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Zoster-Like Lesions from Herpes Simplex Virus in Newborn Rats.* (30442)

SATOSHI TANAKA† AND CHESTER M. SOUTHAM
Sloan-Kettering Institute for Cancer Research, New York City

This paper reports that intradermal inoculation of herpes simplex virus into newborn rats causes a vesicular skin lesion with a segmental distribution. Teague and Goodpasture(1) reported a similar eruption in the skin of rabbits and guinea pigs when herpes simplex virus was inoculated by application to the scarified skin. The appearance of those lesions was very similar to that found in the present studies in rats. We are not aware of any other report of an experimental virus lesion that has a distribution corresponding to sensory branches of the spinal nerves. Because of the similarity of this lesion to herpes zoster of man, it may have value as an experimental model for the study of that skin eruption, in spite of the fact that herpes simplex and herpes zoster are caused by antigenically unrelated viruses.

This study also provides an new example of increasing natural resistance to virus infection with increasing age. This prenomenon was systematically explored in mice by Lennette and Koprowski(2) using herpes simplex and several arboviruses. Recent studies with arboviruses in this laboratory have demonstrated that the phenomenon also occurs in rats(3).

Methods. The virus was the HF strain(4) of herpes simplex (herpes virus hominis) originally obtained from Rockefeller Institute but maintained in this Institute for several years through an unrecorded number of intracerebral passages in adult mice. Its identity was confirmed during the course of these studies by neutralization tests with 2 commercial antisera, and by the production of characteristic lesions on the corneas of rabbits and on the chorioallantoic membranes of embryonated hens' eggs.

Virus stock for these experiments was prepared as the supernate from a 20% homogenate of infected adult mouse brain and stored in sealed glass ampules in a dry-ice (solid CO_2) box until use. The suspending fluid for virus preparations was 0.85% sodium chloride containing 0.75% bovine serum albumin and penicillin (200 U/ml) and streptomycin (0.2 mg/ml).

Control mouse brain preparations were made in the same manner from healthy uninoculated mice from the same source.

Virus infectivity was titrated simultaneously with every rat experiment by intracerebral inoculation of young (16 to 18 g) male Swiss white mice (Millerton Farms, Millerton, N. Y.) with 0.03 ml volumes of 10-fold serial dilutions of the virus preparation. All virus titers are expressed as that

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† Present address: 2nd Surgical Dept., Okayama Univ. Hospital, Okayama City, Japan.

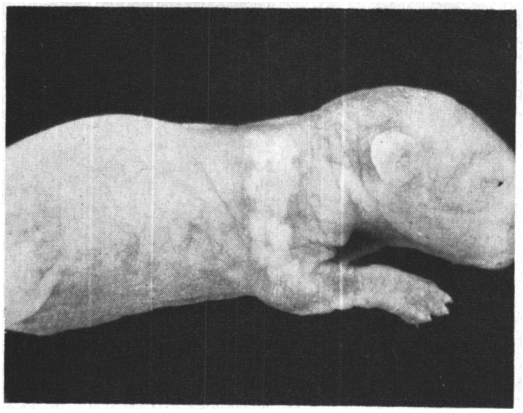

FIG. 1. Zoster-like skin lesions in 7-day-old rat following inoculation of herpes simplex virus intradermally in right dorsal region at age 2 days. The lesion was first apparent 24 hr before this picture was taken, and the rat died 24 hr later.

dilution of infected tissue which was the I.C. LD_{50} for these mice.

The rats were of Wistar stock obtained from Carworth Farms, New City, N. Y. but had been bred for several generations in this laboratory. Those which were injected within 48 hours after birth are referred to as "newborn" in this paper. Each litter was kept with its mother in an individual cage until the end of the experiment when all survivors including the mothers were killed.

"Intradermal" inoculations of 0.03 ml were made in the rats by a 27-gauge needle on a ¼-ml tuberculin-type syringe. Intradermal deposition of at least part of the virus suspension was evidenced by formation of a very thin walled bleb at the injection site, but there is no doubt that some of the inoculum was deposited subcutaneously.

Rats were inspected daily. Deaths occurring within 48 hours after inoculation were considered non-specific, but death and signs of disease occurring thereafter were attributed to the virus.

Fluorescent antibody studies were performed by the direct technique of Coons(5) using an antiherpes serum prepared in this laboratory by injection of herpes virus of the same HF strain as infected mouse brain suspension into rabbits. The serum was adsorbed with 10% normal mouse brain suspension to reduce non-specific reactions and was then conjugated with fluorescein isothiocyanate(6). All specimens were also examined using an anti-Guaroa-virus fluorescent antibody as a control, with consistently negative results. The anti-Guaroa serum was also prepared in this laboratory by injecting rabbits with mouse brain stocks of Guaroa virus. We are indebted to Mr. Roller Bailey for technical assistance in these studies.

Results. Inoculation of herpes simplex virus intradermally on the back, back of neck, or abdomen, of newborn rats caused a vesicular dermatitis with a segmental distribution which resembled herpes zoster (Fig. 1).

The frequency of the vesicular eruption (Table I) was related to the amount of infectious virus which was inoculated. It was nearly 100% at a virus dose of 1,000 or more LD_{50}, was significantly less frequent at 100 LD_{50}, and was rare with lower doses. Vesicles, sometimes preceded by erythema, first appeared in the area of virus inoculation 3 or 4 days after the injection. The intensity and distribution of the skin lesions increased over the next 2 or 3 days, during which time all rats that had the skin lesions became progressively sicker and died. The skin lesion developed as a line or band which

TABLE I. Frequency of Zoster-Like Skin Lesions in Baby Rats and Mice Given Herpes Simplex Virus Intradermally.

Animal	Age when inoculated (days)	Rats, amount of virus inoculated*					
		0–.8	1.0–1.3	1.5–1.8	2.0–2.3	2.5–2.8	3.0–3.3
Rats	2	0/8	1/23	1/12	9/31	14/20	21/24
"	3–7	—	—	—	0/6	—	5/6
"	9–10	—	—	—	—	0/30	0/3
Mice	2	—	0/8	—	0/8	—	0/8

* Amount of virus inoculated is expressed as $\log_{10}$ of number of mice I.C. LD_{50}, as determined by simultaneous intracerebral titration in 16-18 g mice.

TABLE II. Prevention by Antiserum of Zoster-Like Lesions Caused in Newborn Rats by Herpes Simplex Virus. (Virus dilutions were incubated with serum at 37°C for 60 min prior to inoculation.)

Source of antisera	Frequency* of zoster-like skin lesions in newborn rats: Virus dilution	Virus incubations with: Normal serum	Virus incubations with: Antiserum	Mouse I.C. LD_{50} titer after incubation of virus with: Normal serum	Mouse I.C. LD_{50} titer after incubation of virus with: Antiserum
Rabbit	10^{-1}	11/12	1/10	$10^{-4.4}$	$10^{-0.5}$
	10^{-2}	2/12	0/10		
	10^{-3}	0/10	0/10		
Guinea pig	10^{-1}	4/5	0/4	$10^{-4.0}$	$10^{-2.4}$
	10^{-2}	1/5	0/5		
	10^{-3}	0/5	0/5		

* Fraction indicates number of rats with skin eruption over total number of rats in each group.

apparently followed the distribution of spinal nerves. This band usually extended from the dorsal midline of the injected region (cervical, thoracic, or lumbar) to the ventral midline. Bilateral lesions often occurred when the injection was made near the midline of the back, but when lesions were unilateral they never extended beyond the midline. When injections were made high in the neck, the skin lesions involved the face on the same side. When the virus was injected into the skin of the abdomen instead of the dorsum, there was a centripetal spread of the lesions along the dermotomal segment yielding the same final distribution of vesicles as when the inoculum was given on the dorsum.

Susceptibility decreased rapidly with age (Table I). Rats inoculated when 3 to 7 days old developed the skin lesions only when the inoculum contained over 1000 infective doses of the virus. By age 10 days no lesions resulted even from the highest dose. Newborn mice did not develop these skin lesions, nor did they become sick when given as much as 1000 infective doses by the intradermal route, although they are at least as susceptible as adult mice to intracerebral inoculation of this virus.

Infective herpes virus was demonstrated in the vesiculated areas of skin of each of the 5 rats so tested on days 4, 5, and 6 after inoculation. Virus titers ranged from 10^{-2} to greater than $10^{-5.5}$. A 10% suspension of one of these preparations, which titered greater than $10^{-5.5}$ in mice, was also inoculated intradermally into 6 newborn rats, and reproduced the zoster-like lesion in all 6. A 10% suspension of normal-appearing skin from another site of the same rat was simultaneously inoculated into 5 newborn rats of the same litter and did not cause any skin lesions, but this unaffected skin was not tested for infectivity in mice.

Liver, spleen, and nervous tissue from rats with skin lesions were also tested for presence of infective virus on day 5 and day 6 after inoculation. In liver, 3 or 4 rats had infective virus at titers of 10^{-1} to greater than $10^{-3.5}$. In spleen, 2 of 4 rats had infective virus titers of $10^{-2.4}$ and greater than $10^{-3.5}$. Brain from 2 rats which were paralyzed and died on day 5 had virus titers of $10^{-3.5}$ and greater than $10^{-4.5}$. From 2 rats which were paralyzed and killed on day 6, the thoracic and lumbar spinal column was resected together with the paraspinal muscles and the spinal cord and homogenized *in toto*. Virus titers were greater than $10^{-5.5}$ for both.

A parallel study with virus which had been neutralized with either of 2 commercial antiherpes sera and virus incubated with normal control serum (37°C for 60 minutes) demonstrated that infective herpes simplex virus was needed to cause the skin lesion, thus ruling out the possibility that contaminating organisms or normal tissue components caused it (Table II). To confirm further that herpes virus was the causative agent, the skin lesions of affected rats were studied by fluorescein labeled antibody technique. Considering the amount of infective virus and severity of

TABLE III. Comparative Susceptibility of 3- to 7-Day-Old Rats to Herpes Simplex Virus, by Various Routes of Inoculation. (Fractions indicate number of mice which died over total mice in each group, after excluding deaths which occurred within 48 hours after virus inoculation.)

Route of inoculation	Virus dose* 3.2	2.2	1.2	.2
Intracerebral	5/5	6/6	6/6	2/5
Intraperitoneal	5/5	5/5	—	—
Intravenous	6/6	5/5	—	—
Intrathoracic	6/6	6/6	—	—
Intradermal	4/6	3/6	—	—
Subcutaneous	6/6	2/6	—	—
Gastric (by stomach tube)	3/6	0/5	—	—
Mice, I.C.	—	3/3	3/3	2/3

* See footnote to Table I.

tissue damage, the amount of reaction with the fluorescent antibody seemed surprisingly low. However, herpes virus antigen was demonstrated in the skin lesions in scattered cells in the basal epithelial layers of hair follicles and in inflammatory cells. Herpes antigen was also demonstrated by this technique in a few parenchymal cells of the hepatic and spinal cord lesions (see below).

Other routes of virus injection were also studied in 3- to 7-day-old rats, using amounts of virus which would be sufficient to cause skin eruption if administered intradermally. No skin lesions developed except in the rats which were inoculated intradermally. However, the virus caused infection evidenced by morbidity, paralysis, and death when administered by all routes tested. Mortality data, in comparison with mice inoculated intracerebrally, are presented in Table III. By the intracerebral route these rats were just as susceptible as mice. They were also highly susceptible to intraperitoneal, intravenous and intrathoracic inoculation, but somewhat less susceptible by subcutaneous or intradermal routes. They were least susceptible to virus given by stomach tube. Since we cannot exclude the possibility that the tube might have traumatized the mouth and esophagus enough to allow direct entry of virus through wounds, it cannot be concluded that these infections resulted from passage of virus through the gastric or intestinal mucosa.

Histologic study of hemotoxylin-eosin stain sections of skin lesions of rats which died or were killed when moribund (4-6 days after injection) revealed almost complete loss of epithelial elements except hair follicles and glands, and an inflammatory infiltrate with round cells predominating. The livers showed focal areas of hepato-cellular destruction characterized by pyknosis and cell loss with little inflammatory response. Brains and spinal cords showed focal areas of neuronolysis.

Autopsy of rats that died or were killed when moribund showed the same general pattern of pathologic changes for all routes of virus inoculation with the notable addition of ascites in most of the rats which were injected intraperitoneally. The ascites was a clear yellow fluid in volumes sufficient to cause abdominal distention (in the magniture of 10 ml). The liver lesions in many of the rats that had ascites were evident grossly as punctate tiny whitish lesions on the liver surface. A few eosinophilic intranuclear inclusions were observed in the basal epithelial cells of hair follicles, and in neurons in the spinal cord, but these were extremely rare and inconspicuous. (In HEp 2 tissue cultures also, this strain of herpes virus does not produce inclusion bodies.)

Summary and conclusions. Newborn rats are susceptible to infection and death by herpes simplex virus administered by many routes. When the virus is admitted intradermally a segmental skin eruption resembling herpes zoster develops. Hepatitis and encephalitis also occur. The etiologic role of herpes simplex virus in these skin and visceral lesions was demonstrated by neutralization and fluorescent antibody studies.

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