Studies of a Rabbit Herpes-Like Virus[1] (36668)

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The isolation of a latent herpes-like virus (HLV) in primary kidney monolayer cultures prepared from a healthy female New Zealand albino rabbit was reported in 1969 by Nesburn (1). When the latter study was compared with those of earlier workers (2, 3) it was concluded that this new isolate represented a reisolation of virus III of Rivers (4, 5). Nesburn proposed that his recent isolate be named *Herpesvirus cuniculi.*

The increased use of primary rabbit kidney cultures for virus studies, particularly of the herpesvirus group, prompted our interest in the incidence of HLV in geographically separate rabbit populations. The fact that there have been few reports concerning the isolation of HLV from rabbits stimulated our investigations into experimental infection of this virus in its natural host. These include studies of the pathogenicity, mode of transmission and the possible persistence of HLV in experimentally infected rabbits.

Materials and Methods. Tissue cultures. Primary kidney cultures were prepared from 2.5–3 lb New Zealand white rabbits obtained locally. These cultures were planted in Leighton tubes containing a coverslip, using human kidney growth medium (6) with 10% fetal calf serum. When monolayers were confluent the cultures were maintained in Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate and 5% calf serum. Cultures were examined for CPE 1–2 times/week at which time the medium was replaced with fresh maintenance medium. Coverslips were stained with hematoxylin and eosin at 3 week intervals or when virus-induced CPE was suspected. All cultures were maintained at 35° for as long as they remained in good condition which was normally for 2–3 months. Primary rabbit kidney cells were also obtained from commercial sources in Maryland and New York as cell suspensions or monolayer cultures together with a serum sample from the same animal when available. These cells were used either for primary cultures or secondary cultures and were studied as above.

Virus assay and antibody studies. A known strain of rabbit HLV was kindly supplied by Dr. A. B. Nesburn. Virus stocks were prepared in primary kidney cell cultures. Virus titers were determined by the appearance of CPE in primary rabbit kidney cultures inoculated with 10-fold dilutions of virus suspensions. For detection of rabbit HLV antigen in primary rabbit kidney cells grown on coverslips, the indirect fluorescent antibody technique was used. Antiserum to the above rabbit HLV was prepared in rabbits immunized with the virus. Fluorescein-conjugated antiserum to rabbit gamma globulin prepared in goats was purchased from Antibodies, Inc., Davis, CA. For antibody determinations rabbit sera obtained from the various sources mentioned above were tested for the presence of neutralizing antibody to the same strain of rabbit HLV. Serum titers were determined according to their capacity to neutralize 100 $TCID_{50}$ of rabbit HLV as described by Nesburn (1).

Experimental infection. Infection with HLV in rabbits was studied by ip inoculation of tissue culture suspensions of virus containing 10^5–10^6 $TCID_{50}$ into 3-month-old

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rabbits. These infected animals were examined intermittently for the presence of virus in the blood and in the various organs when the animal was sacrificed. Transplacental transmission of rabbit HLV was also studied by examination of tissues obtained from newborn and fetal progeny of infected mothers. Virus in the blood was measured by the inoculation of serial 10-fold dilutions of whole blood into primary kidney cultures. In certain instances, leukocytes were separated from the heparinized blood and were used as sources of virus isolation and titration. For the detection of virus in the tissues, the latter were minced and inoculated into cultures of primary rabbit kidney cells. All inoculated cultures were examined for CPE and the presence of intranuclear inclusions. Kidney tissues obtained from inoculated rabbits were also dispersed with trypsin and planted as primary monolayer cultures.

Results. Natural infection of HLV in rabbits. In the examination of kidney cultures prepared from 218 rabbits from 3 different geographic locations, New York, Maryland and Connecticut, no virus was isolated (Table I). Antigen as determined by fluorescent antibody studies to rabbit HLV was detectable in 4 lots of cultures prepared from 71 rabbits from three different locations but neutralizing antibody titers of 1:5 to 1:40 were demonstrable in 10 of 59 animals obtained from Maryland and in 1 of 20 rabbits from one colony in Connecticut.

TABLE I. Prevalence of Herpes-Like Virus in Rabbits.

Location	No. positive/no. studied		
	Virus isolation	Neutralizing antibody[a]	Antigen[b]
Connecticut			
Guilford	0/104	0/104	1/41
Durham	0/20	1/20	1/7
Branford	0/13	0/13	ND
Maryland			
A	0/59	10/59	2/23
B	0/12	ND	ND
New York	0/10	ND	ND

[a] Antibody titers $\geq$ 1:5 considered positive.

[b] Virus antigen in nucleus of primary kidney cell cultures as determined by fluorescent antibody methods using known antiserum to rabbit HLV.

TABLE II. Virus Isolation and Antibody Titers in Rabbits Experimentally Infected with Rabbit Herpes-Like Virus.

After intraperitoneal inoculation (days)	Virus isolation from blood	Av antibody titer (reciprocal)
0	—	—[a]
1–10	—	—
11–30	+	60
31–50	+	60
50–100	+	40
>100[b]	+ (1.7 log $TCID_{50}$/ml)	40

[a] 1:5 lowest dilution tested.

[b] 117 days expt. terminated.

Experimental infection of HLV in rabbits. Virus was recovered from the blood of ip infected animals as early as 11 days after inoculation and persisted for as long as 117 days, the longest period tested (Table II). Virus titers of 1.7 log $TCID_{50}$/ml of blood were found but no clinical disease which could be attributed to the virus was detected. The recovery of virus from the blood coincided with the appearance of neutralizing antibody titers in the serum. Further studies showed that the virus was present in the white blood cells in a concentration of 1 infectious virus particle/10,000 cells.

The distribution of infectious virus in the various tissues of experimentally infected animals was also determined. It was found that appreciable titers of virus existed in the leukocytes, spleen, liver, lungs, and salivary glands, whereas the concentration of virus in the kidney tissues appeared to be variable (Table III). By increasing the inoculum 10-fold, the opportunities for the recovery of virus from the kidney tissues was also increased. As shown in Table III, rabbits in group A received 4.7 log $TCID_{50}$ of HLV and no virus was isolated from the kidneys or primary cultures prepared from the kidneys of these infected animals, whereas group B

TABLE III. Distribution of Herpes-Like Virus in Tissues of Experimentally Infected Rabbits.

Tissue examined[a]	Av virus infectivity titers log $TCID_{50}$/ml packed cells[b]	
	A	B
Leukocytes	3.4	5.1
Spleen	2.9	3.7
Liver	1.3	1.8
Lung	ND	3.8
Salivary gland	1.3	1.2
Kidney, minced tissue	<0.3	2.5
Kidney, monolayer culture	—	+

[a] Tissues minced and inoculated onto primary rabbit kidney cultures except leukocytes and kidney cell trypsinized for monolayer cultures.

[b] Group A received a virus suspension containing 4.7 log $TCID_{50}$; Group B received a virus suspension containing 6.1 log $TCID_{50}$.

which received 6.1 log $TCID_{50}$ of HLV yielded virus in both the kidneys and the kidney cell cultures.

Attempts to demonstrate transplacental transmission were unsuccessful. A total of 28 newborn or fetal rabbits from 2 infected mothers at four separate deliveries were examined. No virus was isolated from any of the tissues of the progeny even though all of the maternal tissues examined harbored demonstrable titers of infectious virus including the kidneys. Neutralizing antibody titers of the newborn rabbit sera ranged from 1:20 to 1:40 while that of the mother was 1:320 at the time of delivery. No contact infection was detected when pairs of infected and noninfected animals were housed in the same cages after a period of 1–2 months.

Discussion. The fact that HLV was not isolated from any of the 218 rabbits studied is surprising, particularly in those populations in which rabbit HLV antigen was detectable by fluorescent antibody techniques or in those which had neutralizing antibody titers. Nesburn (1) reported that the prevalence of rabbit HLV was very low in the rabbit colony he studied, in that only one HLV isolation was made from more than 100 lots of primary kidney culture used during the 3 years following the original isolation. Our finding that the virus was only recovered from kidney cultures of experimentally infected rabbits which received high doses of virus may account in part for this low rate of isolation, particularly since in both studies kidney cultures were used as the sole source for detection.

The presence of rabbit HLV neutralizing antibody or viral antigen in certain rabbit populations certainly indicates that some previous contact with this virus has occurred. Rivers and Tillett (5) reported that 15% of 200 rabbits examined were immune to experimental infection with virus III and that 4 of 20 sera examined had neutralizing antibody. Of 377 rabbits studied by Andrewes (7), 8 were found to be resistant to virus III infection. Topacio and Hyde (8), studying 79 Maryland rabbits found approximately 17% to be refractory to infection, a value in close agreement with that found in the present studies (Table I).

Rabbit HLV was recovered from the blood for as long as 117 days after intraperitoneal inoculation in the presence of appreciable neutralizing antibody titers (Table II). Rivers and Tillett (5) showed that immunity, following infection with live virus III, lasted for at least 6 months. Andrewes (7) was unable to recover virus from the tissues of immunized rabbits. Our results suggest that immunity as measured by the presence of circulating neutralizing antibody does not eliminate this virus from the tissues. The failure of earlier attempts to isolate virus might be explained in part by the presence of considerable amounts of antibody which mask the presence of infectious virus in the tissues.

The method of transmission of this virus in rabbit populations has not as yet been elucidated. Few studies of this nature have been reported. Topacio and Hyde (8) reported that active immunity followed intranasal inoculation; however, no mention was made as to the significance this method might play in natural infection. Our studies of transplacental transmission have not shown any conclusive results. Studies with other members of the herpesvirus group, herpes simplex (9) and guinea pig HLV (10) showed that the in-

travenous inoculation of pregnant animals with small amounts of virus resulted in no virus crossing the placenta. However, when large amounts were used virus was recovered from fetuses, fetal membranes and fluids (9, 10). While the amounts of rabbit HLV used in the present study are considered to be large, no virus was detected in any of the 28 newborn and fetal rabbits examined. Since neutralizing antibody titers of 1:20–1:40 were found in the fetal sera, it is possible that small amounts of virus would escape detection using the procedures described.

Consideration of primary rabbit kidney for vaccine purposes and its widespread use for virus assay, particularly for members of the herpesvirus group, makes meticulous screening of such cultures for latent HLV essential. The finding that a rabbit HLV persists in and may be recovered from the blood, specifically from the leukocytes, might provide a sensitive method for the detection of these viruses in animals.

Summary. A study of three populations of New Zealand white rabbits showed that some groups have neutralizing antibody to the rabbit HLV isolated by Nesburn [J. Virol. **3**, 59 (1969)]. Primary kidney cultures prepared from such animals failed to yield infectious virus but the primary tissue culture cells of a small number had rabbit HLV antigen as determined by fluorescent antibody techniques. Studies of experimental infection in rabbits showed that this virus may persist and be recovered from the blood for more than 100 days after inoculation. No virus was isolated from the kidneys or monolayer cultures prepared from the kidneys of animals inoculated with small doses of virus.

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