The unscarified corneas of rabbits were inoculated with 50 μl of 2–4 × 10^6 PFU/ml of herpes simplex virus, type 1 (HSV-1), McKrae strain in 10 separate experiments over a 12-month period. Sixty of 104 (57.7%) rabbits survived to postinoculation (PI) day 20. These sixty rabbits were swabbed with dacron-tipped swabs for twenty consecutive days (PI days 20–39). The tear film collected on the swabs was immediately placed in tissue culture tubes with confluent primary rabbit kidney (RK) cell monolayers. The RK monolayers were monitored for cytopathic effects indicative of HSV-1. Fifty-eight of the sixty rabbits (96.7%) inoculated had at least one positive episode. Ninety-three of the 120 eyes (77.5%) of the latently infected rabbits had at least one positive episode. Virus was detected in 72 of the 93 positive eyes (77.4%) between PI days 20 and 29 and in 21 of the 93 positive eyes (22.5%) between PI days 31–39. A total of 2400 swabs were taken and 324 were positive (13.5%). All of the 58 positive rabbits were used later for ocular induction of HSV-1 and all 116 eyes of the latently infected rabbits shed virus for at least four consecutive days during induction. Invest Ophthalmol Vis Sci 26:587–590, 1985

The history of spontaneous HSV-1 shedding in the tear film of latently infected rabbits is a very important aspect of ocular studies of reactivation. Spontaneous HSV-1 ocular shedding must be documented before reliable data concerning induction, reactivation, and inhibition can be accumulated. Kwon et al.1 have shown that the longer the postinoculation (PI) time the fewer the detectable spontaneous sheddings that will occur. Kwon et al.1 used a broad time frame (PI days 40–220) and 10 rabbits. In this report, a very narrow PI range (PI days 20–39) and 60 rabbits are used. Shimomura et al.2 reported the importance of spontaneous HSV-1 ocular shedding in relation to induction of HSV-1 ocular shedding.

A complete, detailed history of spontaneous shedding is important for planning experiments and evaluating results from induced reactivations. If the history is not available, one possibility is that spontaneous shedding may be confused with induced shedding. Another possibility is that if there is no induction following some specific procedure, then without a spontaneous history, there is no proof that the rabbit has the ability to shed virus in the tear film. Spontaneous shedding proves that both HSV-1 latency and reactivation are operable. The following details the spontaneous ocular shedding of herpes simplex virus McKrae strain in rabbit eyes between PI days 20–39 in rabbits used over a 12-mo period in 10 separate experiments.

Materials and Methods. Virus inoculation: Rabbit (2–3 kg) eyes were inoculated with HSV-1, McKrae strain (2–4 × 10^6 PFU/ml). These investigations using rabbits conformed to the ARVO Resolution on the Use of Animals in Research. The virus was grown on rabbit kidney (RK) cells and titrated on African green monkey kidney cells (CV-1). The harvested virus was frozen at −80°C in 3-mI aliquots. A 50 μl suspension of HSV-1 was placed in the lower cul-de-sac of each rabbit eye and the closed eyelid was gently massaged against the unscarified cornea for 1 min. This inoculation resulted in an acute HSV-1 infection which was verified by slit-lamp biomicroscopy. A single 3-mI aliquot was used for each experiment and each of the 104 rabbits were inoculated from the same pooled stock of virus.

Determination of viral shedding: Starting on PI day 20, eye swabs were taken from rabbits every day for twenty consecutive days with a sterile dacron-tipped swab gently rotated in the upper cul-de-sac, across the cornea, and then into the lower cul-de-sac where the swab was allowed to absorb tear film in the fornix for 5 sec. The swabs were then immediately placed in tissue culture tubes with confluent RK monolayers.
and incubated for 24 hr at 37°C in a CO₂ incubator. Subsequently, the swabs were gently squeezed against the side of the tubes to remove excess media. Then, 1 ml of Eagle's Minimal Essential Media (E-MEM) with 7% fetal calf serum (FCS) was added for nutrition and pH adjustment. The tubes were then monitored daily for 14 days for the appearance of cytopathic effects indicative of HSV-1.

Identification of viral isolates: Isolates from ocular swab cultures were identified by a plaque-reduction assay on CV-1 monolayers using HSV-1 specific rabbit antiserum. This procedure is essentially the same as that described by Knotts et al.³ In all cases, the virus shed was identified as HSV-1, McKrae.

Results. One hundred-four rabbits were inoculated with HSV-1, McKrae strain over a 12-month period. The rabbits were swabbed every day for 20 days (PI days 20–39) to determine if the tear film collected would elicit cytopathic effects on RK monolayers indicative of HSV-1. One hundred-four rabbits were inoculated and 60 survived to PI day 20 (60/104, 57.7% survival rate). The range of survivors in the 10 groups was 20% (2/10) to 100% (10/10) (Table 1).

The data obtained from the ocular swabs can be divided into several categories. The first category is the number of positive rabbits. A positive rabbit is defined as one which had at least one positive episode of shedding during the 20 days of swabbing. There were 58 of 60 positive rabbits (96.7%) (Table 1). The range of positive rabbits in the 10 groups was 83.3% (5/6) to 100% (10/10). The next category is the number of positive eyes compared to total eyes. Ninety-three of 120 eyes (77.5%) had at least one positive episode during the swabbing period (Table 1). The range of positive eyes was 66.7% to 90%. All 58 rabbits were used in induction experiments and exhibited induced ocular shedding for at least 4 consecutive days on PI days from 70–180 days (data not shown). The induction procedure was that reported by Shimomura et al.,² which involves iontophoresis of 6-hydroxydopamine followed by topical 2% epinephrine. The final category is total positive swabs compared to the total swabs performed (324/2400, 13.5%) (Table 1). The range for the 10 groups was 6.5% to 27%.

Figure 1 shows that 32.7% (106/324) of all episodes were of 1-day duration. Figure 1 also shows that 73% (237/324) of all episodes were from 1–4 days consecutive shedding. Only 27% (87/324) of all episodes were greater than 4 days. Another category is the data which shows the PI day of the first positive swab obtained from each positive eye. Figure 2 shows that 77.4% (72/93) of the eyes were first positive between

### Table 1. Summary of data on spontaneous ocular shedding of HSV-1 strain McKrae

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Survived/ inoculated</th>
<th>Positive rabbits/total rabbits</th>
<th>Positive eyes/ total eyes</th>
<th>Total positive swabs/total swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7/10 (70)*</td>
<td>6/7 (85.7)</td>
<td>11/14 (78.6)</td>
<td>46/280 (16.4)</td>
</tr>
<tr>
<td>II</td>
<td>5/8 (62.5)</td>
<td>5/5 (100)</td>
<td>8/10 (80)</td>
<td>26/200 (13)</td>
</tr>
<tr>
<td>III</td>
<td>2/10 (20)</td>
<td>2/2 (100)</td>
<td>3/4 (75)</td>
<td>10/80 (12.5)</td>
</tr>
<tr>
<td>IV</td>
<td>5/10 (50)</td>
<td>5/5 (100)</td>
<td>9/10 (90)</td>
<td>54/200 (27)</td>
</tr>
<tr>
<td>V</td>
<td>5/10 (50)</td>
<td>5/5 (100)</td>
<td>8/10 (80)</td>
<td>13/200 (6.5)</td>
</tr>
<tr>
<td>VI</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
<td>16/20 (80)</td>
<td>54/400 (13.5)</td>
</tr>
<tr>
<td>VII</td>
<td>6/10 (60)</td>
<td>6/6 (100)</td>
<td>9/12 (75)</td>
<td>25/240 (10.4)</td>
</tr>
<tr>
<td>VIII</td>
<td>6/10 (60)</td>
<td>6/6 (100)</td>
<td>8/12 (66.7)</td>
<td>17/240 (7.1)</td>
</tr>
<tr>
<td>IX</td>
<td>6/14 (42.8)</td>
<td>5/6 (83.3)</td>
<td>8/12 (66.7)</td>
<td>39/240 (16.3)</td>
</tr>
<tr>
<td>X</td>
<td>8/12 (66.6)</td>
<td>8/8 (100)</td>
<td>13/16 (81.3)</td>
<td>40/320 (12.5)</td>
</tr>
<tr>
<td>Averages</td>
<td>60/104 (57.7)</td>
<td>58/60 (96.7)</td>
<td>93/120 (77.5)</td>
<td>324/2400 (13.5)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percentages.
A summary of individual experimental groups. Included are averages and ranges for each category of data.

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Length of Consecutive Shedding (days)

**Fig. 1.** Spontaneous ocular shedding of HSV-1 in the rabbit: episodes versus length of consecutive shedding (days). This depicts the frequency of the length of consecutive daily spontaneous ocular sheddings of HSV-1 by episodes. An episode is defined as an isolated series of positive eye swabs obtained on one or more consecutive days.
PI days 20–29. Figure 2 shows that 19% (18/93) were first positive on PI day 22, while 8.6% (8/93) were first positive on PI day 25. Every PI day had at least one first positive swab except PI day 30 (Fig. 2). Figure 3 shows the total number of eyes positive per PI day. The frequency of recovery of virus in the tear film varied with PI day (Fig. 3). The range of total eyes positive per day per total eyes positive for the 10 groups per PI day (20–39) is 1.5% (5/324) to 8.3% (27/324).

**Discussion.** Laibson and Kibrick\(^4\) reported the spontaneous ocular shedding (PI days 31–1131) of HSV-1, Rodanus strain. Their data has similarities to our data and that of Kwon et al.\(^1\) These similarities are as follows: (1) 50.8% (57/112) of their rabbit eyes shed for one day duration,\(^4\) while 32.7% (106/324) of our rabbits shed for only one day, and (2) 72.3% (81/112) of all detected viral shedding was from 1–4 days duration,\(^4\) while 73% (237/324) of our rabbits were detected as having positive cultures for 1–4 days duration. Their data reinforces the observation that the higher the PI day the fewer detectable spontaneous positive cultures that can be obtained. Laibson and Kibrick\(^4\) reported that 77% (31/40) of their positive eyes were between PI day 31 and PI day 100. On PI days 401 through 500 only 38% (9/24) positive eyes were found and on PI days 1001 through 1131 no positives were reported.\(^4\) Kwon et al\(^1\) reported that 80% (16/20) of their rabbits shed virus between PI day 40 and PI day 80 and that this frequency decreased to 25% (5/20) during the period between PI days 181 and 220. Kwon et al\(^1\) and the present data was obtained using HSV-1, McKrae strain while Laibson and Kibrick\(^4\) used HSV-1, Rodanus strain.

Gerdes and Smith\(^5\) used HSV-1, McKrae strain, as well as various other HSV-1 and HSV-2 strains. With HSV-1, McKrae strain, 85% (28/33) of their rabbits were positive, while 96.7% (58/60) of our rabbits were positive (Table 1). The tear film samples taken by Gerdes and Smith\(^5\) showed a 5.3% (126/2382) frequency (total positive/total swabs) for the McKrae strain, compared to our tear film samples which showed an average of 13.5% (324/2400) frequency (total positives/total swabs) (Table 1). The HSV-1, Syn +17 strain is similar to HSV-1, McKrae in that 80% (8/10) of the rabbits were detected as positive and 24% (24/1014) of the total tear film samples were detected as being positive.\(^5\) HSV-1, MacIntyre; HSV-2, LJ 359; and HSV-2, HG 52 all showed no detectable virus in tear film.\(^5\) The HSV-2, 186 strain showed 3% (1/31) positive animals and 0.15% (1/660) positive tear film samples per total tear film samples.\(^5\)

Green et al\(^6\) have reported that the frequency of spontaneous positive samples per total samples remains constant from PI days 20–300 and that the range of positive cultures per total cultures is 5–8%.\(^6\) In contrast, Kwon et al\(^1\) and Laibson and Kibrick\(^4\) have reported a decrease in frequency of positive samples per total samples with increasing PI days.

Nesburn et al\(^7\) has correlated clinical slit-lamp biomicroscopy findings with positive viral samples, observed spontaneous dendritic lesions on the corneal epithelium, and then cultured a positive sample from that eye. They accumulated data from PI days 21–179 and reported data for positive rabbits per total rabbits, but no data is given for total positive swabs per total swabs.\(^7\) There were 5% (1/20) positive animals on PI days 21–80; 60% (12/20) on PI days 82–122; and 25% (5/20) on PI days 122–179. This implies that the higher the PI day the fewer detectable spontaneous positive cultures that can be obtained.
represents a 65% (13/20) positive rabbit per total rabbit frequency. 

Spontaneous shedding data often allows conclusions to be made about latency and reactivation data. Spontaneous ocular shedding is proof of viral latency. For example, a virus strain that has a low frequency of spontaneous shedding may also prove to have low inducibility, ie, it cannot be reactivated. Rabbits with only one eye positive during the swabbing for spontaneous viral shedding could be induced to shed in both eyes for at least 4 consecutive days using iontophoresis of 6-hydroxydopamine followed by topical 2% epinephrine (data not shown).

We used HSV-1, McKrae strain in our spontaneous experimentation. This strain gave us 100% ocular infectivity and an average of 57.7% survival (Table 1). However, the range of survival was 20–100%. The only explanation of this broad range is that with biological systems broad ranges and great variance is unavoidable. The rabbits were purchased from the same breeder and the variance of body weight from group to group was negligible throughout the year.

Our experiments were performed in a chronological order (Groups I through X). These experiments were begun January 7, 1983 (Group I) and completed on December 28, 1983 (Group X). A new group was started every 3–6 weeks. The number of rabbits in groups I through X ranged from eight to 14. These experiments were designed to contain at least 100 rabbits spaced over a 12-month period. There was no selection or removal of any rabbit once they were placed in a group and inoculated with HSV-1. Group IV, which had the highest shedding, was inoculated April 4, 1983 and swabbed from April 24 through May 14. Group III had the lowest survival rate (20%), and had 12.5% total positive swabs per total swabs. Groups IV and V showed an identical survival rate (50%), but showed the most diversity in the total positive swabs per total swabs. Group V had the lowest total positive swabs per total swabs (6.5%) while Group IV had the highest total positive swabs per total swabs (27%). The 27% total positive swabs per total swabs for Group IV was statistically significant (P < 0.05) by the Chi square analysis from the total average of 13.5% (324/2400). Analysis of all the other parameters in Table 1 showed no statistically significant differences.

The data in this paper (Fig. 2) shows that there is spontaneous shedding on PI day 39. If an induction experiment is started on PI week 4 or 5, one may confuse spontaneous shedding with induced shedding. We believe that the appropriate time for induction or inhibition studies is after a spontaneous ocular shedding history has been established. We compile this history from PI days 20–39. Therefore, we could begin experiments as early as PI week 7 (PI day 49).

In conclusion, this is a complete and detailed spontaneous ocular shedding history of HSV-1, McKrae strain in New Zealand white rabbits for PI days 20–39. These data demonstrate the importance of obtaining a spontaneous ocular shedding history before other experiments related to ocular HSV-1 shedding, latency, and induced reactivation are performed.

Key words: spontaneous shedding, HSV-1, rabbit eye, tear film

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