New Zealand albino rabbits were inoculated in the right superior cervical ganglion with 25 μl of herpes simplex virus type 1 (HSV-1) (McKrae strain; 10^5 or 10^4 PFU/ml). Positive tear film swabs were detected at least once in 28/32 (88%) of ipsilateral eyes and 6/32 (19%) of contralateral eyes beginning on postinoculation (PI) day 2–6. The average HSV-1 titer in the tear film was 4.0 × 10^3 PFU in ipsilateral eyes and 2.7 × 10^3 PFU in contralateral eyes, determined from eye washes after inoculation of 25 PFU of HSV-1. In selected rabbits, the aqueous humor was positive for virus on PI days 3, 4, 5, 6, and 8. The aqueous humor in ipsilateral eyes showed positive results in 9/11 (82%) of the eyes tapped on PI 3, 13/18 (72%) on PI 4, 5/11 (45%) on PI 5, 1/6 (17%) on PI 6, and 1/2 (50%) on PI 8. No virus was detected in aqueous humor tappings in any contralateral eyes (0/65). Conjunctivitis and iritis (iris hyperemia) appeared in all ipsilateral eyes beginning as early as PI day 1. Conjunctivitis occurred in 1/21 (4.8%) of contralateral eyes. Cells and flare appeared in 18/21 (86%) of ipsilateral eyes and 2/21 (9.5%) of contralateral eyes. Hyphema was noted in 3/21 (14%) of ipsilateral eyes. Of the eyes with iritis, 12/21 (57%) developed corneal edema. Corneal dendritic ulcers were observed in 4/21 (19%) of ipsilateral eyes and 2/21 (9.5%) of contralateral eyes. No ocular fundus changes were seen in any contralateral or ipsilateral eyes. This model will be useful for the study of HSV infection of the autonomic nervous system and HSV iritis produced in an untramatized eye. Invest Ophthalmol Vis Sci 27:1447–1452, 1986

Herpes simplex virus (HSV) acute and latent infections of the superior cervical ganglion (SCG) have been confirmed in experimental animals and humans. Adrenergic agents, which are chemical mediators of the autonomic ganglia, have been reported to have a role in the reactivation of HSV in ocular tissues. However, only one publication has described clinical findings and virus recovery from eyes after direct inoculation to HSV into SCG. Virus recovery from the aqueous humor after ganglionic inoculation has not been reported. This study demonstrates virus recovery from aqueous humor and ocular tear film, and describes clinical findings in rabbit eyes after inoculation of HSV into the right superior cervical ganglion.

Materials and Methods

Virus Strain

McKrae strain HSV-1 was prepared on primary rabbit kidney cell monolayers and titrated on green monkey kidney cell culture (CV-1). HSV-1 titers were diluted to 10^5 and 10^4 PFU/ml.

Animals and Virus Inoculation

New Zealand albino rabbits (2.5–3.0 kg) were inoculated in the right SCG with approximately 25 μl of HSV suspended in Eagle’s minimum essential medium with 7% fetal bovine serum (E-MEM-FBS). The care and maintenance of the rabbits used in these experiments conformed to the ARVO Resolution on the Use of Animals in Research. The surgery was similar to that described by Mintsios et al. All rabbits were anesthetized with a combination of xylazine hydrochloride (4 mg/kg) and ketamine hydrochloride (20 mg/kg). The neck area of the rabbit was shaved, and the skin was washed with 70% ethyl alcohol. The neck skin was cut along the midline and the neck muscles were separated by blunt dissection. The bifurcation of the carotid artery was exposed, and the right SCG was identified. Approximately 25 μl of HSV-1 in E-MEM-FBS was injected...
directly into the right SCG by means of a 30-gauge needle. The same procedure was used to inject control rabbits with the same volume of E-MEM-FBS only. Excess fluid was gently removed with a sterile swab. Subcutaneous tissue and skin were closed separately with 4-0 silk sutures. The procedure resulted in little or no bleeding. An antibiotic (ampicillin sodium) in powder form was placed in the wound area before the skin was closed, for prophylactic purposes. Aseptic technique was used for all procedures.

Clinical Examination

Rabbits were examined by slit-lamp biomicroscopy daily for 14 days after surgery; surviving rabbits were examined at subsequent intervals until the end of the experiment. The posterior pole of the eye was examined by indirect ophthalmoscopy.

Recovery of HSV

HSV shedding into the ocular tear film was determined from eye swabs taken daily for 14 days after inoculation, except as noted below. Eye washes were substituted for swabs on 5 consecutive days (PI 3–7) in three rabbits, to determine HSV titer. Eye swab§ and eye wash§§ procedures have been described.

For aqueous humor sampling, the rabbits were anesthetized as for surgery. Approximately 0.1 ml of aqueous humor was taken from the upper limbal area with a 26-gauge needle. Special care was taken not to harm the corneal endothelium, iris, and lens. The corneal surface was washed with E-MEM (no serum) prior to insertion of the 26-gauge needle. After removal of the aqueous humor, the needle was wiped with sterile gauge. At least 3 days were permitted to elapse before the next aqueous humor tapping, except as described below. No eye swabs were performed the day after aqueous humor tapping.

Identification of Viral Isolates

Randomly selected viral isolates from ocular swabs, eye washes, and aqueous humor taps were identified by a plaque-reduction assay on CV-1 monolayers using HSV-1 specific hyperimmune rabbit antiserum. In all cases, the virus shed was identified as HSV-1.

Experimental Design

Table 1 shows an overview of the experimental design. In all cases, only the right SCG received the 25 μl injection; 56 rabbits received HSV-1 and two rabbits received only E-MEM-FBS. Of the 56 rabbits inoculated with HSV-1, 45 received 10⁵ PFU/ml (2500 PFU) and 11 received 10³ PFU/ml (25 PFU). The two control rabbits that received only E-MEM-FBS survived for more than 3 months with no clinical problems. The mortality rate was 33/45 (73%) for the rabbits receiving 10⁵ PFU/ml and 11/11 (100%) for the rabbits receiving 10³ PFU/ml, by day 21. In the 10⁵ PFU/ml HSV-1 group, 21 rabbits were evaluated daily by slit-lamp biomicroscopy and indirect ophthalmoscopy. Slit-lamp examination was performed on other rabbits as necessary. Ocular tear film swabs and aqueous humor tappings were performed on 32 rabbits (21 received 10⁵ PFU/ml HSV-1 and 11 received 10³ PFU/ml HSV-1). Four of the rabbits receiving 10³ PFU/ml were tapped on 4 consecutive days (PI 4–7). Aqueous humor tapping was performed for 4 consecutive days (PI 3–6) in three additional rabbits inoculated with 10³ PFU/ml of HSV-1.

Aqueous humor tapping was performed on selected days, including PI 2 (one rabbit inoculated with 10⁵ PFU/ml), PI 3 (11 rabbits inoculated with 10³ PFU/ml) PI 4 (11 rabbits inoculated with 10⁵ PFU/ml and seven with 10³ PFU/ml), PI 5 (seven rabbits inoculated with 10² PFU/ml and four with 10⁵ PFU/ml), PI 6 (two rabbits inoculated with 10⁵ PFU/ml and four with 10⁴ PFU/ml), PI 7 five rabbits inoculated with 10⁵ PFU/ml and four with 10⁴ PFU/ml), PI 8 (two rabbits inoculated with 10³ PFU/ml), PI 10 (two rabbits inoculated with 10⁵ PFU/ml and one with 10³ PFU/ml), and PI 14 (two rabbits inoculated with 10³ PFU/ml).

Aqueous humor tapping was performed only once for each rabbit between PI 1 and PI 5, except for the rabbits tapped on consecutive days during this period.
Table 2. Eyes positive for virus demonstrated by ocular tear film specimens after inoculation of HSV into the right superior cervical ganglion

<table>
<thead>
<tr>
<th>HSV Inoculum</th>
<th>Eye</th>
<th>Incidence (Eyes Positive/Total Eyes)</th>
<th>Average Day of First Positive After Inoculation</th>
<th>Average Duration of Positive Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Day) (Range)</td>
<td>(Days) (Range)</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral</td>
<td>10⁵ PFU/ml: 18/21 (85.7%)</td>
<td>3.0 (2-4)</td>
<td>2.4 (1-6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10³ PFU/ml: 3/21 (14.3%)</td>
<td>6.3 (5-7)</td>
<td>2.0 (1-4)</td>
</tr>
<tr>
<td></td>
<td>Contralateral</td>
<td>10⁵ PFU/ml: 10/11 (92.2%)</td>
<td>4.4 (3-6)</td>
<td>2.2 (1-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10³ PFU/ml: 3/11 (33.7%)</td>
<td>3.3 (3-4)</td>
<td>4.3 (1-6)</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral</td>
<td>Total: 28/32 (87.5%)</td>
<td>3.5 (2-6)</td>
<td>2.3 (1-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral: 6/32 (18.8%)</td>
<td>4.8 (3-7)</td>
<td>3.2 (1-6)</td>
</tr>
</tbody>
</table>

**Results**

**Virus Detection**

The ocular tear film for 28 ipsilateral eyes of 32 HSV-1 inoculated rabbits showed positive cytopathic effects at least once (Table 2). The first positive swabs occurred, on the average, on PI 3.5 (range: PI 2-6). The duration of consecutive positive swabs averaged 2.3 days. In the contralateral eyes, six eyes from 32 rabbits showed positive cytopathic effects at least once; the earliest averaged PI 4.8, and duration averaged 3.2 days. There were no significant differences between the positive eye swabs from rabbits inoculated with 10⁵ PFU/ml HSV-1 and those receiving 10³ PFU/ml HSV-1.

Positive cytopathic effects were demonstrated in nine samples of aqueous humor from 11 ipsilateral eyes on PI 3, 13 of 18 eyes on PI 4, 5 of 11 eyes on PI 5, 1 of 6 eyes on PI 6, and 1 of 2 eyes on PI 8 (Table 3). Titers were not determined. Aqueous humor tappings performed for 4 consecutive days in seven rabbits showed positive cytopathic effects for an average of 2 consecutive days (Table 4).

**Clinical Findings**

Twenty-one rabbits inoculated with 10³ PFU/ml HSV-1 were examined by slit-lamp biomicroscope and indirect ophthalmoscope (Table 5). Characteristic manifestations were conjunctivitis and iritis. All rabbits inoculated with HSV-1 showed ipsilateral conjunctival injection and iris hyperemia. Conjunctival injection was found as early as PI day 1 in ipsilateral eyes; the average was 1.4 days PI. Severity reached a maximum, on the average, on PI day 4.1, decreasing thereafter over an average of 10.2 days. One rabbit showed contralateral conjunctival injection on PI day 2. Control eyes, which received only E-MEM-FBS, with no virus, also showed ipsilateral conjunctival injection on PI day 1, but it was markedly less severe than in the inoculated rabbits, and disappeared about PI day 7.

Iritis and iris hyperemia occurred first, on the average, on PI 2.4. Severity increased over the next 2 days, with the development of flare, cells, pupillary membrane formation, precipitates on the corneal endothelial surface and lens surface, posterior synechiae, and/or fibrin-like clots with or without hemorrhage. Cells in the anterior chamber remained for an average of 2.9 days but precipitates were observed for the entire clinical course in seven eyes. All other findings disappeared within 14 days. Three rabbits showed contralateral iris hyperemia and two showed cells in the anterior chamber for a few days. No iritis developed in any of the control eyes (0/4).

Four ipsilateral eyes and two contralateral eyes of the 21 inoculated rabbits showed corneal dendritic ulcers. With the development of iritis, 12 of the eyes

Table 3. Ipsilateral eyes positive for virus demonstrated by aqueous humor tapping on selected days after inoculation of HSV into the superior cervical ganglion

<table>
<thead>
<tr>
<th>Titer of HSV Inoculum</th>
<th>Postinoculation Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>10⁵ PFU/ml</td>
<td>0/1†</td>
</tr>
<tr>
<td>10³ PFU/ml</td>
<td>N.D.</td>
</tr>
<tr>
<td>Total</td>
<td>0/1 (0)</td>
</tr>
</tbody>
</table>

* No positives in contralateral eyes.
† Number positive/total tapped.
N.D.: Aqueous tap not done.
demonstrated stromal edema, which improved when the iritis improved.

In three rabbits that received $10^3$ PFU/ml in the right SCG, eye washes were done for 5 consecutive days (PI days 3–7). The contralateral eyes were positive twice in the same rabbit on PI days 4 and 5; the titers were 0.8 and $4.5 \times 10^3$ PFU/eye. All ipsilateral eyes were positive at least once in all three rabbits on PI days 3, 4, 5, and 6. The average PFU was $4 \times 10^3$ in the ipsilateral eyes. The highest titer was $1.7 \times 10^4$ and the lowest was 5 PFU/eye.

**Discussion**

In this study, 28/32 (88%) of the ipsilateral eyes and 9/11 (82%) of the aqueous humor taps from the ipsilateral eyes on PI 3 showed positive cytopathic effects for HSV. Additionally, all ipsilateral eyes inoculated with HSV-1 showed severe conjunctival infection and iris hyperemia, resulting from the invasion of HSV. Similarly, Mintsioulis et al.\textsuperscript{15} reported clinical findings with HSV directly into the SCG. The establishment of latency in the SCG after HSV inoculation into the anterior chamber or onto the cornea has been rarely reported in cases of iritis in humans\textsuperscript{21–23} and rarely in animal models, even when HSV was inoculated directly into the anterior chamber.\textsuperscript{20,23,24} To our knowledge, our study is the first report of HSV recovery from the aqueous humor of rabbits inoculated with HSV directly into the SCG. The establishment of latency in the SCG after HSV inoculation into the anterior chamber or onto the cornea has been reported.\textsuperscript{1–4} Furthermore, adrenergic agents, chemical mediators of the autonomic nervous system, have been shown to induce reactivation of latent HSV.\textsuperscript{6–14} All of these findings suggest that the SCG is a reservoir for latent HSV infection.

Almost all of the clinical findings seen in this study occurred on the ipsilateral side, and could be attributed to direct ocular invasion of the virus from the SCG. The observed iritis was similar to that seen when HSV is inoculated directly into the anterior chamber.\textsuperscript{20} It was not clear if the iritis in the two contralateral eyes was a result of direct HSV invasion from the SCG or a by-product of some type of immune reaction.\textsuperscript{24–29}

### Table 4. Ipsilateral eyes positive for virus demonstrated by aqueous humor tapping for 4 consecutive days after inoculation of HSV into the superior cervical ganglion*

<table>
<thead>
<tr>
<th>Titer of HSV Inoculum</th>
<th>Postinoculation Day</th>
<th>Average Duration (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>$10^3$ PFU/ml</td>
<td>2/3 (67%)</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>$10^2$ PFU/ml</td>
<td>N.D.</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>2/3 (67%)</td>
<td>7/7 (100%)</td>
</tr>
</tbody>
</table>

* No positives in contralateral eyes.
† Number positive/total tapped.
†† One rabbit died after aqueous humor tap on postinoculation day 5. N.D.: aqueous tap not done.
Conjunctival injection found as early as PI day 1 in ipsilateral eyes may have been caused by direct HSV invasion or by autonomic nerve stimulation during HSV inoculation. In the controls, in which only E-MEM-FBS was injected into the SCG, moderate conjunctival injection was found on PI day 1, but disappeared by PI day 7. In the HSV-inoculated rabbits, the severity increased for about 10 days, after which the condition diminished by 14 days. The increased severity and positive ocular tear film swabs in most of the sampled rabbits suggest that the conjunctivitis was induced by the virus from the ganglion.

Corneal dendritic lesions were observed in 4/21 (19%) of ipsilateral eyes and in 2/21 (9.5%) of contralateral eyes. The reason for the low frequency of corneal dendritic lesions is not clear. The average HSV titer in the eye washes was $4.0 \times 10^3$ PFU/ml in rabbits receiving $10^3$ PFU/ml. However, it has been reported\textsuperscript{28} that a relatively large amount of viral inoculum is necessary to produce dendritic keratitis. Also, the conjunctiva may provide an immuno-defense mechanism against ocular infection,\textsuperscript{28,29} and, therefore, the pathogenic activity of HSV may be reduced in this system.

In these experiments, the mortality rate at PI day 21 was 100% (11/11) for those rabbits receiving 25 PFU and 73% (33/45) for those receiving 2500 PFU. The difference was not statistically significant ($P > 0.05$ by chi square analysis). This mortality rate renders long-term studies impractical. Future experiments will involve low viral titer and perhaps a less virulent strain of HSV. Mintsioulis et al\textsuperscript{15} inoculated HSV into the rabbit SCG and found a high mortality rate with no clinical signs except encephalitis. We did not detect HSV from the tear film or aqueous humor after PI day 8. No ocular pathology was observed after PI day 14 except for mild conjunctival injection. No fundus changes were observed in any eyes. Similar observations have been reported by Mintsioulis et al.\textsuperscript{15}

The animal model described in this study may be useful in studies of HSV infection. The advantages of this model are similar to those cited by Lubin et al\textsuperscript{30}: (1) anterior uveitis is produced consistently after inoculation of HSV at a distant site; (2) ocular structures are left undisturbed by the inoculation procedure and mild uveitis from the procedure alone is, therefore, avoided; (3) it is not necessary to use intravenous or intracameral injection, so that the immune system should not be as immediately overwhelmed by presentation of an antigen overload to the spleen; and (4) the inflammatory reaction seems to be specific to ocular tissue.

**Key words:** aqueous humor, clinical course, HSV-1, superior cervical ganglion, tears

### References

from the superior cervical and vagus ganglions of human beings. 
6. Laibson PR and Kibrick S: Reactivation of herpetic keratitis by 
7. Laibson PR and Kibrick S: Reactivation of herpetic keratitis in 
rabbit. II. Repeated reactivations in the same host. Arch 
8. Schmidt JR and Rasmussen AF: Activation of latent herpes sim-
9. Kwon BS, Gangarosa LP, Burch KD, deBack J, and Hill JM: 
Induction of ocular herpes simplex virus shedding by iontopho-
resis of epinephrine into rabbit cornea. Invest Ophthalmol Vis 
10. Kwon BS, Gangarosa LP, Green K, and Hill JM: Kinetics of 
ocular herpes simplex virus shedding induced by epinephrine 
11. Hill JM, Kwon BS, Shimomura Y, Colborn GL, Yaghmai F, 
and Gangarosa LP: Herpes simplex virus recovery in neural tis-
sues after ocular HSV shedding induced by epinephrine ionto-
phoresis to the rabbit cornea. Invest Ophthalmol Vis Sci 24:243, 
1983.
12. Shimomura Y, Gangarosa LP Sr, Kataoka M, and Hill JM: HSV-
1 shedding by iontophoresis of 6-hydroxydopamine followed by 
quantitation from rabbit neural tissues after epinephrine-induced 
14. Hill JM, Shimomura Y, Kwon BS, and Gangarosa LP: Ionto-
phoresis of epinephrine isomers to rabbit eyes induced HSV-1 
15. Minsoulis G, Dawson CR, Oh JO, and Briones O: Herpetic eye 
disease in rabbits after inoculation of autonomic ganglia. Arch 
virus in the central nervous system of rabbits and mice. J Exp 
17. Ehinger B: Ocular and orbital vegetative nerves. Acta Physiol 
18. Kaufman HE, Brown DC, and Ellison ED: Herpes virus in the 
lacrimal gland, conjunctiva and cornea of man—a chronic in-
encephalitis in mice after ophthalmic inoculation. J Infect Dis 
130:16, 1974.
20. Whittum JA, McCalley JP, Niederkorn JY, and Streilein JW: 
Ocular disease induced in mice by anterior chamber inoculation 
associated with viral disease. Trans Am Ophthalmol Soc 55:333, 
1957.
22. Pavan-Langston D and Brockhurst RJ: Herpes simplex panu-
23. Sundmacher R and Neumann-Haellein D: Herpes simplex virus-
positive and -negative keratouveitis. In Immunology and Im-
munopathology of the Eye, Silverstein AM and O'Connor GR, 
24. Kimura SJ: Herpes simplex uveitis: A clinical and experimental 
26. Darougar S, Treharne JD, and Monnickendam MA: Herpes 
simplex virus infections of the eye. In Recent Advances in Clinical 
Virology, Waterson AP, editor. Edinburg, Churchill Livingstone, 
27. Narang HK and Codd AA: Progression of herpes encephalitis 
in rabbits following the intra-ocular injection of type I virus. 
28. Easterbrook M, Wilkie J, Coleman V, and Dawson CR: The 
effect of topical corticosteroids on the susceptibility of immune 
animals to reinoculation with herpes simplex. Invest Ophthalmol 
29. Chandler JW and Gillette TE: Immunologic defense mechanisms 
30. Lubin JR, Albert DM, Essex M, Noronha F, and Riis R: Ex-
perimental anterior uveitis after subcutaneous injection of feline 