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.[8] Other investigators estimated that less than an hour is required for their reformation after being torn from the basal lamina by suction.[9] 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,[10] 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 as well as in vitro,[11] could reach the epithelial cells via these capillaries.

Key words: corneal epithelium, cell migration, mouse

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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 induces 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

Table 1. HSV-1 ocular shedding in rabbit eyes after dextro(+) epinephrine or levo(−) epinephrine iontophoresis

Rabbit no.	Epi	Eye	Days pre- and post-epinephrine iontophoresis										
			−3	−2	−1	0 ↓	1 ↓	2 ↓	3	4	5	6	7
1	d(+)	OD	−	+	+	+	−	+	−	+	C	−	+
2	d(+)	OD	−	−	−	+	+	+	C	+	+	−	+
3	d(+)	OD	−	−	−	−	+	−	−	−	C	−	−
4	d(+)	OD	−	−	−	−	−	−	−	−	−	−	+
5	d(+)	OD	−	−	+	−	+	+	−	+	−	+	−
1	l(−)	OS	−	−	−	+	−	−	−	+	+	+	+
2	l(−)	OS	−	−	−	+	−	−	+	−	−	−	+
3	l(−)	OS	−	−	−	+	−	+	+	+	+	+	+
4	l(−)	OS	−	−	−	−	−	+	−	−	−	−	−
5	l(−)	OS	−	−	−	−	−	+	+	−	−	−	−
			−3	−2	−1	0	1	2	3	4	5	6	7

+: HSV-1 ocular shedding; −: no shedding; C: contamination; l: iontophoresis of dextro(+) or levo(−) epinephrine.

All right eyes received iontophoresis of 0.01% dextro(+) epinephrine, and all left eyes received iontophoresis of 0.01% levo(−) epinephrine. A direct current (0.8 mAmp) was applied for 8 min. Iontophoresis was begun on PI day 150.

in latently infected rabbits. The data suggest that the mechanism of induction of HSV-1 ocular shedding by epinephrine is correlated to the receptor potency of levo(−) epinephrine. Invest Ophthalmol Vis Sci 26:1299–1303, 1985

The mechanism for reactivation of latent infections of herpes simplex virus (HSV) is unknown. Epinephrine iontophoresis to the cornea induced a high frequency (75–100%) of HSV type-1 (HSV-1) ocular shedding from latently infected rabbits.[1-4] Adrenergic receptors are generally stereospecific or stereoselective. The biological activity of the dextro(+) enantiomorphs of adrenergic agonists has been estimated to be lower than that of the levo(−) enantiomorphs.[5,6] However, Rowland and Potter[6] have reported that in the rabbit eye the dextro(+) and levo(−) isomers of epinephrine vary from the rule of stereospecificity. The present studies were conducted to investigate whether or not a stereospecific or stereoselective activation is involved for HSV reactivation by the two isomers of epinephrine.

Materials and Methods. *Animals and virus infection:* Twenty-five New Zealand albino rabbits (2–3 kg) were used. The rabbits were inoculated bilaterally on the unscarified cornea with a 50-μl suspension of HSV-1 McKrae strain (1×10^6 PFU/ml). This procedure results in an acute HSV-1 infection (verified by slit-lamp biomicroscopy). The care and condition of the rabbits used in these investigations conformed to the ARVO resolution on the Use of Animals in Research.

Determination of viral shedding: HSV-1 ocular shedding was detected from eye swabs taken with sterile, dacron-tipped applicators as previously described.[1-4] In the present study, rabbits were used that had shed virus from both eyes spontaneously at least once during postinoculation (PI) days 20–39. Ocular sheddings were determined for 4 consecutive days before the initial iontophoresis and for 7 consecutive days after the initial iontophoresis.

Reactivation of latent HSV (iontophoresis): Epinephrine iontophoresis was performed to induce HSV-1 ocular shedding in the latently infected rabbits. The procedures were described previously.[1-4] In all these reports,[1-4] only the levo(−) form of epinephrine was used. Three iontophoretic conditions were employed: [1] levo(−) or dextro(+) epinephrine (0.005%) at 0.4 mAmp for 4 min; [2] levo(−) or dextro(+) epinephrine (0.01%) at 0.8 mAmp for 8 min; [3] levo(−) or dextro(+) epinephrine (0.001%) at 0.8 mAmp for 8 min. Iontophoresis was performed once a day for 3 consecutive days. The iontophoretic conditions (epinephrine concentration, mAmps, and time) were derived from previous reports,[1-4] unpublished experimental data, and Faraday's law. Levo(−) epinephrine was obtained from Sigma and dextro(+) epinephrine was supplied by Dr. A. E. Soria (Sterling-Winthrop Research Institute; Rensselaer, NY). Epinephrine solutions were prepared with double-distilled and double-deionized water.

Identification of viral isolates: The specificity of HSV-1 in positive cultures from ocular swabs was identified by a plaque-reduction assay on CV-1 cells using an HSV-1 hyperimmune rabbit antiserum. In all cases, the isolated virus was identified as HSV-1.

Results. Table 1 shows the pattern of HSV-1 shedding pre- and post-iontophoresis of 0.01% dextro(+) epinephrine or 0.01% levo(−) epinephrine to the rabbits beginning on PI day 150. A direct current (0.8 mAmp) was applied for 8 min. All right eyes (OD) received iontophoresis of dextro(+) epinephrine,

Table 2. Induction of HSV-1 ocular shedding by iontophoresis of 0.005% levo(−) epinephrine at 0.4 mAmp for 4 min

Rabbit no.	Eye	Days pre- and post-iontophoresis												
		−3	−2	−1	0	↓	1	↓	2	↓	3	4	5	6	7
6	OD	−	−	−	−		−		−		+	+	+	+	−
	OS	−	−	−	−		−		+		−	−	−	−	−
7	OD	−	−	−	−		−		−		−	+	+	−	−
	OS	−	−	−	−		−		−		−	+	+	+	−
8	OD	−	−	−	−		−		+		−	−	−	−	−
	OS	−	−	−	−		−		−		+	+	+	−	−
9	OD	−	−	−	−		−		+		+	+	+	−	−
	OS	−	C	−	−		−		−		−	+	−	−	−
10	OD	−	−	−	−		−		+		−	−	−	−	−
	OS	−	−	−	−		−		+		+	+	+	+	−
		−3	−2	−1	0		1		2		3	4	5	6	7

+: HSV-1 ocular shedding; −: no shedding; C: contamination; ↓: iontophoresis of dextro(+) or levo(−) epinephrine.

All eyes beginning on day 60 PI received iontophoresis of 0.005% levo(−) epinephrine for 3 consecutive days. A direct current (0.4 mAmp) was applied for 4 min. Eye swabs were taken daily for 4 days before and for 7 days after the initial iontophoresis.

and left eyes (OS) received iontophoresis of levo(−) epinephrine. One hundred percent of the eyes (10/10) shed virus after dextro(+) or levo(−) epinephrine iontophoresis. The mean duration of shedding by dextro(+) epinephrine was 2.8 ± 0.80 days (arithmetric mean ± standard error of the mean), while that by levo(−) epinephrine was 3.0 ± 0.89 days. There was no statistical difference between the two groups ($P > 0.8$ by Student's t-test). There were five spontaneous sheddings prior to dextro(+) epinephrine iontophoresis, while three spontaneous sheddings were detected before levo(−) epinephrine iontophoresis.

Table 2 shows the results of 0.005% levo(−) epinephrine iontophoresis on 10 eyes (5 rabbits) beginning on PI day 60. A direct current (0.4 mAmp) was applied for 4 min. HSV-1 ocular shedding was detected in 100% of the eyes within 7 days after the first iontophoresis. The mean duration of shedding for all eyes was 2.5 ± 0.48 days.

Table 3 displays the HSV-1 ocular sheddings following 0.005% dextro(+) epinephrine iontophoresis on 10 eyes (5 rabbits) beginning on PI day 53. The iontophoresis was at 0.4 mAmp for 4 min once a day for 3 consecutive days. HSV-1 ocular shedding was detected in 50% of the eyes (5/10) within 7 days after the first iontophoresis. The mean duration of shedding for all eyes was 1.1 ± 0.41 days. The shedding frequency of 100% caused by 0.005% levo(−) epinephrine iontophoresis (Table 2) was significantly ($P < 0.05$ by χ^2 test) different than the frequency of 50% caused by 0.005% dextro(+) epinephrine iontophoresis (Table 3). There also was a statistical (*P*

Table 3. HSV-1 ocular shedding before and after iontophoresis of 0.005% dextro(+) epinephrine at 0.4 mAmp for 4 min

Rabbit no.	Eye	Days pre- and post-iontophoresis												
		−3	−2	−1	0	↓	1	↓	2	↓	3	4	5	6	7
11	OD	−	−	−	−		−		−		−	−	−	−	−
	OS	−	−	−	−		−		+		+	+	−	−	−
12	OD	−	−	−	−		−		+		+	+	−	−	−
	OS	−	−	−	−		−		−		−	−	−	−	−
13	OD	−	−	C	−		−		−		−	−	−	−	−
	OS	−	−	−	−		−		+		+	−	−	−	−
14	OD	−	−	−	−		−		−		−	−	−	−	−
	OS	+	−	−	−		−		−		−	−	−	−	−
15	OD	−	−	−	−		−		−		+	+	−	−	−
	OS	−	−	−	−		−		−		−	+	−	−	−
		−3	−2	−1	0		1		2		3	4	5	6	7

+: HSV-1 ocular shedding; −: no shedding; C: contamination; ↓: iontophoresis of dextro(+) or levo(−) epinephrine.

All eyes received iontophoresis of 0.005% dextro(+) epinephrine for 3 consecutive days. A direct current (0.4 mAmp) was applied for 4 min. All rabbits were used beginning on PI day 53.

Table 4. HSV-1 ocular shedding in latently infected rabbits

Group	PI day	Epinephrine	Ionotophoretic condition	Positive eyes / Total eyes	Positive swabs / Total swabs	Average duration of shedding (days) (mean ± SEM)
A (Table 1)	150	0.01% dextro(+)	0.8 mAmp for 8 min	5/5 (100%)	14/32 (44%)	2.8 ± 0.80
B (Table 1)	150	0.01% levo(−)	0.8 mAmp for 8 min	5/5 (100%)	15/35 (43%)	3.0 ± 0.89
C (Table 3)	53	0.005% dextro(−)	0.4 mAmp for 4 min	5/10 (50%)	11/70 (16%)	1.1 ± 0.41*
D (Table 2)	60	0.005% levo(−)	0.4 mAmp for 4 min	10/10 (100%)	25/70 (36%)	2.5 ± 0.48†
E	47	0.001% dextro(+)	0.8 mAmp for 8 min	1/10 (10%)	1/70 (1.4%)	0.1
F	47	0.001% levo(−)	0.8 mAmp for 8 min	1/10 (10%)	1/70 (1.4%)	0.1

* Significantly different ($P < 0.05$) from group A; significantly different ($P < 0.05$) from group B; significantly different ($P < 0.05$) from group D.

† No significant difference ($P > 0.50$) from group B.

< 0.05 by Student's t-test) difference between the mean durations of shedding caused by 0.005% levo(−) epinephrine (2.5 ± 0.48 days) and 0.005% dextro(+) epinephrine iontophoresis (1.1 ± 0.41 days).

Table 4 is a summary of Tables 1, 2, and 3 with two other groups of rabbits. Groups E (dextro) and F (levo) were used beginning on PI day 47 and received 0.001% epinephrine at 0.8 mAmp for 8 min. After iontophoresis, groups E and F each had only one positive shedding and these results are consistent with spontaneous reactivation.[1] Groups E (dextro) and F (levo) had three episodes of ocular shedding prior to iontophoresis. Groups A, B, and D are statistically different ($P < 0.05$) from groups C, E, and F. Group C is statistically different ($P < 0.01$) from groups E and F.

Discussion. Both dextro(+) and levo(−) epinephrine (0.01%) iontophoresis (0.8 mAmp, 8 min) induced HSV-1 ocular shedding with regard to mean duration of shedding, positive swabs per total swabs, and positive eyes per total eyes. At a lower concentration of levo(−) epinephrine (0.005%), iontophoresis (0.4 mAmp, 4 min) induced ocular shedding significantly ($P < 0.05$) higher than dextro(+) epinephrine (0.005%). At 0.001% epinephrine at 0.8 mAmp for 8 min neither the levo(−) nor the dextro(+) enantiomorphs induced shedding. Previously, systemic (intramuscular injection) administration of levo(−) epinephrine induced 30% viral shedding in latently infected rabbits[1] and topical application of 2% levo(−) epinephrine to the eyes of latently infected rabbits induced 60% viral shedding.[7] The duration of shedding of HSV-1 after systemic or topical levo(−) epinephrine was generally for only 1 day.

Epinephrine iontophoresis to the eyes of latently infected rabbits results in reactivation of HSV-1 in both the trigeminal and superior cervical ganglia (SCG).[3,4] The trigeminal ganglia (TG) is sensory while the SCG is autonomic. The iris contains primarily α-adrenergic receptors, the ciliary body contains primarily β-adrenergic receptors, and the cornea has mostly sensory fibers and some sympathetic fibers. If the cornea is one site of the action of epinephrine for the reactivation of HSV-1, then this is a structure where a sympathetic fiber might influence a sensory fiber. In fact, Laties and Jacobowitz[8] have suggested that the corneal adrenergic fibers could influence the sensory transmission. Therefore, the epinephrine-activated sympathetic nerve fibers may act to stimulate the sensory fibers, reactivating HSV-1 in both the TG and SCG and ultimately leading to HSV-1 shedding into the tear film.

Our data suggest that the mechanism(s) of epinephrine induction of HSV-1 ocular shedding might be related to the receptor affinity of the stereoisomer. Indeed, chemical sympathectomy (using iontophoresis of 6-hydroxydopamine) augmented the effect of the topical application of 2% levo(−) epinephrine, eliciting a 100% frequency of HSV-1 ocular shedding in rabbits for a 4–6 day duration.[7] The present data demonstrate that a very low concentration (0.005%) of levo(−) epinephrine administered by iontophoresis can induce 100% of eyes to shed at an average duration of 2.5 days (Table 2). We suggest that induction of HSV-1 ocular shedding by levo(−) epinephrine is a receptor mediated event and is stereoselective.

Key words: epinephrine, isomers, iontophoresis, HSV-1, rabbit eye, reactivation

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Experimental Granulomatous Uveitis: An Electron Microscopic Study of Pigment Containing Giant Cells

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An experimental granulomatous uveitis in the Brown Norway rat is characterized by large numbers of giant cells and epithelioid cells containing uveal pigment. Ultrastructurally, the epithelioid cells and the giant cells exhibited melanosomes and individual melanin granules in the absence of phagocytic membranes and compound pigment granules. These observations support the view that the pigment containing giant cells may develop from uveal melanocytes. Invest Ophthalmol Vis Sci 26:1303–1305, 1985

Fuchs was sufficiently disturbed by the differences between the pigment-containing epithelioid and giant cells of sympathetic ophthalmia and the typical melanophages observed with tissue necrosis to suggest that the former may not represent phagocytes but rather represent modified uveal melanocytes.[1,2] Ikui redirected attention to this possibility when he described transitional forms between uveal melanocytes and pigment containing epithelioid cells.[3] The so-called epithelioid cells of Dalen-Fuchs nodules have clearly been demonstrated to represent modified retinal pigment epithelial cells.[3–5]

Brown Norway rats sensitized to lens protein develop an extensive granulomatous choroiditis in addition to phacoanaphylactic endophthalmitis after lens injury.[6] There are many pigment containing epithelioid and giant cells in the choroid of these animals. We have been unable to demonstrate any cross reaction between the antilens antisera of these animals with either uveal melanocytes in tissue reaction or the melanosome fraction obtained after centrifugation of disrupted choroidal tissue by the indirect Coombs method. This model appears to be a useful preparation to evaluate the development of pigment-containing epithelioid cells in the choroid in the absence of early detectable immune responses directed against the uveal melanocytes.

The purpose of this report is to examine the pigment-containing epithelioid cells of the choroid for evidence of phagocytosis such as compound pigment granules or phagocytic membrane surrounding melanosomes and alternatively for evidence of melanogenesis that would be expected if these cells are altered uveal melanocytes.

Materials and Methods. Ten male Brown Norway rats were given four subcutaneous injections of fresh saline soluble whole rabbit lens protein at 2-wk intervals. Each injection contained 10 mg lens protein in 0.5 ml saline with 0.5 ml of complete Freund's adjuvant (Difco; Detroit, MI).

The lens of the animals were disrupted with a modified Zeigier knife through a limbal incision. Both eyes were injured 1 wk after the last sensitizing injection. The eyes were removed 7 days after injury, the right eyes were fixed in 4% paraformaldehyde solution and embedded in paraffin. Sections stained with hematoxylin and eosin as well as with Periodic Acid-Schiff reaction and Giemsa were examined. The left eyes were fixed in 2% gluteraldehyde solution and processed for epon embedding. One micron thin sections were prepared for light microscopic examination. The pigment-containing epithelioid cells and giant cells were studied for ultrastructural details with JEOL 101 electron microscope.

These investigations conformed to the ARVO Resolution on the Use of Animals in Research.