account for the appearance and disappearance of corneal LC. While LC are felt to have an immunologic role in processing antigen,\(^*\) and in corneal transplantation,\(^*\) we believe these cells also play some role in tissue damage and/or repair. The appearance of LC in the cornea may be important after certain types of corneal injury and infection. Roussel et al\(^*\) suggested that the perpetuation of corneal inflammation may be mediated by LC, and Rubsamen et al\(^*\) proposed a role for LC in corneal transplant rejection. Methods for controlling the number of LC in the cornea may prove useful in these situations.

Corneal LC are seen in large numbers after chronic inflammation and trauma.\(^*\) In our studies, corneal tissue was also damaged by suture placement and by UV irradiation. LC appear to be active after a variety of corneal perturbations. The exact role for LC in nonimmunologic disturbances of the cornea will require further investigation.

Key words: Langerhans cell, guinea pig, cornea, nylon suture, UV-irradiation

Acknowledgment. The authors thank Ramsey Hemady for his excellent technical assistance.


Measurement of Centripetal Migration of Normal Corneal Epithelial Cells in the Mouse

Robert C. Buck

Fine punctate marks were made in normal corneas of mice using a needle rotating in a mixture of India ink and thorium dioxide. After 7 days, the marker was visible in the stroma and also in epithelial cells which had moved away from the stromal marks and towards the center of the cornea. The mean distance between these labels at the end of 7 days was 94 \(\mu m \pm 14\) (SEM). The median distance migrated was about 17 \(\mu m\) per day. This figure represents the distance through which superficial and wing cells had migrated; the distance migrated by basal cells was not determined. Invest Ophthalmol Vis Sci 26:1296–1299, 1985

Recently Thoft and Friend\(^*\) have proposed an "XYZ hypothesis" of corneal epithelial cell maintenance. They review the evidence, which is indirect, that normal corneal epithelial cells are in a state of constant centripetal migration, as well as proliferation and desquamation. However, no experimental work has been carried out on the specific question of the possible migration of the epithelial sheet in the normal cornea.

In order to test this hypothesis, certain of the methods used to study morphogenetic movements of cells and cell sheets during embryonic development could be applied to the cornea. Markers have been developed for this purpose and include carmine or Nile blue dyes,\(^*\) carbon or India ink implantation,\(^*\) and \(H^3\)-thymidine, the labeled cells being traced by autoradiography.\(^*\) Fortunately, the corneal epithelial cells are highly phagocytic towards the colloidal carbon of India ink, and this simple method can be used to trace their movement.

Materials and Methods. Swiss mice weighing 20–25 g were anesthetized by an intraperitoneal injection

References

of sodium pentobarbitol. Into each eye was then instilled a drop of a mixture of Pelican India ink (Gunther Wagner) and equal parts of a colloidal solution of thorium dioxide (Thorotrast; Testagar, Detroit), and the excess was blotted away. A short, 1-mm diameter steel needle with a very sharp point mounted in the chuck of a high speed drill was pressed lightly against the cornea, and power was applied for about 10 sec. A tiny black spot marked the position of the "tatoo" in epithelium and underlying stroma.

Preliminary experiments showed that an interval of 7 days was satisfactory for checking the result because the label was still seen in the epithelial cells as well as in the stroma. Animals allowed to live for 2 wk showed ink marks only in the stroma; the labeled epithelial cells had largely desquamated.

The animals were killed by cervical dislocation 7 days after marking the cells. The eyes were enucleated and immersed for 4 hr in half-strength Karnovsky fixative. The corneas were excised and cut into four sections which were flattened between a weighted coverslip and a microscope slide so that they could be examined even by the oil immersion lens.

The ink marks were identified, the specimen was rotated to give a consistent position of the limbus, and a photograph was taken. The distance between the leading edge of the migrating epithelial cells and the closest edge of the stromal tatoo was measured. Humane treatment of the mice was provided in accordance with the ARVO Resolution on the Use of Animals in Research.

**Results.** Most of the tatoos were not satisfactory because ink had not marked the stroma. In order to obtain a measure of migration, it was necessary to have ink in the stroma to indicate the starting point, and also ink in a cluster of the migrating cells.

In almost all cases, when a stromal tatoo was observed in the cornea after 7 days, a group of cells having particles in their cytoplasm extended from the stromal tatoo towards the center of the cornea (Fig. 1). Epithelial cells containing the ink in phagocytic vacuoles were easily distinguished from stromal cells by their shape and by the level in the cornea, determined by their plane of focus using the oil immersion lens (NA 1.0). Prior to the onset of migration the spot was small and dense (Fig. 2).

Observations were made on 21 tatoo marks in 16 mice. The mean distance migrated in 7 days was 94 \( \mu \text{m} \pm 14 \) (SEM). This figure is significantly different from 0 (the null hypothesis), having a \( P \) value of \(<0.01\). This mean includes two specimens in which no ink could be found in the epithelium and one specimen in which migration appeared to be 30 \( \mu \text{m} \) in the opposite direction from the rest of the group.

If these three are excluded, the mean distance migrated becomes 111 \( \pm 13 \mu \text{m} \) (SEM). The median distance was 123 \( \mu \text{m} \). It seems that the best estimate for distance migrated is about 17 \( \mu \text{m} \)/day.

Although it was not the purpose of this research to study the movement of conjunctival epithelium, ink marks were made in conjunctiva close to the limbus in several animals. Only six satisfactory tatoos were observed in conjunctiva, and none of these showed any movement towards or away from the limbus after 7 days. The question of conjunctival epithelium crossing the limbus in the normal eye requires further study.

In order to determine which layers of the epithelium were involved in the migration, some specimens, after being studied by light microscopy, were prepared in the usual way for transmission electron microscopy. They were oriented so that sections would be cut through the marked cells of both stroma and epithelium. The thorium dioxide, being electron dense, had been added to the ink for this purpose. Phagocytic inclusions were observed in all layers of the epithelium,
The present results confirm migration in the normal cornea, although the rate of migration is only about \( \frac{1}{10} \) of that seen earlier in the peripheral part of the corneas repairing a central defect.

Previous studies suggesting centripetal cell movement have generally been based on observations in which some insult was made to an appreciable area of corneal epithelium, which might therefore be said to be reacting to injury. Mann \(^7\) studied the migration in living rabbits of pigmented epithelial cells as they responded to lesions of the cornea made close to the limbus, and by repeatedly scraping the cornea, she was able to draw these pigmented cells far from the limbus. Other examples given by Thoft and Friend \(^1\) suggest centripetal cell migration, such as observations on sex chromatin of donor grafts and the movement of epithelial dots after grafting. It is difficult to evaluate how much effect, if any, such invasive procedures might have on migration, and to decide including basal cells (Fig. 3), although they appeared to be larger and more numerous in wing and superficial cells. However, this tedious method does not lend itself to the measurement of migration. Obviously, the probability of finding thorium inclusions in thin sections is not high, and it is not possible to determine whether the distance migrated by cells observed to have inclusions is representative of all migrating basal cells.

**Discussion.** I earlier demonstrated \(^5\) by the use of ink labeling that during the resurfacing of a small central epithelial defect of the cornea, mass movement of epithelium in the cornea peripheral to the lesion resulted. Subsequently, I observed that the hemidesmosomes of these peripheral cells were arranged in rows. \(^6\) An analysis comparing the angle of these rows to the radial axis of the cornea showed a highly significant correlation, and I concluded that the pattern in rows was an expression of migration in a radial direction. However, a similar pattern of hemidesmosome rows was also seen in the normal cornea suggesting that normal epithelium too might be constantly undergoing centripetal migration.
whether the migration represents a normal phenomenon in these cases.

Although a quantitative evaluation of the movement of basal cells could not be carried out by the method used here, basal cells containing thorium inclusions were observed to lie in centripetal positions relative to the stromal marks. Whether they moved as far as the more superficial cells remains to be determined.

That they do move centripetally, as anticipated from the study of the orientation of the hemidesmosomes, opens up the question of the participation of hemidesmosomes in their migration. They cannot be static. During the repair of suction blisters of skin, hemidesmosomes apparently undergo a rapid dislocation and relocation, which takes place in less time than is required for the epidermal cell to migrate through its own length. Other investigators estimated that less than an hour is required for their reformation after being torn from the basal lamina by suction. Thus, the relatively slow rate of migration of the normal corneal epithelium would pose no problem for the repeated dislocation and re-attachment of its hemidesmosomes to the basal lamina.

The significance of centripetal epithelial migration for the maintenance of corneal epithelial integrity is, of course, a matter for speculation. The convergence of cells towards the center, by which central cells are replaced with those derived from peripheral cornea, could possibly reflect the gradient from peripheral cornea to central cornea of certain substances derived from the limbal capillaries. According to Maurice, substances spread through the cornea by diffusion from the perilimbal capillaries. The gradient for large molecules is steep, serum albumin being several times more concentrated at the periphery than at the center. Whether these molecules confer any advantage on the peripheral epithelial cells is unknown, but possibly such substances as growth factors, known to have pronounced effects on corneal epithelial cells in vivo, could reach the epithelial cells via these capillaries.

Key words: corneal epithelium, cell migration, mouse


References


**Iontophoresis of Epinephrine Isomers to Rabbit Eyes**

**Induced HSV-1 Ocular Shedding**

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Iontophoresis of 0.01% levo(−) epinephrine for 8 min at 0.8 mAmp once daily for 3 consecutive days induced ocular shedding of herpes simplex virus type 1 (HSV-1) in latently infected rabbits. In the present experiment, we tested dextro(+) and levo(−) epinephrine for their comparative effects on induced HSV-1 ocular shedding. One hundred percent of the eyes shed virus after either 0.01% dextro(+) or levo(−) epinephrine iontophoresis (8 min, 0.8 mAmp). However, the shedding frequency caused by 0.005% levo(−) epinephrine was significantly higher (P < 0.05) than that by 0.005% dextro(+) epinephrine when the iontophoresis was conducted at 0.4 mAmp for 4 min. Iontophoresis of 0.001% levo(−) epinephrine for 8 min at 0.8 mAmp once daily for 3 consecutive days and iontophoresis of 0.001% dextro(+) epinephrine for 8 min at 0.8 mAmp once daily for 3 consecutive days did not induce HSV-1 ocular shedding.