural cellular elements, including keratocytes, Langerhans cells, Schwann cells, and lymphocytes. These nonneural cell types can not be distinguished on confocal microscopy of unstained normal living human cornea. However, objects similar to those observed (Fig 1) previously were imaged by confocal microscopy and hypothesized to be keratocyte nuclei.[14,16,17] It is unclear if the observed movement of these elements represents cell body motion or shifting of intracellular contents (within a cell body that is fixed in position).

Before the availability of the scanning slit confocal microscope,[17] the human subepithelial nerve plexus was not observed in vivo. The subepithelial nerve plexus has a distinct morphology and location that make it readily distinguishable from both deeper stromal nerves and overlying epithelial nerves when observed by scanning slit confocal microscopy. The current investigation suggests that stromal nerves, including the subepithelial plexus, remain stable in position and topology over extended periods. This allows the use of these nerves as landmarks to track cellular movement in the cornea.

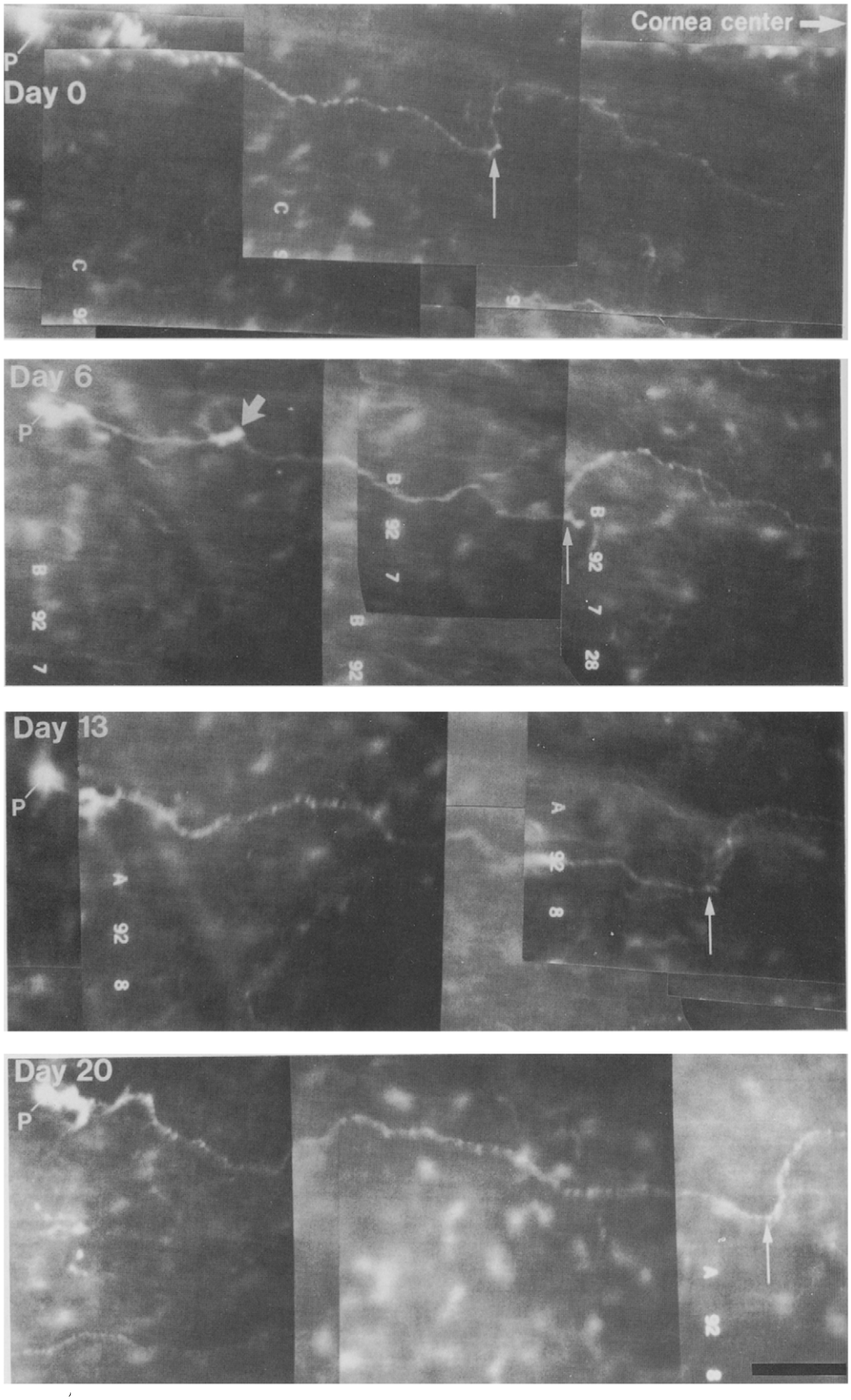


Figure 4. Photographic montages of basal epithelial nerve plexus obtained at days 0, 6, 13, and 20 in a 63-year-old man. There is centripetal movement of the kink in the nerve (thin arrow). A bright object (thick arrow) in contact with the nerve is observed at day 6. The corneal apex is to the right. P = entry point of the nerve into the epithelium with an associated bright object. Bar = 100 μm.

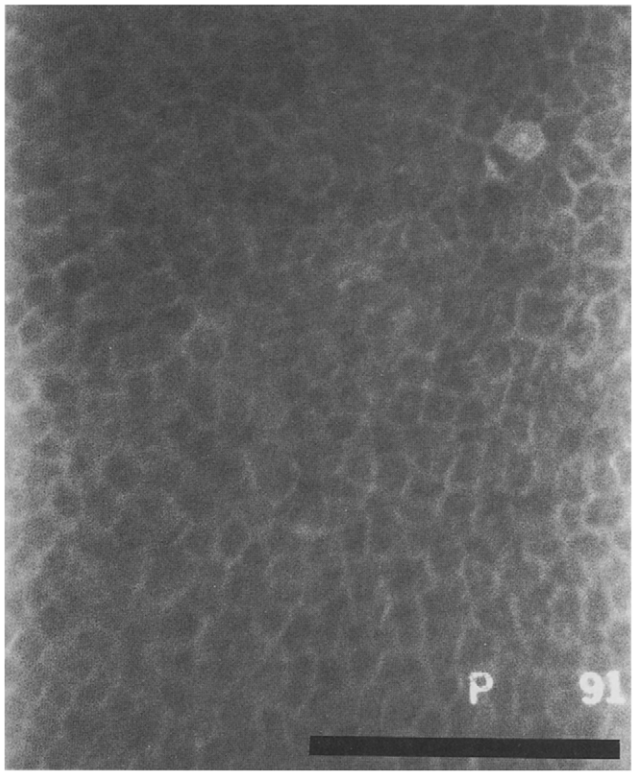


Figure 5. Basal epithelial cells are observed as white outlines (10–20 μm in diameter) with dark contents. The faint white punctate objects within some of the cell outlines may be nuclei. Bar = 100 μm.

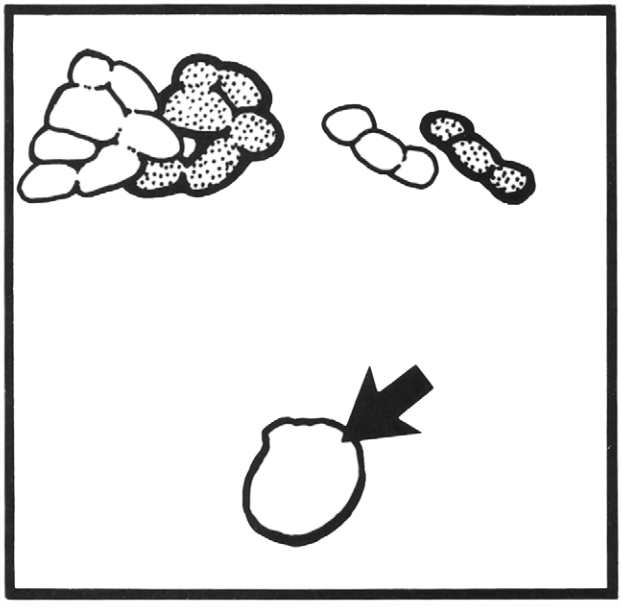

Figure 7. Line drawing of Figure 6. Two epithelial cell clusters (light outline with clear fill, 0 hours; heavy outline with speckled fill, 24 hours) shift relative to the bright object overlying a nerve entry point (arrow).

With scanning slit confocal microscopy, finely detailed images of the basal epithelial nerve plexus were obtained with resolution comparable or even superior to that obtained with histopathologic staining[8,11] and scanning pinhole confocal microscopy[16] (e.g., beads and connecting elements were visible). Confocal (scanning pinhole and scanning slit) microscopic images of the human basal epithelial nerve plexus are similar in appearance to stained histopathologic specimens,[8,11] despite the distortions in histopathologic preparations induced by cell death, fixation, and staining.

It is likely that the observed basal epithelial cell and nerve movement are normal phenomena in the healthy living human cornea. The current finding of basal epi-

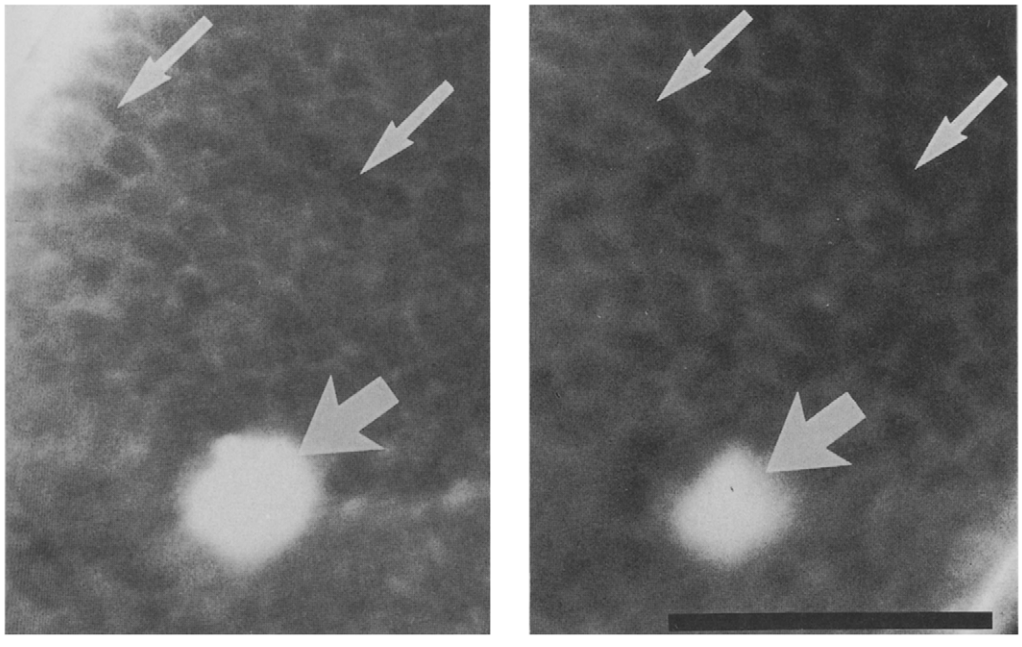

Figure 6. Photomicrographs taken 24 hours apart in the cornea of a 35-year-old man. The corneal apex is to the right. Basal epithelial cell outlines in these photographs allow identification of recognizable cell clusters (thin arrows). Centripetal slide of these clusters occurred relative to the bright object (thick, short arrow) at a site of nerve entry into the epithelium. Bar = 100 μm.

thelial centripetal slide is the first direct confirmation of the "X-Y-Z" hypothesis. Centripetal sliding of the basal epithelial cells is probably due to a higher mitotic rate and lower superficial cell slough rate in the corneal periphery relative to the inferocentral cornea.[1-3] The observed variations in velocity of epithelial cell and nerve movement (range, 1.7–32 μm daily) raise the possibility that epithelial migration rate may be affected by age, sex, contact lens wear, and location within the cornea.

Axonal growth (addition of plasma membrane) was long thought to occur exclusively at neurite growth cones.[18] Recently, axon growth along the neurite rather than at the growth cone was demonstrated experimentally in cultured Xenopus neurons,[19] but it has never been previously observed in vivo. In the human cornea basal epithelial nerve plexus, we observed what appeared to be an addition of new membrane occurring along the neurites at or near the point of nerve entry into the epithelium, rather than at the distal tip of the axon at a growth cone.

We were unable to simultaneously measure basal epithelial nerve and epithelial cell migration velocities, but it is possible that they migrate centripetally in tandem. Basal epithelial cells are tightly bound to each other by desmosomes and by pronounced interdigitation of lateral cell membranes.[7] Basal epithelial nerves are in close apposition to and closely intertwined with the basal epithelial cells, with almost every basal epithelial cell in contact with a nerve.[5] Given the tight connections between basal epithelial cells, the close relation between nerves and basal epithelial cells, the relatively stable topology of the basal epithelial nerve plexus as it slides centripetally, and the comparable rates of migration observed in basal epithelial cells and nerves, we suspect that the nerves and basal epithelial cells are migrating together as a unit. An alternative hypothesis—that nerves and epithelial cells are sliding at different rates—is also possible, but requires that basal epithelial cells continually pass through and over the basal and anterior epithelial nerves.

Observations in this study suggest that in the human corneal basal epithelium, new nerve material is produced at or proximal to the site of entry of the nerve into the epithelium. The basal epithelial nerve then slides centripetally, possibly in tandem with centripetally sliding basal epithelial cells. We have not performed serial observations of the inferocentral cornea, where wave fronts of centripetally sliding basal epithelial cells collide. In that area, individual basal epithelial cells and the nerve fibers associated with them presumably migrate anteriorly, degenerating or sloughing into the tear film.

Bright objects were observed moving centripetally along the length of a basal epithelial nerve. The objects were too large to be swellings associated with axonal transport. The objects were likely to be entire cells or portions (e.g., nuclei) of cells. Their movement relative to the nerve suggests that they may be actively motile. The objects may have been guided by a neurotropic factor, or may have been taking advantage of the potential space around the nerve as a path of least resistance to movement through the basal epithelium.

It is possible that the nonpigmented bright objects observed to migrate along the basal epithelial nerves are bodies or nuclei of Langerhans (dendritic) cells. Lymphocytes are another nonpigmented cell type present in the normal corneal basal epithelium, but they have not been observed in direct contact with epithelial nerves.[20] Langerhans cells are present in the normal peripheral human cornea, are motile, and have been found contiguous to nerves of the basal epithelial plexus in histopathologic specimens.[7,11,21] Furthermore, in a guinea pig model, Langerhans cells migrating through the basal corneal epithelium in response to trauma took on a markedly elongated linear appearance, morphologically consistent with movement along basal epithelial nerves.[22]

The large bright objects observed at the entry point of the nerve into the epithelium (Figs 2, 3, 6, and 7) may be one or, in view of their large size, multiple cells. The objects could be Langerhans cells, lymphocytes, or anterior extensions of Schwann cells. Poorly characterized cellular structures have been observed in association with basal epithelial nerves on light microscopy.[11,23] In a transmission electron microscopic study, cellular elements were noted to surround basal epithelial nerves at their entry point into the epithelium in 3 of 18 human corneas.[9] The serial images obtained in the current investigation raise the possibility that the bright objects at the nerve entry sites were a source of the objects observed migrating along the nerves (Fig 3).

In this investigation, we demonstrated that individual cells in the living human corneal epithelium can be followed noninvasively over time. With confocal microscopy, we have the unique potential to study the effects of disease and therapeutic intervention on the cellular level, including influences on individual corneal epithelial cells, keratocytes, and nerves.

**Acknowledgment.** The authors thank Heinz Rosskothen for performing the skilled instrument fabrication for this project.

## References

1. Thoft RA, Friend J. The X, Y, Z hypothesis of corneal epithelial maintenance [letter]. Invest Ophthalmol Vis Sci 1983;24:1442–3.
2. Sharma A, Coles WH. Kinetics of corneal epithelial maintenance and graft loss. A population balance model. Invest Ophthalmol Vis Sci 1989;30:1962–71.
3. Lemp MA, Mathers WD. Renewal of the corneal epithelium. CLAO J 1991;17:258–66.
4. Buck RC. Measurement of centripetal migration of normal corneal epithelial cells in the mouse. Invest Ophthalmol Vis Sci 1985;26:1296–9.
5. Duke-Elder S, Wybar KC. The Anatomy of the Visual System. In: Duke-Elder S, ed. System of Ophthalmology Vol. II. St. Louis: CV Mosby, 1961;92–131.
6. Tervo T, Vannas A, Tervo K, Holden BA. Histochemical evidence of limited reinnervation of human corneal grafts. Acta Ophthalmol 1985;63:207–14.
7. Klyce SD, Beuerman RW. Structure and function of the cornea. In: Kaufman HE, McDonald MB, Barron BA, Waltman SR, eds. The Cornea. New York: Churchill Livingstone, 1988;3–54.

8. Ueda S, del Cerro M, LoCascio JA, Aquavella JV. Peptidergic and catecholaminergic fibers in the human corneal epithelium. An immunohistochemical and electron microscopic study. Acta Ophthalmol Suppl 1989;192: 80–90.
9. Ueda S, del Cerro M, Rao GN, Aquavella JV. The epithelial innervation in human cornea: a light and electron microscopic study. In: Cavanagh HD, ed. The Cornea: Transactions of the World Congress on the Cornea III. New York: Raven Press, 1988;chap. 11.
10. Matsuda H. Electron microscopic study on the corneal nerve with special reference to its endings. Jpn J Ophthalmol 1968;12:163–73.
11. Schimmelpfennig B. Nerve structures in human central corneal epithelium. Graefes Arch Clin Exp Ophthalmol 1982;218:14–20.
12. Harris LW, Purves D. Rapid remodeling of sensory endings in the corneas of living mice. J Neurosci 1989;9:2210–14.
13. Koester CJ, Roberts CW, Donn A, Hoefle FB. Wide field specular microscopy. Clinical and research applications. Ophthalmology 1980;87:849–60.
14. Koester CJ, Auran JD, Rosskothen HD, et al. Clinical microscopy of the cornea utilizing optical sectioning and a high-numerical-aperture objective. J Opt Soc Am [A] 1993;10:1670–9.
15. Auran JD, Koester CJ, Rapaport R, Florakis GJ. Wide field scanning slit in vivo confocal microscopy of flattening-induced corneal bands and ridges. Scanning 1994;16:182–6.
16. Cavanagh HD, Petroll WM, Alizadeh H, et al. Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease. Ophthalmology 1993;100:1444–54.
17. Masters BR, Thaer AA. Real-time scanning slit confocal microscopy of the in vivo human cornea. Appl Opt 1994;33: 695–701.
18. Jacobson M. Developmental Neurobiology, 3rd ed. New York: Plenum Press, 1991;chap. 5.
19. Popov S, Brown A, Poo M. Forward plasma membrane flow in growing nerve processes. Science 1993;259:244–6.
20. Scheiffarth OF, Stefani FH, Gabriel N, Lund O-E. T lymphocytes of the normal human cornea. Br J Ophthalmol 1987;71:384–6.
21. Pau H, Conrads H. Die Bedeutung der Langerhansschen Zellen für die Nerven des Hornhautepithels. Graefes Arch Ophthal 1957;158:427–33.
22. Kelley JG, Ohashi Y, Friedlaender MH. Langerhans cell alterations in the guinea pig cornea. Invest Ophthalmol Vis Sci 1985;26:1293–6.
23. Scharenberg K. The cells and nerves of the human cornea. A study with silver carbonate. Am J Ophthalmol 1955;40: 368–79.