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Genital Infection of Female Hamsters with Herpesvirus Hominis Type 2 (HVH-2) (39114)

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Herpesvirus hominis Type 2 is a common infection of the human and is transmitted by venereal contact (1, 2). In addition to being potentially painful and recurrent, infection of the mother's genital tract close to the time of delivery exposes the infant to a special danger (3); a review of 148 cases of neonatal herpetic infection indicated that 71% died, severe neurological or ocular disorders occurred in 15%, and only 14% appeared to be free of sequella. Several studies have indicated an association between HVH-2 infection and cervical carcinoma (4, 5, 6). From the above evidence, it is concluded that HVH-2 is an important pathogen, and that a need exists for a model infection in a convenient laboratory animal. Such a model would permit study of the disease process and could be applied to the evaluation of potential antiviral drugs.

Burnstein (7) demonstrated that vaginal infection of hamsters with herpes simplex virus resulted in a vaginal exudate followed by posterior paralysis. He isolated virus from vaginal washings and the spinal cord. Based on his report, we decided to evaluate the genital infection of the female hamster as a model for HVH-2 infection with the goal of establishing a system for the evaluation of therapeutic agents. This report summarizes our findings on the incidence, severity, and pathogenesis of this infection.

Methods. Hamsters. Female hamsters (35 g) were purchased from the Lakeview Hamster Colony. They were placed in cages (five animals/cage) in a room with male hamsters. They were allowed food and water *ad libitum*, and at the time of virus inoculation they weighed 45–60 g.

Virus. Herpesvirus hominis Type 2 (HVH-2) (strain 35D) was generously supplied by Dr. Lawrence Chien, University of Alabama. This virus was propagated in primary rabbit kidney cells and assayed 6×10^6 PFU/ml by the plaque method on rabbit kidney monolayers using standard techniques (8).

Virus inoculation. Virus (0.1 ml) was administered intravaginally by tuberculin syringe fitted with a Temflex #24 vinyl-covered 22-gauge needle.

Vaginal swabs. Cotton tipped swabs on $\frac{1}{8}$-in wooden dowels, wetted with Hanks solution were used to swab the vagina. Swabs were placed in tubes containing 2 ml Eagles medium with 3% fetal bovine serum and antibiotics and quickly frozen. Samples were stored at −40° until the time of virus assay.

Tissue homogenates. Tissues were removed surgically from anesthetized animals and stored at −40°. At the time of assay, the samples were homogenized [10% wt/vol in saline (0.9% NaCl)] using Kontes glass homogenizers. After removing the debris by centrifugation, the supernatants were diluted and the virus titers determined by the plaque method.

Pretreatment. Where indicated, hamsters were pretreated by gently flushing the vaginas with 2 ml saline. The saline solution was administered by tuberculin syringe fitted with a vinyl-covered 22-gauge needle (described above). Alternatively, the vagina was swabbed with a dry cotton swab 30 min prior to virus inoculation.

Results. Intravaginal infection of female hamsters produces illness which progresses from a vaginal discharge with vaginitis to paralysis of the hind legs and finally death. During this time the animals appear to consume less food. Encephalitis does not usually precede death. In several experiments 40–70% of the animals developed illness and died. In an effort to enhance the susceptibility to infection, vaginas were

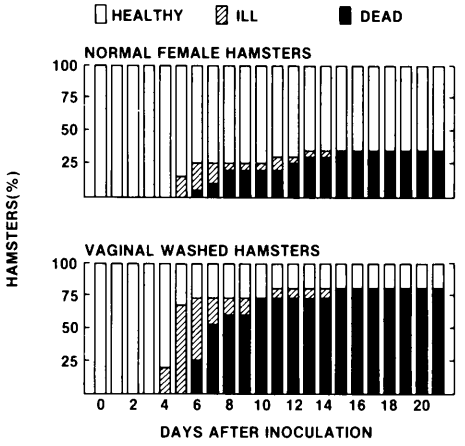

FIG. 1. Response of female hamsters to intravaginal inoculation of HVH-2. Hamsters were inoculated intravaginally with 0.1 ml HVH-2 containing 6×10^5 PFU. The normal hamsters received no pretreatment, whereas the vaginal-washed hamsters were pretreated with a 2 ml saline (0.9% NaCl) wash 30 min prior to virus inoculation. Illnesses (including vaginitis, discharge, and paralysis) and deaths were recorded daily.

flushed with saline or were swabbed 30 min prior to virus inoculation. These pretreatments resulted in an enhancement of the infection. Genital inoculation of normal (nonpretreated) hamsters resulted in 40% (6/15) death; whereas 83% of the group given a saline vaginal wash 30 min prior to infection succumbed to disease (Fig. 1). These results are consistent with those from several other experiments where 40–67% of the normal hamsters and 80–100% of the pretreated hamsters developed illness and died.

A comparison of the development of disease in normal hamsters with a second group, prewashed with saline 30 min prior to virus inoculation, is shown in Table I. The hamsters were marked so that the individual animals in each group were followed. Virus was recovered from the vagina from four of 10 of the normal hamsters. Seven hamsters became ill (including three of four from which virus was recovered from vaginal

TABLE I. RESPONSE OF INDIVIDUAL FEMALE HAMSTERS TO GENITAL HVH-2 INOCULATION[a]

(hamster number)	Vaginal swab virus titer (PFU/ml) on day				Illness (day of onset)	Death (day)
	2	3	4	5		
Normal female hamsters						
1	—	—	—	—	12	17
2	—	2.5×10^3	—	2.5×10^2	5	6
3	—	3.5×10^3	5.4×10^3	8.0×10^1	4	5
4	—	—	3.0×10^1	—	5	11
5	—	—	—	—	13	—
6	—	—	—	—	7	9
7	—	—	—	4.0×10^1	—	—
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	—	—	—	—	7	9
Vaginal-washed hamsters						
1	—	—	—	—	—	—
2	—	—	—	—	—	—
3	7.0×10^1	6.8×10^3	8.0×10^1	4.0×10^1	4	6
4	4.0×10^1	—	1.2×10^4	1.8×10^2	5	7
5	—	9.0×10^1	1.4×10^4	3.0×10^2	6	13
6	5.5×10^3	1.6×10^3	4.3×10^2	—	4	7
7	8.0×10^1	3.0×10^1	—	—	4	10
8	3.7×10^2	1.0×10^3	1.0×10^1	—	5	11
9	2.1×10^2	—	9.0×10^1	1.5×10^3	5	9
10	1.5×10^2	1.3×10^4	7.0×10^1	2.0×10^2	4	6

[a] Vaginal swab virus titers in normal and vaginal-prewashed hamsters were determined on the days indicated. The day of onset of illness and the day of death were recorded for each hamster.

swabs), and six of the ill animals died during the 21-day observation period. In groups given a vaginal prewash, virus was recovered from vaginas of eight of 10 hamsters. All eight hamsters became ill 4–6 days after virus inoculation and died 2–7 days later.

To better define the disease process, vaginally prewashed hamsters were inoculated with virus, and vaginal swabs, whole blood, spleen, kidney, liver, spinal cord, and brain were collected from five animals daily. Homogenates (10% wt/vol) were prepared from each sample, including blood, and the virus titers were determined. The development of illness and death was observed daily on a separate, but identically treated group of 15 hamsters. The first appearance of illness (vaginitis) was apparent on Day 3, with the first death occurring on Day 7. In all, 15 of 15 hamsters died within 21 days with a mean survival time of 9.2 days.

Virus was recovered from the vaginal swabs of all animals through Day 4, and from four of five swabs on Day 5 (Table II). The first evidence of spinal cord infection was apparent on Day 2, when virus was recovered in one of five spinal cord homogenates. All spinal cord homogenates yielded virus on Days 3 and 4, while four of five homogenates yielded virus on Day 5. During this time the mean log virus titer of the homogenates gradually increased. It was not possible to demonstrate virus in brain homogenates prior to Day 5, but on that day low virus titers were present in four of five homogenates. Virus could not be detected in homogenates from other organs, except on Day 5 when virus was recovered in low titer from one of the kidney homogenates.

Discussion. The present study shows that young female hamsters, approaching sexual maturity, can be infected intravaginally with HVH-2. The earliest signs of disease are vaginitis with discharge occurring 3–4 days after virus inoculation. This was followed, usually within 1 day, by paralysis of the hind legs. The animals died 2–7 days later. This response to HVH-2 infection is similar to that described by Burnstein (7) for hamsters infected with herpesvirus.

Washing or swabbing the vagina with saline just prior to infection, increases susceptibility to infection. Virus is recovered earlier and with increased frequency in vaginal swabs from pretreated animals than

TABLE II. PATHOGENESIS OF GENITAL HVH-2 INFECTION IN VAGINAL-WASHED HAMSTERS[a]

(hamster number)	Virus titers(PFU/ml) on day				
	1	2	3	4	5
Vaginal swabs					
1	1.1×10^3	2.4×10^3	2.1×10^3	2.9×10^3	<10
2	2.0×10^3	2.3×10^3	2.0×10^1	9.0×10^1	1.6×10^3
3	5.8×10^4	7.0×10^1	3.4×10^4	1.5×10^3	1.2×10^3
4	8.0×10^2	1.1×10^3	2.2×10^4	2.2×10^1	4.0×10^2
5	3.3×10^1	9.6×10^3	2.3×10^3	4.8×10^1	3.8×10^3
Spinal cord					
1	<10	<10	3.2×10^2	5.0×10^2	<10
2	<10	<10	1.0×10^2	4.9×10^1	4.0×10^3
3	<10	<10	1.1×10^3	1.3×10^2	6.7×10^2
4	<10	<10	5.7×10^1	3.0×10^1	6.5×10^2
5	<10	1×10^2	5.0×10^1	3.4×10^3	2.0×10^3
Brain					
1	<10	<10	<10	<10	<10
2	<10	<10	<10	<10	2.0×10^1
3	<10	<10	<10	<10	1.0×10^1
4	<10	<10	<10	<10	5.0×10^1
5	<10	<10	<10	<10	5.0×10^1

[a] On the days indicated, the virus titers in vaginal swabs, and homogenates prepared from spinal cord and brain were determined. Five animals were harvested at each time.

from nonpretreated controls. Also, the number of animals that become ill and die is increased by the pretreatment. The mechanism whereby the infection rate is increased by vaginal washing or swabbing was not investigated in the present study. One possibility, however, is that an antiviral substance may have been removed from the vagina by the pretreatment; it has been shown that such a substance with antiviral activity for herpesvirus is present in vaginal secretions of humans (9).

Baker *et al.* (10) have shown that young adult female mice (25 days old) are more susceptible to intravaginal infection with HVH-2 virus than are 46- or 100-day-old mice. Although the age of mice was not considered, it has been shown that pregnancy also increases susceptibility to intravaginal HVH-2 inoculation; 100% of pregnant, but only 50% of nonpregnant mice became infected and died in response to HVH-2 infection (11). The role of pregnancy in susceptibility to infection was not evaluated in the present study.

Vaginal washing prior to HVH-2 inoculation serves to synchronize the infection in that recovery of virus from the vagina correlates well with the development of illness and death; those animals from which virus is isolated from the vagina subsequently become ill 4–6 days following inoculation and die 2–7 days later, whereas in the nonpretreatment group, virus isolation, the time of development of illness, and death are poorly correlated. Maximum virus titers (ca. 10^4 PFU/ml) in the vaginal swabs occur 3–4 days after inoculating vaginally prewashed hamsters. The earliest evidence of virus invasion of the spinal cord was observed on Day 2, with increasing titer through Day 5. The brain remains free of detectable virus until Day 5, and then relatively low titers are found. These data confirm those of Burnstein (7), who observed similar vaginal virus titers through Day 5, with decreasing titers thereafter. Histopathologic lesions were found in the spinal cord but not in brain sections. Virus isolations from other organs were essentially negative, and as in the intravaginal infection of the mouse (11), it would appear that spread of the virus from the vagina to the spinal cord occurs via the neural route.

Intravaginal HVH-2 infection of the female hamster after prewashing with saline or swabbing is presently being utilized for the evaluation of antiviral drugs. The genital HVH-2 model described herein can be considered in addition to those already described, which include the intravaginal infection of nonpregnant mice (10, 12), pregnant mice (11), guinea pigs (13), nonhuman primates (4, 16), and rabbits (17), and provides an alternative model of an HVH-2 vaginal infection in a convenient laboratory animal. The ability to produce similar disease states in different species is an important consideration in the evaluation of antiviral agents.

Summary. Intravaginal inoculation of female hamsters (40–65 g) with HVH-2 (6×10^5 PFU) results in an infection of 40–67% of the animals. The illness is characterized by vaginitis with discharge, paralysis, and finally death. Washing the vagina with saline 30 min prior to virus inoculation enhances the infection so that 80–100% of the animals eventually die. Virus can be isolated from the vagina within 1–2 days after inoculation. Evidence of infection of the spinal cord is apparent by the third day whereas virus could not be isolated from the brain until Day 5. Attempts to demonstrate virus in whole blood, spleen, kidney, or liver homogenates prepared from infected animals were negative.

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1. Nahmias, A. J., Dowdle, W. R., Naib, Z. M., Josey, W. E., McLone, D., and Domescik, G., *Brit. J. Vener. Dis.* **45**, 294 (1969).
2. Hutfield, D., *Brit. J. Vener. Dis.* **44**, 241 (1968).
3. Nahmias, A. J., Alford, C. A., and Korones, S. B., *Advan. Pediat.* **17**, 185 (1970).
4. Melnick, J. L., and Rawls, W. E., *Ann. NY Acad. Sci.* **174**, 993 (1970).
5. Aurelian, L., *Cancer Res.* **33**, 1539 (1973).
6. Duff, R., and Rapp, F., *J. Virol.* **8**, 469 (1971).
7. Burnstein, T., *Nature* **205**, 1244 (1965).
8. Renis, H. E., Hollowell, C. A., and Underwood, G. E., *J. Med. Chem.* **10**, 777 (1967).

9. Pannu, J. S., and Sigel, M. M., *Proc. Soc. Exp. Biol. Med.* **114,** 763 (1963).
10. Baker, M. B., Larson, C. L., Ushijima, R. N., and Anderson, F. D., *Infect. Immun.* **10,** 1230 (1974).
11. Overall, J. C., Jr., Kern, E. R., Schlitzer, R. L., Friedman, S. B., and Glasgow, L. A., *Infect. Immun.* **11,** 476 (1975).
12. Nahmias, A. J., Naib, Z. M., Josey, W. E., Clepper, A. C., *Obstet. Gynecol.* **29,** 395 (1967).
13. Lukas, B., Wiesendanger, W., and Schmidt-Ruppin, K. H., *Arch. Gesamte Virusforsch.* **44,** 153 (1974).
14. London, W. T., Catalano, L. W., Nahmias, A. J., Fucillo, D. A., and Sever, J. L., *Obstet. Gynecol.* **37,** 501 (1971).
15. Felsburg, P. J., Heberling, R. L., and Kalter, S. S., *Arch. Gesamte Virusforsch.* **39,** 223 (1972).
16. Felsburg, P. J., Heberling, R. L., Brach, M., and Kalter, S. S., *J. Med. Primatol.* **2,** 50 (1973).
17. Larson, C. L., Ushijima, R. N., Karim, R., Baker, M. B., and Baker, R. E., *Infect. Immun.* **6,** 465 (1972).

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