In Vitro Growth and Differentiation of Rabbit Bulbar, Fornix, and Palpebral Conjunctival Epithelia

Implications on Conjunctival Epithelial Transdifferentiation and Stem Cells

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Purpose. The anterior surface of the eye is covered by several physically contiguous but histologically distinguishable epithelia overlying the cornea, limbus, bulbar conjunctiva, fornix conjunctiva, and palpebral conjunctiva. It is important to determine whether the different phenotypes of these epithelia are the result of intrinsic divergence, extrinsic modulation, or a combination of both. Based on keratin expression and cell kinetic criteria, the authors previously suggested that corneal epithelial stem cells may actually reside in the limbal basal layer.

Methods. In this article, the relationship between the corneal–limbal epithelial cells and conjunctival epithelial cells was analyzed by comparing their growth and differentiation properties in an identical cell culture environment.

Results. Using Dispase instead of trypsin to dissociate the cells, the authors were able to grow all five rabbit ocular surface epithelia in the presence of 3T3 feeder cells. They found that corneal and limbal cells synthesize identical keratins, including large amounts of the K3 and K12 markers of corneal-type differentiation. By contrast, all three conjunctival epithelia shared another keratin pattern, with large amounts of simple epithelial keratins but only minute amounts of K3/K12 keratins.

Conclusions. This observation, coupled with previous findings that the “transdifferentiation” of conjunctival epithelial cells to corneal epithelium appears to be both incomplete and reversible, provides strong evidence that (1) the limbal–corneal epithelial cells form a lineage distinct from the conjunctival lineage and (2) conjunctival transdifferentiation actually represents a process of environmental modulation. In addition, of the three types of conjunctival epithelial cells, fornix cells were found to have a much greater proliferative potential than bulbar and palpebral cells. This observation, coupled with recent finding that fornix is enriched in slow-cycling (label-retaining) cells, raises the possibility that conjunctival epithelial stem cells may preferentially reside in the fornix.

bits, the most frequently used experimental animals for studying ocular surface healing, fornix epithelium has the highest density of goblet cells, and palpebral epithelium appears to be most highly stratified. Collectively, these three conjunctival epithelia are thought to play an important role in forming a physical protective barrier of the conjunctival surface and, by their goblet cell secretions, in contributing to the formation and maintenance of a "tear film" of the ocular surface.1

Another important function that has been attributed to conjunctival epithelium is that it can "transdifferentiate" into a corneal epithelium if the latter is depleted by wounding.2-9 This process was studied in detail.6,7 It was shown that, after the rabbit corneal epithelium was removed using n-heptanol, the remaining conjunctival epithelial cells migrate rapidly onto the denuded corneal surface, forming a monolayer of cells covering the wound. After 12-24 hr, the cells proliferate and begin to stratify. Concurrently, the goblet cells, which have comigrated onto the corneal stroma, disappear. The process continues, and by 4 weeks postwounding, there forms an epithelium histologically similar to normal corneal epithelium.

The analysis of epithelial keratins has generated useful information about, not only the differentiation, but also the lineage of epithelial cells. Keratins are a group of water-insoluble cytoskeletal proteins that form the desmosome-associated 10-nm intermediate filaments in almost all epithelia.10-13 There are a total of approximately 30 keratins that can be divided into an acidic (Type I) and a neutral-to-basic (Type II) subfamily.14-18 Detailed analysis of keratins in a number of epithelial cell types helped to establish that the expression of these keratins follows a set of rules.15,16 Most notable is that each basic keratin tends to coexpress with a particular acidic keratin, forming a so-called keratin "pair."16,19 In addition, each keratin pair tends to be expressed in a tissue-restricted and differentiation-dependent fashion. Perhaps most important for ocular surface epithelia is the K3/K12 keratin pair, which is synthesized in large quantities in corneal, snout, and some oral mucosal epithelia but only in small amounts in conjunctival epithelia.20,21 Moreover, immunofluorescent staining and cell separation experiments showed that these two keratins are expressed in the suprabasal cell layers of cultured rabbit corneal epithelial structure, indicating that they represent markers for an advanced stage of corneal epithelial differentiation.20,22,23 Using a monoclonal antibody (AE5), we demonstrated that K3 is expressed suprabasally in limbal epithelium but uniformly in central corneal epithelium.20 This finding implies that the (K3-positive) basal cells of central corneal epithelium may have attained a more advanced state of differentiation than the (K3-negative) basal cells of limbal epithelium. Based on this and several other considerations, we proposed that corneal epithelial stem cells are not uniformly distributed in the basal layer of the entire corneal epithelium but are restricted to the basal layer of limbal epithelium.20,24

The distinct phenotypes of conjunctival and corneal epithelia might be the result of differences in their genetic program (intrinsic divergence). Alternatively, the two epithelia might be genotypically identical but attain different phenotypes as a result of responding to different mesenchymal influences (external modulation).25-29 If the latter is true, then the two epithelia should behave identically if they were provided artificially with an identical growth environment. Taking this experimental approach, we have previously shown that rabbit corneal, skin, and esophageal epithelial cells behave differently even when they are placed in identical cell culture and in vivo environments. This finding allowed us to conclude that, although external signals can modulate to a significant extent the differentiation programs of keratinocytes, the three cell types are intrinsically divergent.30

Using a similar cell culture approach in conjunction with keratin analysis, we address here the issue of conjunctival transdifferentiation. An obstacle to this approach in the past has been our inability to grow rabbit conjunctival epithelial cells using a protocol that has worked well for rabbit corneal and limbal epithelial cells (Sun et al., unpublished).30 We have overcome this problem through our observation that rabbit conjunctival epithelial cells are unusually sensitive to trypsinization. By avoiding excessive trypsinization, we were able to isolate and grow all three conjunctival epithelial cells and to compare the in vitro growth and differentiation of these keratinocytes with those of corneal and limbal cells isolated and grown under identical conditions. Our results indicate the following. First, the in vitro phenotypes of corneal and limbal epithelial cells are identical, except that limbal cells grow better than the central corneal epithelial cells. This result confirms and extends the work of another group31,32 and lends further support to the concept that corneal epithelial stem cells reside in the limbal zone. Second, the in vitro phenotypes of the three conjunctival epithelial cells are identical but are distinct from that of corneal–limbal epithelium. A notable difference is that only the latter synthesizes a large quantity of K5 keratin. This provides the strongest evi-
ence thus far available suggesting that conjunctival epithelium represents a cell lineage(s) that is intrinsically distinct from the corneal–limbal epithelial lineage. Third, of the three conjunctival epithelia, fornix cells have a much greater in vitro proliferation potential do than bulbar and palpebral epithelia. This result, in conjunction with our recent observation that fornix epithelium is particularly enriched in label-retaining cells,³³ raises the possibility that the fornix contains a relatively high concentration of conjunctival epithelial stem cells. These results have important implications concerning the homeostasis and regeneration of anterior ocular surface epithelia.

MATERIALS AND METHODS

All animals used in this study were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Isolation and Cultivation of Rabbit Conjunctival Epithelial Cells

New Zealand white rabbits weighing 3–4 kg were killed by intravenous injection of T-61 euthanasia solution. Under sterile conditions, cuts were made into the subcutaneous space around lid margin without cutting through the conjunctival sac so that the whole eye could be removed with eyelid still attached. The eyes were soaked in Dulbecco's minimum essential medium supplemented with tenfold antibiotic concentrations for 30 min at 25°C. The whole sheet of conjunctival epithelial tissue was then trimmed off the Tenon's tissue, and a specific zone of the conjunctiva (bulbar, fornix, or palpebral) was conformed to the profile of a sterile paraffin sheet with a central depression of approximately 4-mm diameter. The lowered conjunctival regions were then subjected to Dispase II (1.2 units/ml; Boehringer Mannheim, Indianapolis, IN) treatment at 37°C for 3 hr under 5% CO₂ and 95% air. The detached epithelium was isolated by light scraping and was dissociated into single cells and small clumps by pipetting. The cells were then plated at a concentration of 2 X 10⁵ per 35-mm dish in the presence of mitomycin C-treated 3T3 feeder cells, with twice per week medium changes.

Isolation and Cultivation of Corneal–Limbal Epithelial Cells

With the aid of a dissecting microscope, an outline was made on the cornea with a 12-mm trephine. The corneal button and a 2-mm wide limbal tissue were cut out with scissors. The corneal endothelium (on Descemet's membrane) was peeled off with forceps. The central cornea (5-mm diameter) was then cut out with a 5-mm trephine and incubated with Dispase II. The isolated keratinocytes were grown as described. All cultures were passaged by light trypsinization (0.125% trypsin in 0.01% ethylenediaminetetraacetic acid and phosphate-buffered saline at 37°C for 5 min) when the cells reached 80–90% confluence and were plated with fresh 3T3 feeder cells with a split ratio of 1:3.

3T3 Feeder Cell Preparation

Confluent 3T3 cultures were treated with 4 μg/ml of mitomycin C for 2 hr at 37°C under 5% CO₂ and 95% air, trypsinized, and plated as a feeder layer at slightly more then one-third of their confluent density (approximately, 2 × 10⁴ cells/cm²) along with corneal or conjunctival epithelial cells.²⁰,³⁰,³⁴

Histologic and Electron Microscopic Analysis

Specimens were fixed with 10% formalin in phosphate-buffered saline, embedded in JB4 plastic embedding medium, cut at 3 μm, and stained with alcian blue–periodic acid-Schiff and hematoxylin (Gill). Electron microscopy was performed as described.³⁵

Tritiated Thymidine Incorporation

Secondary keratinocyte cultures were plated without 3T3 feeder cells and pulse-labeled with ³H-thymidine (5 μCi/ml) for 2 hr at 37°C, washed with phosphate-buffered saline, fixed with 10% formalin in phosphate-buffered saline for 30 min, and processed for autoradiography as described.²⁴,³⁶,³⁷

![Figure 1](https://lovos.arvojournals.org/ projekt/1816.png)
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FIGURE 2. Morphology of cultured rabbit central corneal epithelial cells. Keratinocytes were isolated from central corneal buttons (5-mm diameter) using Dispase and plated in Dulbecco's minimum essential medium containing 20% fetal bovine serum in the absence (A and E) and presence (B–D) of mitomycin C-treated 3T3 feeder cells. (A) A primary culture grown in the absence of feeder cells with relatively wide intercellular space and large cell size frequently associated with keratinocyte senescence. (B, C) Primary cultures grown in the presence of 3T3 feeder cells; note the formation of expanding epithelial colonies containing small (basal-like) cells. (D) A secondary culture. (E) A secondary culture stained with AE5 antibody (recognizing K3 keratin) showing that most of the superficial cells are K3 positive. Abbreviations are: E: epithelial cells; F: 3T3 fibroblast feeder cells. A–D are the same magnification. Scale bar equals 50 μm.

Immunofluorescent Staining
Secondary cultures of keratinocytes growing on glass cover slips were fixed with methanol-acetone (1:1) for 30 min and stained with monoclonal antikeratin antibodies using the indirect immunofluorescent staining technique.10

Keratin Analysis
Primary cultures of rabbit ocular keratinocytes were grown to confluence. The 3T3 feeder cells were selectively removed using ethylenediaminetetraacetic acid, and the keratinocytes were extracted with a buffer containing 10 mmol/l Tris HCl (pH 7.4) and a mixture of protease inhibitors to remove the water-soluble proteins. The insoluble cytoskeleton was then solubilized with 9.5 mol/l urea, 10 mmol/l Tris HCl (pH 7.4), and analyzed by sodium dodecyl sulfate polyacrylamide and two-dimensional gel electrophoresis coupled with immunoblotting as described previously.19,20,30,39
RESULTS

Isolation of Rabbit Ocular Keratinocytes

We isolated epithelial cells from several ocular anterior surface compartments, defined according to the following morphologic and anatomic criteria. The central cornea "button" covered a 5-mm radius, which was at least 5 mm away from the limbal zone and was therefore free from any limbal or conjunctival cell types. The limbal sample was defined as a 2-mm wide...
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In Vitro Growth and Differentiation of Corneal and Limbal Epithelial Cells

In previous investigations, we studied the in vitro growth and differentiation of the entire corneal–limbal epithelium. Consistent results were obtained...
using isolated central corneal and limbal keratinocytes, both of which gave rise to stratified epithelial colonies (Figs. 2, 3) that expressed large amounts of K3 keratin as revealed by AE5 antibody staining (Figs. 2, 3) and by sodium dodecyl sulfate polyacrylamide gel electrophoresis coupled with immunoblotting (Fig. 4). One difference between the two cell populations, however, was that limbal cells grew better than central corneal epithelial cells (Figs. 2, 3, 5). The limbal cells in general proliferated more rapidly (Fig. 5A), remained relatively small in average cell size (Fig. 3), reached confluence earlier, and under our experimental conditions, could be subcultured three times (Fig. 6). By contrast, central corneal epithelial cells grew more slowly (Fig. 5A), became enlarged and appeared senescent relatively early in vitro, and could be subcultured only once (Fig. 6). These results are consistent with the human data obtained by others3132 and lend further support to the idea that corneal epithelial stem cells reside in the limbal zone.

In Vitro Growth and Differentiation of Bulbar, Fornix, and Palpebral Conjunctival Epithelial Cells

Using the same techniques, we studied the in vitro growth and differentiation of rabbit conjunctival epithelial cells. Like corneal–limbal epithelial cells, conjunctival epithelial growth was greatly facilitated by the presence of 3T3 feeder cells (Figs. 7–9). All three conjunctival epithelial cell types gave rise to stratified colonies. A small portion of the cells stained positively with AE5 antibody, indicating the synthesis of K3 keratin (Figs. 7–9). Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis revealed that the total keratin patterns of the three conjunctival cell types were indistinguishable from one another, with abundant K5/K14 “basal cell” keratins, the K6/K16 “hyperproliferation” keratins, and K8/K18 and K19 “simple epithelial” keratins (Fig. 4).16 Little K3/K12 “corneal-type” keratins were present. Such a keratin pattern was relatively similar to those of in vivo conjunctival epithelium and was strikingly different from that of in vivo corneal–limbal epithelia, which contained abundant K3/K12 keratins and little simple epithelial K8/K18 keratins (Sun et al., unpublished data).20,22,42,43

Although the three regions of conjunctival epithelium exhibited almost identical in vitro morphologic and biochemical phenotypes (Figs. 4, 7–9), they had different growth potential. Bulbar and palpebral epithelia grew relatively poorly and could be subcultured only once (Figs. 5, 6). Under the same conditions, the fornix epithelial cells grew extremely well and could be subcultured three times, indicating that fornix epithelium had a much higher proliferative potential than its neighboring conjunctival epithelia (Fig. 10). These results are schematically summarized in Figure 11.

DISCUSSION

A central issue that we address in this article is whether the conjunctival and corneal epithelia are equipotent, ie, whether they are genetically identical but exhibit different in vivo phenotypes as a result of different environmental modulation. This concept might explain the frequently cited phenomenon of “conjunctival epithelial transdifferentiation,” in which conjunc-
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3° FIGURE 6. In vitro growth of rabbit anterior ocular surface keratinocytes. Rabbit corneal (C), limbal (L), bulbar conjunctival (B), fornix conjunctival (F), and palpebral conjunctival (P) epithelial cells were plated (at $2 \times 10^5$ cells/35-mm dish) in the absence (−3T3) and presence (+3T3) of feeder cells. 1°, 2°, and 3° (of +3T3 cultures) denote primary, secondary, and tertiary cultures, respectively. Cultures were fixed after 6–9 days, stained with Rhodanilile blue, and photographed. Note the superior growth potential of limbal cells and fornix conjunctival epithelial cells.

tival epithelial cells migrate onto the denuded corneal surface and become morphologically indistinguishable from the bona fide corneal epithelium. The importance of this concept is clearly illustrated by a current clinical practice in which a piece of conjunctival epithelium is transplanted as a source to repair a damaged corneal epithelium. By growing various ocular keratinocytes under an identical cell culture condition, we experimentally "normalized" their environmental influences. Any morphologic or biochemical differences persisting among these cultured ocular keratinocytes would then have to reflect intrinsic divergence. Using this approach, we generated useful information regarding the lineage relationships among several ocular keratinocytes.

Additional Evidence Supporting the Limbal Stem Cell Theory

The notion that corneal epithelial stem cells reside in the limbal zone was supported by the following evidence. First, as discussed earlier, the K3 keratin, a marker for an advanced stage of corneal epithelial differentiation, was present in the basal cells of central corneal epithelium but not in those of the limbal epithelium. Second, a population of cells that are slow cycling (an important property of stem cells) exists in the limbal basal layer; moreover, these cells can be preferentially stimulated to divide by wounding and by topical application of O-tetradecanoylphorbol-13-acetate (TPA). By contrast, no label-retaining cells can be identified in central corneal epithelium. Third, complete removal of limbal epithelium by repeated abrasion or surgery results in defective corneal epithelium regeneration. Fourth, corneal epithelial cells undergo centripetal migration. Our finding that rabbit limbal epithelial cells grew better than central corneal epithelial cells (Figs. 5, 6) and similar data on human cells added further support to this theory (for a different view, see reference 55). Interestingly, some recent evidence suggests that more AE5-negative stem-like cells are present in the superior and inferior portions of the limbus.

The Corneal–Limbal Lineage Is Distinct From the Conjunctival Epithelial Lineage

The finding that cultured corneal and limbal epithelial cells synthesize the same keratin set (with abundant K3/K12 corneal epithelial differentiation markers), which is clearly different from the keratin pattern shared by all three cultured conjunctival keratinocytes...
(bulbar, fornix, and palpebral; Fig. 4), provides clear evidence that (1) our isolated bulbar conjunctival epithelial cells are not significantly contaminated by limbal keratinocytes; (2) the corneal and limbal epithelia are closely related; (3) the three conjunctival keratinocytes are closely related; and (4) the corneal-limbal compartment is intrinsically divergent from the conjunctival compartment.

An important corollary of the last notion is that corneal and conjunctival epithelia are not equipotent. This conclusion is supported by several other pieces of experimental evidence. First, the glycogen content and several other biochemical properties of conjunctiva-derived "corneal epithelium" remain abnormal long after the completion of the transdifferentiation process. Second, the conjunctiva-derived corneal epithelium can respond to corneal vascularization by forming goblet cells and by expressing immunoglobulin A secretory component—two markers of normal conjunctival epithelium. Third, although by light microscopy the conjunctiva-derived corneal epithelium appears normal, some goblet cells persist, and electron microscopic studies showed that the epithelium has much wider intercellular space than the corneal epithelium. Fourth, it was found that, in humans, the conjunctiva-derived corneal epithelium is frequently associated with persistent epithelial defects, recurrent erosion, stromal neovascularization, necrosis, and retarded healing rate. Fifth, it has been shown that the complete removal of corneal-limbal epithelium by physical means frequently results in the failure of conjunctiva-initiated regeneration of corneal epithelium.
FIGURE 8. Morphology of cultured rabbit fornix conjunctival epithelial cells. (A) A primary culture grown in the absence of feeder cells; all other panels show cells grown in the presence of feeder cells. (B) A primary culture. (C) A tertiary culture. (D) AE5-staining of a secondary culture (no feeder) showing a few positive cells. (E, F) Ultrastructure showing stratification, keratin bundles (K), desmosomes (D), and relatively large intercellular space (*). A-C are of the same magnification. Scale bars equal to 50 μm in C and D, 1 μm in E, and 0.5 μm in F.

neal epithelium. Sixth, although treatment of corneal-limbal surface with n-heptanol for 1 min (a classic procedure) can effectively remove all corneal epithelial cells, it does not remove all limbal epithelial basal cells—the putative corneal epithelial stem cells. This raises the possibility that some of the earlier data on transdifferentiation may represent the transient covering of the denuded corneal surface by conjunctival epithelial cells, followed by long-term regeneration of bona fide corneal epithelium by the few surviving (limbal) corneal epithelial stem cells. Seventh, it has been shown that surgical removal of limbal epithelium results in defective corneal epithelial regeneration, and that human limbal epithelium provides a much better tissue source than the conjunctival epithelium for corneal epithelial regeneration. Taken together, these data strongly suggest that limbal–corneal epithelium and conjunctival epithelium represent two separate cell lineages that are intrinsically divergent.
Can Conjunctival Epithelium Transdifferentiate to Become Corneal Epithelium?

The term "transdifferentiation" has been used in the literature to describe the conversion of one differentiated cell type to another. A well-known example is the regeneration of a lens from the iris pigmented epithelium of embryonic and adult newt.65-66 Another example relates to adult rat bladder epithelium which, when combined with the mesenchyme of embryonic urogenital sinus, can be reprogrammed to form a prostatic epithelium that is ultrastructurally, biochemically, and histochemically indistinguishable from a bona fide prostatic epithelium.67,68

These transdifferentiation events, which are usually complete and not readily reversible, should be distinguished from the environmental modulation of cellular phenotypes. Although the modulation process can be accompanied by significant changes in cellular morphology and gene expression, it usually does not result in the complete conversion of one cell type to another and the process is readily reversible (for studies on the environmental modulation of keratinocyte differentiation, see references 19, 30, 35, and 69-72). Such a distinction is important because transdifferentiation represents an unusual (and rare) process in which "differentiated adult cells reverse their commitment and engage in a new pathway of differentiation thus violating the general rule of stability of determination in differentiated animal somatic cells."64

As discussed earlier, conjunctival epithelial cells can migrate onto a denuded corneal surface to form an epithelium that resembles corneal epithelium by...
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Passage

FIGURE 10. In vitro growth potential of cultured rabbit palpebral (P), bulbar (B), and fornix (F) conjunctival epithelial cells. Keratinocytes were grown in the presence of 3T3-feeder cells and subcultured (at 2 × 10^5 cells/35-mm dish) as described in Figure 9, and the time it took for a culture to reach more than 90% confluence was plotted.

light microscopy and may even acquire some of the biochemical features of corneal epithelium, including elevated synthesis of K3 keratin. However, there is ample evidence that this conversion is both incomplete and readily reversible. Therefore, this process reflects environmental modulation rather than transdifferentiation.

Regardless how we describe this process, the notion that the limbal–corneal cells are intrinsically divergent from the conjunctival cells has two important implications as follows. First, transplantation of corneal and conjunctival epithelia should be preferably done using homologous tissues; heterologous tissue transplants may still be useful, however, for short-term wound coverage. Second, the fact that corneal epithelial stem cells reside in the limbus, the transitional zone between cornea and conjunctiva, raises the question as to whether such stem cells can give rise, not only to corneal epithelium, but also to conjunctival epithelium. However, this seems to be incompatible with our finding that cultured limbal–corneal epithelial cells behave so differently from any of the cultured conjunctival keratinocytes (Fig. 4). We therefore favor the alternative possibility that conjunctival epithelium, as a compartment distinct and separate from the corneal–limbal compartment, is governed by its own stem cell population(s).

Significance of AE5-Staining in Cultured Conjunctival Epithelial Cells

We have previously shown that K3 keratin is present not only in corneal epithelium, but also in snout, lip, and some other mucosal epithelia and in the transitional zone between palpebral conjunctival epithelium and eyelid epidermis (Fig. 11). Therefore, this keratin is clearly not "cornea-specific," even though it, along with its usual partner K12 keratin, is synthesized in the upper cell layers of cultured corneal epithelial colonies and might be regarded as a marker for an advanced stage of "corneal-type differentiation." Immunofluorescent staining and immunoblotting data showed that conjunctival epithelial cells normally express relatively small amounts of K3 keratin. Our analysis of keratins synthesized by cultured rabbit ocular keratinocytes (Fig. 4) confirmed this differential synthesis of K3/K12 keratins. The small proportion of cultured rabbit conjunctival epithelial cells stained positively by AE5 antibody (Figs. 7–9) could represent contamination by the neighboring AE5-positive limbal cells or the cells located in the transitional zone between the palpebral and eyelid skin. Some of them could, however, represent bona fide conjunctival epithelial cells undergoing elevated synthesis of K3 keratin.

FIGURE 11. A schematic diagram summarizing the distribution of K3 keratin, goblet cells, and label-retaining cells in anterior ocular stratified epithelia. Data on rabbit and mouse models, as described in this and several other articles, were combined to generate this diagram. Abbreviations are: E: epidermis; T: transitional zone between palpebral conjunctiva and epidermis; P: palpebral conjunctiva; F: fornix conjunctiva; B: bulbar conjunctiva; L: limbus; C: cornea; AC: anterior chamber; and LS: lens. Strongly AE5-stained epithelial regions are hatched. Note the presence of strongly AE5-stained epithelial regions is hatched. Note the presence of strongly AE5-stained cells in cornea–limbus and in the transitional zone between eyelid epidermis and palpebral conjunctiva. Other conjunctival epithelial cells stain only weakly, although a few strongly stained cells can occasionally be seen. Round dots denote goblet cells, the highest density of which is in the fornix region. Dashed lines denote the relative densities of label-retaining cells; note that the distribution of these label-retaining cells coincides well with that of keratinocytes with a high in vitro proliferative potential (Figs. 5, 6, 10). This diagram was not drawn to scale.
Relationship Among Bulbar, Fornix, and Palpebral Conjunctival Epithelia: A Hypothesis

Cultured keratinocytes of rabbit bulbar, fornix, and palpebral conjunctiva exhibited similar morphologic and biochemical features (Figs. 4, 7–9). Although this result does not prove that the three cell types are equipotent, it is intriguing to note that fornix cells can be subcultured three times; under identical experimental conditions, bulbar and palpebral cells can be subcultured only once or not at all (Figs. 6, 10). This indicates that fornix cells have a much greater proliferative potential than bulbar and palpebral cells. This finding, in conjunction with our recent observation that fornix epithelium contains many more label-retaining cells than other regions of the conjunctival epithelium (Fig. 11),

raises the intriguing possibility that the three conjunctival epithelia are equipotent and are maintained by the same population of stem cells. More experiments are needed, however, to test this theory.

Key Words
conjunctival epithelium, growth, differentiation, stem cells, cell culture

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