Further Studies on the Mode of Transmission of Herpes-Like Virus in Guinea Pigs[1] (35911)

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In a previous paper (1), we reported that guinea pigs infected with a herpes-like virus (HLV) could transmit the virus to their fetuses. The frequency of the transplacental transmission appeared to be related to the amount of inocula animals received, and the length of infection in the animals. Further studies were designed to investigate whether the HLV could be spread by intranasal or oral inoculation, or by direct contact. In addition, virus growth in infected animals, and the frequency of virus infection in the offspring of infected guinea pigs, as reflected by age, were also determined. The results of these studies are reported below.

Materials and Methods. Virus strain. The HLV isolated from strain 2 guinea pigs was used (2). The virus stock suspension had an infectivity of approximately 5.7 log $TCID_{50}$/ml.

Source of animals. The Hartley strain guinea pigs, which were used throughout the study, were obtained from commercial sources (2). A total of six strain 2 guinea pigs, three male and three female, were kindly supplied by the Animal Care Division of the National Institutes of Health; two separate colonies have been propagated in our laboratory.

Animal inoculation. All animals were tested for virus infection in their blood prior to inoculation. Animal inoculation was performed while the animals were under light ether anesthesia as follows:

Intracardiac inoculation. Varying concentrations of virus suspension were inoculated into the heart of the guinea pigs. Blood samples were taken from the heart 3 hr after inoculation, and at intervals thereafter, for determination of the growth of HLV in the blood of guinea pigs.

Oral inoculation. Guinea pigs were fed with a virus suspension, which had an approximate titer of 5.3 log $TCID_{50}$. They were bled on the third and sixth week of infection. Failure to isolate any virus from the blood and from tissues at time of sacrifice was considered a definite negative for the oral inoculation experiments.

Intranasal inoculation. A virus suspension containing approximately 5.3 log $TCID_{50}$ was dripped into the nares of the guinea pig. The inoculated animals were bled at different time intervals and the blood was used for virus isolation. Animals in this experiment were observed for 45 days.

Contact infection. Healthy female guinea pigs were housed in the same cage with infected male guinea pigs. An additional group of 4 uninfected guinea pigs was housed in a cage adjacent to a cage which contained 4 previously infected animals. Contact was maintained for a period of 3 months or longer.

Virus isolation and titration. Whole blood obtained by cardiac puncture was used for HLV isolation throughout the study. In cases where the animals were sacrificed, spleen, lung, kidney, and urine were also used.

The heparinized blood was serially diluted in Hanks' balanced salt solution. Two-tenths milliliter of each dilution was inoculated into feeder-layer cultures (1). The appearance of cytopathic effect (CPE) was the indication for a positive virus isolation.

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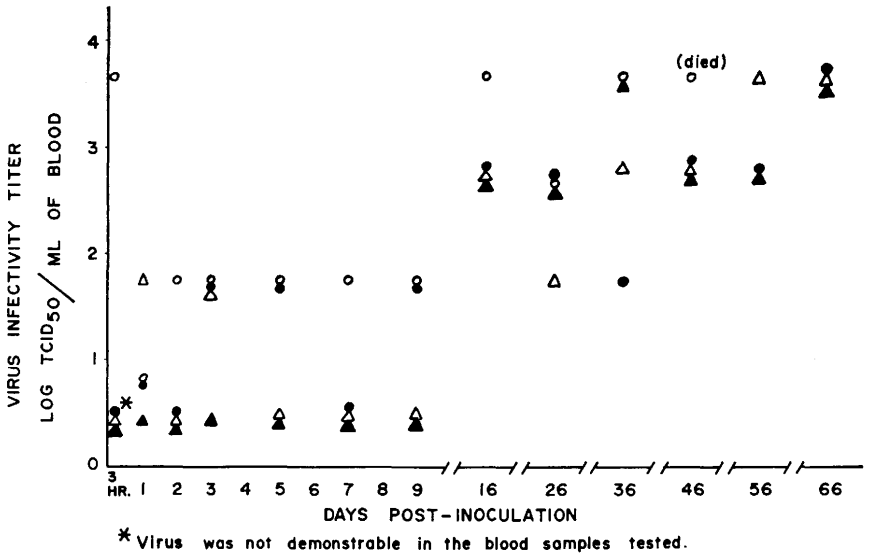

FIG. 1. Growth of HLV in the blood of guinea pigs after intracardiac inoculation: Animal A (○) received 5.7 log $TCID_{50}$; animal B (●) received 4.7 log $TCID_{50}$; animal C (△) received 3.7 log $TCID_{50}$; animal D (▲) received 2.7 log $TCID_{50}$.

The isolation of virus from spleen, kidney, and lung tissues was made by mincing the tissues into fine pieces and inoculating them into feeder-layer cultures as previously described (*1*). Urine samples were centrifuged and the cell pellets were washed twice before inoculation into feeder-layer cultures.

Results. Growth of HLV in the blood stream. The growth of the HLV in the blood stream of 4 guinea pigs is shown in Fig. 1. The animals, A, B, C, and D were inoculated intracardially with 5.7, 4.7, 3.7, and 2.7 log $TCID_{50}$ of virus, respectively. Three hours after inoculation, animal A had a virus titer of 3.7 log $TCID_{50}$/ml of blood whereas neither B, C, nor D had any detectable virus. Virus was demonstrated in the blood of animals A, B, and C but not in D at 24 hr after inoculation. Detectable virus in the blood of animal A was found 2, 3, 5, 7, and 9 days after inoculation; virus isolation results in animals B and C on the aforementioned days were irregular; no virus was detected in the blood of animal D during the first 9 days postinoculation. By day 16, all 4 animals had virus titers of 2.7 log $TCID_{50}$ or higher/ml of blood. Virus infectivity titers remained stable at detectable levels until they were sacrificed on day 66. Spleen and kidney of these animals also yielded virus, yet no virus was isolated from the urine samples of any of these animals at the time of sacrifice. In addition, urine samples from 14 intraperitoneally infected Hartley guinea pigs and one from a naturally infected strain 2 animal were also tested for the presence of virus in the urine. HLV was isolated from the one strain 2 animal but not from any of the experimentally infected guinea pigs.

Oral inoculation. Two experiments were performed and the results are shown in Table I. There were 3 guinea pigs inoculated in Expt. 1. HLV was isolated from blood, spleen, and kidney of one of the three animals when they were sacrificed on day 45. Three out of 4 animals in Expt. 2 showed virus in their blood 3 weeks after inoculation; the negative one remained negative when sacrificed in week 6.

Intranasal inoculation. The results of intranasal inoculation are also shown in Table I. Animals inoculated with 5.3 log $TCID_{50}$ of virus were found to be uniformly susceptible to HLV infection as a combined total of 8 out of 8 guinea pigs were infected. Animals were also infected when they received 3.3 log $TCID_{50}$ of the inocula but they were not infected when 1.3 log $TCID_{50}$ of virus was

TABLE I. Isolation of HLV in Guinea Pigs After Intranasal and Oral Inoculations.

Route of inoculation	Expt. no.	Inoculum log $TCID_{50}$	No. of animals inoculated	No. showed virus
Oral	1	5.3	3	1[a]
	2	5.3	4	3
Intranasal	1	5.3	3	3[a]
	2	5.3	4	4
	3	5.3	1	1
		3.3	2	2
		1.3	2	0

[a] Isolations were made from both blood and tissues; only blood was used for isolation from the others.

given. One of the animals in a group of four in Expt. 2, which received 5.3 log $TCID_{50}$ of the inocula, first showed detectable virus in its blood stream on day 14; two additional animals showed virus on day 17; the remaining one had detectable virus on day 22.

Contact infection. A total of 3 separate experiments were performed (Table II). Virus was not isolated from the three female contact guinea pigs in Expt. 1, but virus was isolated from the blood and spleen of the female animal in experiment 2 which was housed with 2 infected males. In Expt. 3, one out of 4 noninfected guinea pigs became infected when housed in a cage adjacent to one containing infected animals.

Isolation of HLV from the offspring of experimentally and naturally infected guinea pigs. Guinea pigs inoculated intraperitoneally (1, 2), and intracerebrally (3) were kept for long-term observation, *i.e.*, 6–24 months. Some of the animals were pregnant and gave birth to apparently healthy offspring which were subsequently tested for the presence of virus in their blood. The results of these tests are listed in Table III. A total of 47 offspring from intracerebrally inoculated parents were tested; none of them had any detectable virus. Of the 60 offspring from the intraperitoneally infected parents, only six showed virus infection when tested at less than 2 months of age. In the 2–6 months group, 17 out of a total of 29 guinea pigs had detectable virus (59%).

HLV was not isolated from the 9 offspring less than 2 months of age of naturally infected strain 2 animals. In the 2–6 months old age group, the virus recovery rate was 5 out of 22. Thirteen out of 20 (65%) had virus in their blood when they were tested at 6 months of age or older.

Discussion. The present results, together with a previous report (1), show that the HLV of guinea pigs can be transmitted by transplacental, oral, intranasal, and contact routes. The results also reveal that approximately 1000 $TCID_{50}$ are necessary to initiate an intranasal infection, and 500 $TCID_{50}$ to establish an intracardiac infection.

The establishment of HLV infection in guinea pigs required about 2 weeks when the virus was given intranasally. Inoculation of

TABLE II. Experimental Design of Contact Infections.

Expt. no.	Mode of contact	No. of animals		Results[a]
		Infected	Contact	
1	Same cage	1	3	0/3
2	Same cage	2	1	1/1
3	Separate but adjacent cages	4	4	1/4

[a] Number of contact animals showed virus infection per total number of contact animals tested.

TABLE III. Frequency of HLV Recovery in the Blood of Offspring of Experimentally and Naturally Infected Guinea Pigs.

Animal species	Parents		Offspring			
	Route of inoculation[a]	Dosage log $TCID_{50}$	Age when tested (months)	No. Tested	No. of HLV isolations	(%)
Hartley	ic	4.0	1–3	47	0	0
	ip	5.0–6.0	<2	60	6	10
			2–6	29	17	59
Strain 2	Natural infection	None	<2	9	0	0
			2–6	22	5	22
			>6	20	13	65

[a] ic = intracerebral; ip = intraperitoneal.

the virus into the heart also had a "lag" period when a lesser amount of virus was inoculated. The lag period in the group of animals inoculated by the intracardiac route was most prominent in the animals receiving a small dose of virus. The appearance of the HLV in the blood stream could be interpreted as the synthesis of new virus in the animal body. However, HLV titers in the blood never increased beyond 3.7 log $TCID_{50}$/ml of blood. The mechanism involved in keeping the virus at such a steady level in the blood is not clear.

As high titers of HLV were found in the blood and also in the kidneys, it was anticipated that urine might harbor infectious virus and might serve as a means of disseminating the HLV in guinea pigs. Although one naturally infected strain 2 guinea pig showed virus in its urine, none of the 14 experimentally inoculated Hartley guinea pigs tested had recoverable virus. The failure to find HLV in the urine of infected guinea pigs is in contrast to cytomegalovirus (CMV) infections in humans, in which instance the isolation of CMV from the urine is a basic diagnostic aid (4).

The present study demonstrated that contact infection with HLV could be achieved in guinea pigs. The presence of virus in the blood of one of the four noninoculated guinea pigs in a cage adjacent to one containing infected animals indicated that droplet or other airborne infection actually does occur. Other investigators have observed the transmission of guinea pig HLV to cage-mates (5). Marek's disease herpesvirus was also transmitted by contact (6), as well as by dust and litter in the housing facilities (7). The transmission of canine herpesvirus was achieved by contact (8), by aerosol (9), and by transplacental transmission (10). The aerosol method was also successful in studies of the transmission of feline herpesvirus infection in kittens (11). Thus, the transmission mechanism of HLV in guinea pigs is similar to that of herpesviruses of chickens, dogs, and cats.

The finding of a higher incidence of HLV infection in older offspring than newborns is worth noting. This observation is consistent with the report by Cole and Kuttner (12) that 84% of full grown guinea pigs showed inclusions in their submaxillary glands; whereas only a small number, 3 of 43, of animals aged less than 1 month showed similar infection. The increased incidence of virus infection in the older offspring was probably due to one of the following: (i) insufficient virus concentration in the blood for detection during early life; (ii) virus multiplication in the animals as their age increased; and/or (iii) contact infection by aerosol or other routes. The failure to isolate virus from the offspring of intracerebrally inoculated parents was puzzling since infectious virus was isolated from the blood, spleen, and brains of the inoculated animals (3).

Summary. Hartley guinea pigs can be infected with HLV by various routes of inocu-

lation. These animals were found to be uniformly susceptible to intranasal inoculation of 3.0 log $TCID_{50}$ of virus. Infection could also be achieved by oral inoculation. Four out of 7 animals inoculated orally had virus in their blood. Contact infection was achieved in 2 out of 7 animals that were housed with or adjacent to infected ones. Incidence of virus infection in newborns from both intraperitoneally inoculated or naturally infected parents was low, but the frequency increased to 59% or higher when the offspring were 6 months of age or older. No virus was isolated from the offspring of intracerebrally inoculated guinea pigs.

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