MULTIPLICATION OF CERTAIN NEUROTROPIC VIRUSES IN THE RABBIT EYE FOLLOWING INTRAOCULAR INOCULATION

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A number of viruses have been studied following inoculation into the eyes of experimental animals. In general, it has been found that the intraocular inoculation of certain viruses is effective in initiating infection of the central nervous system in susceptible hosts, with or without the production of specific reactions in the ocular tissues.

Thus, the viruses of poliomyelitis (1, 2), rabies (3), vesicular stomatitis (4), fox encephalitis (5), and equine encephalomyelitis (6) produce infection of the central nervous system in susceptible hosts following intraocular inoculation. Corneal edema and opacity are produced when the viruses of influenza (7), fox encephalitis (8), and equine encephalomyelitis (9) are introduced into the anterior chamber of the rabbit eye. These reactions are specifically inhibited by antiserum. It has been shown that influenza virus does not multiply in the rabbit eye and the reaction has been attributed to toxic properties of the virus (7). Similarly, the corneal opacity produced in rabbits by the viruses of Eastern and Western equine encephalomyelitis is not the result of growth of virus in the eye and large amounts must be injected to produce the reaction (9). Herpes simplex virus may be passed serially on the scarified cornea of rabbits, each passage producing a fatal encephalitis (10). Histological sections indicated, however, that the virus did not enter the eye after inoculation onto the cornea, but passed to the conjunctiva and thence to the brain via the sensory branches of the fifth cranial nerve. Herpes simplex virus inoculated into the vitreous cavity produces edema and separation of the retina with inclusion bodies in the retinal cells (10). Inclusion bodies have also been observed in the corneal endothelium of the eyes of foxes following injection of fox encephalitis virus into the anterior chamber (8).

These studies have not provided definitive evidence that the eye per se is capable of supporting virus multiplication. The presence of inclusion bodies, although suggestive, cannot be considered as conclusive evidence of virus multiplication (11). Evans and Bolin suggested that limited growth of equine encephalomyelitis virus may occur in the rabbit eye after inoculation of large amounts of a highly virulent strain into the anterior chamber (9). However, the possibility that the virus recovered from the eye represented residual virus from the large inoculum could not be ruled out in their experiments.

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The present paper reports the results obtained in a quantitative study of three neurotropic viruses following inoculation into the rabbit eye. Two of the viruses, lymphocytic choriomeningitis virus and Semliki Forest virus, were found to multiply readily in the eye. No evidence of multiplication of the Lansing strain of poliomyelitis virus in the rabbit eye was obtained in these experiments.

**Materials and Methods**

**Viruses.**—Semliki Forest virus (SFV) was originally obtained from Dr. K. C. Smithburn in 1945 as the 102nd mouse brain passage. SFV is a neurotropic virus lethal for mice by various routes of inoculation, and for guinea pigs, rabbits, rhesus monkeys by intracerebral inoculation (12). It has been maintained by intracerebral passage in mice without detectable change in pathogenicity. Virus suspensions were prepared by inoculating 0.04 ml. of a $10^{-8}$ dilution of SFV mouse brain extract intracerebrally into a group of mice. After 2 days the mice were killed with ether and 20 per cent extracts of the brains were prepared by grinding in alundum and suspending in 10 per cent tryptose phosphate broth-saline. The suspensions were cleared by centrifugation at 3000 R.P.M. for 15 minutes.

The WE strain of lymphocytic choriomeningitis virus (LCM) was obtained from Dr. T. F. McNair Scott. A 10 per cent mouse brain extract was inoculated intracerebrally into mice and after 5 days the brains were removed and extracted as described above.

The Lansing strain of poliomyelitis virus was obtained from Dr. D. Bodian and maintained in this laboratory by cotton rat passage. Twenty per cent saline extracts of a 7th cotton rat passage were used in these experiments.

All virus suspensions were stored in a dry ice cabinet.

**Technique of Intracocular Inoculation.**—Domestic albino rabbits 4 to 8 months of age were used. Inoculations were performed under light ether anesthesia while the eye was held with fine-toothed conjunctival forceps. Anterior chamber inoculations were made by passing a ½ inch 27 gauge needle through the cornea near the sclera. After aspirating approximately 0.04 ml. of aqueous humor, 0.04 ml. of virus suspension was injected. For inoculation into the vitreous, the needle was inserted into the sclera several millimeters behind the corneal-scleral junction, directed posteriorly, and 0.04 ml. of the virus suspension was injected into the vitreous.

**Virus Titrations.**—At varying intervals following intraocular inoculation of virus the rabbits were killed by intravenous air injection. The skin surrounding the eye was wetted with alcohol and iodine and cut away. The eye was then removed aseptically, with care not to penetrate the chambers. Any connective tissue and muscle adhering to the eyeball were removed. The eye was then opened in a mortar and the eye fluids and tissues ground with alundum. The sclera resisted grinding and was discarded; the remaining material usually weighed about 2 gm. This was suspended in 10 per cent tryptose phosphate broth-saline to a final concentration of 10 per cent by weight. The extract was cleared by centrifugation at 3000 R.P.M. for 10 minutes and the sediment was discarded.

The brains and optic nerves of most of the rabbits were also obtained and extracts prepared as described for the eyes. Penicillin, in a final concentration of 200 units per mL., and streptomycin, in a final concentration of 200 micrograms per mL., were added to all extracts. Blood agar and thioglycollate cultures of the eye extracts were taken at the time of sacrifice. No bacterial growth has been encountered in these experiments.

Serial tenfold dilutions of the rabbit tissue extracts and the original virus suspensions were made in broth-saline and inoculated intracerebrally into groups of four to six mice. The virus titers were calculated according to the method of Reed and Muench (13) and expressed as the logarithm of the LD$_{50}$ per 0.04 gm. of tissue extracted.
Neutralization Tests.—In some instances serum and eye extracts were tested for virus-neutralizing substances. Serial tenfold dilutions of the virus were mixed in equal volume with either a 1:10 dilution of serum or eye extract to be tested. After incubating 30 minutes at 37°C. the mixtures were inoculated intracerebrally into mice. For control the virus dilutions were mixed with normal rabbit serum or an extract of a normal rabbit eye (diluted 1:10). The neutralization index was then determined by comparing the LD₉₀ titers of the control and experimental mixtures.

EXPERIMENTAL

Semliki Forest Virus

Quantitative Study of SFV Multiplication in the Rabbit Eye

It was necessary to determine at the beginning whether virus which could be recovered from the eye represented multiplication of virus or merely residual virus from the original inoculum. For this purpose varying amounts of SFV were inoculated into the eyes of a group of rabbits. Six days later the virus content of the eyes was determined.

Serial tenfold dilutions of SFV mouse brain extract, from 10⁻⁰.⁵ through 10⁻⁷.⁵ inclusive, were inoculated into the vitreous of one eye of a group of six rabbits, each rabbit receiving a separate dilution. The LD₉₀ titer of the SFV extract was 10⁻⁹.⁰ on intracerebral inoculation in mice. The rabbits were sacrificed after 6 days, the inoculated eyes removed, and extracted as described under Methods. The LD₉₀ titer of the eye extracts was then determined in mice.

The results are summarized in Table I. The expected yield of virus is that amount which would have been recovered if no loss or multiplication of virus had occurred in the eye. The inoculation of a volume of 0.04 ml. into the rabbit eye weighing on the average 2.0 gm., excluding the sclera, resulted in an initial 50-fold dilution of virus. Therefore, the expected LD₉₀ of virus per 0.04 gm. of eye tissue would be the inoculum log LD₉₀-log 1.7. It will be seen that a

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>Yield of virus from eye 6 days after inoculation</th>
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<tbody>
<tr>
<td>2.0</td>
<td>0.3 &lt;1.0</td>
</tr>
<tr>
<td>3.0</td>
<td>1.3 4.3</td>
</tr>
<tr>
<td>4.0</td>
<td>2.3 4.7</td>
</tr>
<tr>
<td>5.0</td>
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<tr>
<td>6.0</td>
<td>4.3 3.5</td>
</tr>
<tr>
<td>7.0</td>
<td>5.3 3.3</td>
</tr>
</tbody>
</table>

All figures given as log LD₉₀.

* 0.04 ml. of SFV mouse brain extract inoculated into the vitreous.

† The amount of virus which could be expected from the original inoculum; i.e., if no loss or multiplication of virus had occurred in the eye. Calculation based on dilution of the inoculum in the eye (see text).
1000-fold increase in virus occurred following the inoculation of log 3.0 \( LD_{50} \). Multiplication of virus is also apparent after inoculation of log 4.0 and 5.0 \( LD_{50} \), although the difference between the expected and actual yields of virus becomes progressively less.

The data indicate that the minimum amount of SFV required to initiate multiplication in the eye is between 100 and 1000 \( LD_{50} \). The actual yield of virus remains remarkably constant even though the amount of virus inoculated into the eye varies from 1000 to 100,000 \( LD_{50} \). It will be noted, however, that the inoculation of very large amounts of virus (log 6.0 and 7.0 \( LD_{50} \)) resulted in a decreased yield of virus from the eye. Under the latter conditions it is not possible, by comparing the expected and actual yields of virus, to state whether multiplication of virus occurred. If the virus recovered were merely residual seed virus, it would be expected that the actual yield of virus would increase in proportion to the amount of virus inoculated into the eye. This was clearly not the case; less virus was obtained from the eye following the larger inocula than expected on the basis of residual seed virus.

The eye of the rabbit inoculated with the \( 10^{-2.0} \) dilution of SFV mouse brain extract developed an iritis during the first 48 hours after inoculation, which subsided in the next 24 hours. The cornea, however, became opaque. This reaction was not noted in the eyes inoculated with the greater dilutions of the SFV extract. Blood agar and thioglycolate broth cultures of the eyes at time of sacrifice were sterile. In other experiments normal mouse brain extract failed to elicit the corneal reaction.

**Distribution of Virus in Various Parts of the Eye**

The following experiment was designed to determine the distribution of the virus in the rabbit eye following inoculation of SFV.

A 10 per cent SFV mouse brain extract (titer in mice = \( 10^{-9.0} \)) was inoculated into the vitreous of both eyes of a rabbit. Two days later the animal was sacrificed. One eye was removed and extracted in broth, as previously described, to provide the "whole eye" extract. The aqueous humor of the other eye was aspirated and serial tenfold dilutions made by volume. The eye was then opened and the vitreous removed. The retina and choroid were scraped with a sharp scalpel. The vitreous and retinal scrapings were then extracted by weight. All the extracts were then tested intracerebrally in mice for virus content.

The virus titers of the various materials were as follows: "whole eye" extract = \( 10^{-4.7} \), vitreous = \( 10^{-4.8} \), retinal extract = \( 10^{-4.7} \), and aqueous humor = \( 10^{-1.5} \). It is thus apparent that the virus is widely distributed in the various structures of the eye tested, with the least amount of virus recovered from the aqueous humor (also see Table II). The iritis described in the preceding experiment was present in both eyes of this rabbit 24 to 48 hours after inoculation.
Recovery of SFV at Varying Intervals after Inoculation into the Eye

(a) Inoculation into the Anterior Chamber.—In the following experiment SFV was inoculated into the anterior chamber of rabbit eyes. At varying times thereafter a rabbit was sacrificed, the eyes were removed, extracted, and tested for virus by intracerebral inoculation of the extracts into mice. The titers of the virus recovered were then charted according to time after inoculation and curves drawn to indicate the trend of multiplication or persistence of virus.

A 10 per cent SFV mouse brain extract in 0.04 ml. volume was inoculated into the anterior chamber of both eyes of a group of eight rabbits. The LD₅₀ titer of the original inoculum was $10^{-1.4}$ in mice. Tests for virus in the eyes were made at intervals from 2 to 12 days after inoculation. The aqueous humor was aspirated from both eyes and pooled. Usually 0.2 to 0.4 ml. was obtained. The eyes were then removed and extracted in broth and the extracts from the two eyes pooled. The brain of each rabbit was also removed and extracted. Serial tenfold dilutions of the aqueous humor and of the eye and brain extracts were then titered intracerebrally in mice.

The results of the virus titrations are summarized in Table II. Although virus was inoculated into the anterior chamber, recovery of virus from the aqueous humor was inconstant and in relatively low titer. Extracts of the eyes, however, yielded virus regularly and in higher titer during the first 6 days. After 8 to 12 days, no virus could be detected in the eye. Extracts of the brains of these rabbits contained no virus (not shown in the table). Tests for virus-neutralizing substance in the eye extracts obtained 8, 10, and 12 days after inoculation were negative.

<table>
<thead>
<tr>
<th>Time after inoculation *</th>
<th>Yield of virus† Aqueous humor</th>
<th>Eye extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
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</tr>
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<td>2</td>
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<td>10</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>12</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

* 0.04 ml. of a 10 per cent SFV mouse brain extract inoculated into anterior chamber
† Figures given as log LD₅₀ of virus.
During the first 48 hours after inoculation the eyes showed a moderate to severe iritis with a clouding of the cornea. After 72 hours the iritis subsided but the corneal reaction progressed, in some instances to complete opacities. A rise in the rectal temperature from a normal of less than 103.6°F. to 105-106°F. was noted during the first 2 to 3 days after inoculation. The temperature gradually returned to normal during the next 2 to 3 days. The animals showed no other objective signs of illness.

(b) Inoculation into the Vitreous.—Fig. 1 presents the results of an experiment designed to determine the fate of SFV during the first 48 hours after inoculation into the vitreous. Approximately 3000 LD_{50} of SFV in 0.4 ml. volume was inoculated into the vitreous of both eyes of six rabbits. The eyes were removed 1, 4, 12, 24, 48 hours, and 5 days after inoculation. They were extracted and titered separately in the usual manner. The expected yield of virus from the eye, based on the dilution factor of the eye extract (1:50), was 60 LD_{50}. It will be seen that no loss of virus could be detected 1 hour after inoculation. However, a decreased amount of virus was obtained at 4 and 12 hours. A striking increase in the titer of virus occurred between 24 and 48 hours and remained elevated for 5 days. If the titer of virus at 12 hours after
inoculation is taken as the starting point of multiplication, an increase of at least 1000-fold occurred in the virus content of the eye within 48 hours after inoculation.

In the next experiment tests for virus were made at intervals up to 55 days after inoculation. In addition, serum and eye extracts were tested for neutralizing substances.

Four hundredths ml. of a $10^{-4.5}$ dilution of SFV mouse brain extract ($10^{-4.3}$ LD$_{50}$ titer in mice) was inoculated into the vitreous of both eyes of a group of twelve rabbits. At varying intervals from 2 to 55 days a rabbit was sacrificed. The eyes were removed and extracted separately; the extracts in this experiment included the aqueous humor. The optic nerves and brains were also removed and extracted similarly. The extracts were tested for virus in mice as previously described. The eye extracts were then preserved in the frozen state and those which yielded no virus were tested for neutralizing substances. Serum obtained from each rabbit prior to sacrifice was also tested for neutralizing substances.

All the rabbits in this experiment showed the iritis described in the previous experiment. One rabbit died 12 hours after inoculation, one after 4 days, and one developed a hemiparesis after 18 days. The latter rabbit was sacrificed 3 days after the onset of the paralysis, but an extract of the brain contained no detectable virus.

Fig. 2 presents the results of this experiment. The amount of virus recovered from the eye extracts during the first 10 days after inoculation was very similar.
to that observed in the preceding experiment (compare Table II). It will be seen that virus was obtained from the eyes up to 33 days after inoculation.

Virus was recovered from the optic nerve 2 days after inoculation in a titer of $10^{-1.9}$, and from the brain of rabbits sacrificed on the 2nd and 6th days in titers of $10^{-2.9}$ and $10^{-2.5}$ respectively. Virus was not recovered from the optic nerves or brains of any of the other animals. In this experiment both eyes of the animals were inoculated, extracted, and titered separately, and considered as duplicate determinations. In other experiments only one eye was inoculated. In repeated tests of this sort no crossing of virus from one eye to the other was observed. Furthermore, the amount of virus recovered from an eye was essentially the same whether the other eye was inoculated or not.

**TABLE III**

<table>
<thead>
<tr>
<th>Inoculum $^*$</th>
<th>Yield of virus from eye 5 days after inoculation</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected $^*$</td>
<td>Right eye</td>
</tr>
<tr>
<td>0.4</td>
<td>&lt;0.1</td>
<td>2.0</td>
</tr>
<tr>
<td>1.4</td>
<td>&lt;0.1</td>
<td>2.4</td>
</tr>
<tr>
<td>2.4</td>
<td>0.7</td>
<td>3.4</td>
</tr>
<tr>
<td>3.4</td>
<td>1.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* All figures given as log LD$_{50}$.

* See footnotes under Table I.

Tests for neutralizing antibodies were made on the sera of all the rabbits in this experiment. Neutralizing antibodies began to appear about the 14th day after inoculation at which time the neutralization index was 30. The neutralization index of the sera obtained 21, 28, 42, and 55 days after inoculation was 30, 100, 1000, and 3000 respectively. Neutralization tests with the eye extracts obtained 42 and 55 days after inoculation indicated traces of neutralizing substances, but the index was less than 10. These findings are in agreement with the work of Duke-Elder who studied the relation of serum and vitreous antibody levels in the eyes of horses (14).

**Lymphocytic Choriomeningitis Virus**

**Multiplication of LCM Virus in the Rabbit Eye**

Studies were next undertaken to determine whether LCM virus would also multiply when injected into the rabbit eye. Varying concentrations of LCM virus were inoculated into the vitreous and the eyes tested for virus 5 days later.
A mouse brain extract containing the WE strain of LCM virus, having a titer of $10^{-4.4}$ on intracerebral inoculation in mice, was used. Serial tenfold dilutions ($10^{-2}$ through $10^{-3}$ inclusive) of the extracts were inoculated in 0.04 ml. amounts into the vitreous of both eyes of a group of four rabbits. The rabbits were sacrificed after 5 days, the eyes removed, and extracted separately. The extracts were then tested for virus by intracerebral inoculation into mice.

The results of the virus titrations are summarized in Table III. The expected yield of virus was calculated as described in the experiment shown in Table I. The data clearly indicate that multiplication of LCM virus occurs in the rabbit eye following inoculation of virus ranging from log 0.4 to 3.4 LD$_{50}$. In contrast to the findings with SFV (compare Table I) much smaller amounts of virus are capable of initiating virus multiplication in the eye. The visible reaction of the eye following inoculation with LCM virus was much less than that with SFV, consisting only of a mild hyperemia which rapidly disappeared within 24 hours after inoculation.

**Recovery of LCM Virus at Various Intervals Following Inoculation into the Vitreous**

Approximately 10 LD$_{50}$ of LCM virus was inoculated into the vitreous of both eyes of a group of five rabbits. The eyes were tested for virus 1, 4, 12, 24, and 48 hours after inoculation. The results of this experiment can be summarized briefly. No virus could be detected in the extracts of the eyes taken 1, 4, and 12 hours after inoculation. At 24 hours one eye contained virus in titer of $10^{-2.4}$, while the titer of the other eye extract was less than $10^{-1.0}$. Both eyes of the rabbit sacrificed at 48 hours contained virus in titers of $10^{-3.2}$ and $10^{-4.0}$ re-
It is apparent, therefore, that rapid multiplication of LCM virus occurs between 24 and 48 hours after inoculation.

In the following experiment LCM virus was inoculated into the vitreous and the eyes were tested at varying intervals from 2 to 28 days for virus content.

The vitreous of both eyes of seven rabbits was inoculated with 0.04 ml. of a 10 per cent LCM mouse brain extract. The extract had a titer of $10^{-4.4}$ on intracerebral inoculation in mice. The rabbits were sacrificed 2, 4, 6, 8, 14, 21, and 28 days after inoculation. The eyes were removed and extracted individually in the usual manner. The brain of each rabbit was also removed and extracted.

The results of this experiment are presented in Fig. 3. The titer of virus recovered from the eye declined steadily and gradually after the 2nd day following inoculation. Virus was present in one eye 21 days after inoculation, but none could be detected after 28 days. The brain extract of the rabbit tested 6 days after inoculation yielded virus in low titer ($10^{-1.9}$). No virus was demonstrated in the brains of the remaining rabbits. None of the rabbits had any obvious illness during the course of this experiment and the eyes showed only a slight transitory reaction as already described.

**Lansing Poliomyelitis Virus**

The Lansing strain of poliomyelitis virus was inoculated into the vitreous of both eyes of eight rabbits. The 10 per cent cotton rat brain extract used as the inoculum had a $\text{LD}_{50}$ titer in mice of $10^{-2.9}$. Six of the rabbits were sacrificed after 10 days and two after 20 days. The eyes of all the rabbits were extracted and tested separately for virus content. No visible reaction occurred in the eyes following inoculation and the rabbits showed no evidence of illness during the period of observation. Ten per cent extracts of the eyes inoculated intracerebrally into mice failed to cause death of 50 per cent of the mice. No evidence was obtained, therefore, that Lansing virus multiplied in the rabbit eye and further experiments of this type were not undertaken.

**DISCUSSION**

These studies afford conclusive evidence that both lymphocytic choriomeningitis and Semliki Forest virus multiply in the rabbit eye after intraocular inoculation. The growth curve of virus in the eye revealed several interesting characteristics. The amount of SFV recovered 1 hour after inoculation was comparable to that inoculated into the eye. However, the data indicated a decrease in the virus content of the eye at 4 and 12 hours after inoculation (Fig. 1). This was followed by a phase of rapid multiplication of virus which reached the maximum titer about 48 hours after inoculation. The virus remained in high titer up to 6 days. Thereafter the virus was recovered in variable amount up to about 5 weeks after inoculation (Fig. 2). The course of events following inoculation of lymphocytic choriomeningitis virus was similar, except...
that a more gradual and constant decline of recoverable virus occurred over a period of 3 weeks (Fig. 3). The significance of the initial loss of virus inoculated into the eye is not understood at the present time. Recently Schlesinger (15) reported a comparable situation with equine encephalomyelitis virus, in which only 3.5 to 10 per cent of the expected amount of virus could be recovered from mouse brains 1 hour after intracerebral inoculation.

The amount of Semliki Forest virus required to initiate infection in the rabbit eye appeared to be about 1000 mouse LD50 of virus. Less than 10 LD50 of lymphocytic choriomeningitis virus, however, was sufficient to induce multiplication in the eye. It is of interest that the actual yield of both viruses from the eye remains remarkably constant even though the amount of seed virus inoculated into the eye varies over a wide range. The inoculation of very large amounts of SFV, however, apparently resulted in a decreased yield of virus from the eye (Table I). It seems likely that this is another manifestation of the interference phenomenon as described by Henle and Henle (16) in studies on the yield of influenza virus from the chick embryo following inoculation of varying concentrations of virus. It is not known whether the virus recovered following inoculation of large amounts of SFV represents newly formed virus, residual seed virus, or a combination of the two. However, the finding that the amount of virus recovered from the eye remains at a constant level even though progressively increasing amounts of virus are inoculated, together with the apparent loss of most of the seed virus, (Table I, Fig. 1), suggests that at least a large part of the virus obtained represents newly formed virus.

No evidence of multiplication of either LCM or SFV viruses outside the eye was obtained in these experiments. Little or no virus could be recovered from the optic nerves or brains of the rabbits, although virus was present in high titer in the eyes. Furthermore, the animals showed no signs of illness other than a transitory fever. However, when large amounts of SFV (10^7 or 10^8 LD50) were inoculated into the eye a severe iritis followed by corneal opacity was produced. This response appears to be similar to the "toxic" reactions observed after intraocular inoculation of influenza (7), fox encephalitis (8), and equine encephalomyelitis (9). In these instances multiplication of virus could not be demonstrated, although the reactions produced were specifically inhibited by antiserum. As with SFV, the reactions were produced only after the injection of large amounts of virus. Smaller amounts of SFV failed to elicit any detectable reaction in the eye, although virus multiplication occurred. No ocular reactions have been observed with LCM virus other than a mild transitory hyperemia.

The site of multiplication of virus in the eye has not been determined in the present experiments. The finding that SFV was obtained irregularly and in low titer from the aqueous humor even after inoculation into the anterior chamber indicates that multiplication does not take place in the cells associated with this chamber. However, virus passes readily from the anterior chamber to other
parts of the eye. No striking difference could be detected in the virus content of the vitreous, an extract of the retinal cells, and an extract of the whole eye 2 days after the inoculation of SFV into the vitreous cavity. As previously noted, multiplication of a virus in one eye of a rabbit is independent of any detectable influence on virus multiplication in the other eye. Furthermore, no crossing of virus from the inoculated to the uninoculated eye of the same animal has been observed. This is in agreement with the work of Green, Evans, and Yanamura (8) who found that although corneal opacity was produced by the inoculation of fox encephalitis virus into one eye of a rabbit, the uninoculated eye remained normal.

No evidence was obtained in these experiments to indicate that the Lansing strain of poliomyelitis virus multiplied in the rabbit eye. It is well known that the rabbit is refractory to infection with this virus by any route of inoculation. The rabbit is also considered to be resistant to infection with lymphocytic choriomeningitis virus (17). Nevertheless, the present experiments clearly show that multiplication of LCM virus occurs in the rabbit eye as an inapparent infection following intraocular inoculation. Thus, one cannot predict that a virus will not multiply in the eye after intraocular inoculation in a host not considered susceptible to infection with the virus by another route.

SUMMARY

Lymphocytic choriomeningitis virus and Semliki Forest virus readily multiply in the rabbit eye following inoculation into the vitreous. Less than 10 mouse LD_{50} of LCM virus was sufficient to induce multiplication in the eye, whereas, approximately 1000 LD_{50} of SFV was required to initiate infection.

Both viruses multiplied in the rabbit eye as an inapparent infection. Little or no virus could be recovered from the optic nerves or brains of the rabbits, although virus was present in high titer in the eyes. The animals showed no signs of illness other than a transitory fever.

Large amounts of SFV induced a severe iritis followed by corneal opacity. This reaction appeared to be due to "toxic" properties of the virus. No ocular reactions have been observed with LCM virus other than a mild transitory hyperemia. The yield of virus from the eye following inoculation of large amounts of SFV was less than when more dilute inocula were used.

Maximum multiplication of both viruses occurred about 48 hours after inoculation into the vitreous. SFV remained in high titer for about 4 days and thereafter could be recovered in variable amounts up to about 5 weeks after inoculation. LCM virus could be recovered in gradually decreasing amounts up to about 3 weeks after inoculation.

Serum-neutralizing antibodies appeared in high titer (neutralization index up to 3000) following inoculation of SFV into the rabbit eye. However, only traces of antibody could be detected in extracts of the eyes.
SFV was obtained irregularly and in low titer from the aqueous humor following inoculation into either the vitreous or anterior chamber. No significant difference in the virus content of the vitreous, an extract of the retinal cells, and an extract of the whole eye could be detected 48 hours after inoculation of SFV into the vitreous.

The Lansing strain of poliomyelitis virus failed to cause any detectable reaction in the rabbit eye and no evidence of multiplication of the virus was obtained in these experiments.

BIBLIOGRAPHY