

Transmission of Street Rabies Virus Strain (V308) to Suckling Hamsters During Lactation. (22015)

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It has been assumed that a bitch infected with rabies transmitted the disease to the puppies by injuring them by biting or other mechanical injuries. There is still the question as to whether or not rabies virus may be present in the milk and if so whether the puppies can become infected from this source. Specific inclusion bodies called Negri bodies (1) are present in the central nervous system of animals dying with rabies. These are most frequently demonstrable within the nerve cells and are eosinophilic bodies, usually spherical, 1 to 30 μ in size. Their inner structure shows basophilic granules. The Syrian hamster infected with rabies virus shows Negri bodies in the brain in greater numbers and of larger size than those demonstrable in the Swiss albino mouse (2). The Syrian hamster was, therefore, selected for these experiments not only because of its Negri body "production" but because of ease in control and care.

Materials and methods. The virus for the present study was obtained from a dog brain sent to this laboratory for routine rabies examination. The hippocampus was removed aseptically, ground with alundum in a mortar, and diluted with physiological saline to a 10% suspension. Three-hundredths ml of this 10% suspension was injected intracerebrally into each of ten 3-week-old Swiss albino mice (Carworth Farm, Webster strain). On the 14th day after injection, several mice developed tremors and paralysis and became moribund. These brains were then removed aseptically. Impressions made from the Ammons horn of each mouse were stained with Seller's stain (3) and examination by the light optical microscope showed numerous Negri bodies throughout the tissue. The Ammons horn from one of these mice contained a larger number of Negri bodies than the others and it was ground with alundum and diluted to a 20% suspension with physiological saline. Each of ten 3-week-old Swiss albino mice was injected intracerebrally with this rich virus

suspension. Titer of the virus was $10^{-3.5}$ when titrated intracerebrally in Swiss albino mice of the same age. The 10 Swiss albino mice which were infected intracerebrally showed rabies symptoms within 6 to 10 days. Their brains which were removed aseptically contained Negri bodies and were ground and diluted to a 10% suspension in physiological saline. This virus-bearing mouse brain suspension was used to initiate the present study. For this series of experiments, diagnosis of rabies in hamsters and mice was made by the following criteria: (1) clinical observations of each hamster, (2) demonstration of Negri bodies in the Ammons horn of the suckling hamster, adult hamster and Swiss albino mouse, (3) reinoculation of the positive and negative suckling hamster brain and uninoculated controls intracerebrally into 3-week-old Swiss albino mice and confirmation by demonstration of Negri bodies in the brain smears. The presence of Negri bodies was demonstrated microscopically in touch preparations of the Ammons horn stained with Seller's stain. Ten lactating hamsters and their sucklings were placed in individual metal boxes with bedding. The sucklings were about 12 days old and are usually weaned at 16 to 18 days. Therefore, these were removed from the parent 6 days after her inoculation. The lactating hamster from each group (#1 to #9) was given 0.1 ml of the above described mouse brain bearing virus via the flank muscle. The area of injection was sterilized with iodine for one hour before the mother from each of the 9 groups was placed with her sucklings. The No. 10 group was kept as a room control. During the next 6 days, the family of each group was not disturbed. Dog checkers were fed to each of the 10 groups daily along with a large bottle of water for drinking (Table I). At the end of the sixth day, after inoculation of the parent, the sucklings from each group were weaned and placed in special glass jars. The lactating hamsters showed no rabies

symptoms at this time and were held for further observation. Seven of the 9 inoculated lactating adults developed rabies symptoms between the 14th and 22nd day after inoculation. Their brains contained numerous Negri bodies. The 10 groups of weaned hamsters from the 9 infected lactating animals and the control hamster were fed, watered and checked twice daily (Table II) for rabies symptoms.

Results. Two days after weaning or 8 days after injection of the adults, several hamsters from Groups 1, 2 and 3 (Table II) showed rabies symptoms and their brains contained numerous Negri bodies. Brains from all suckling hamsters showing rabid symptoms were ground in a mortar with physiological saline to a 20% suspension and injected intracerebrally into each of 6 Swiss albino mice. Mice showing rabies symptoms were examined for Negri bodies as shown in Table II. At the end of 21 days all suckling hamsters showing no rabies symptoms were sacrificed and their brains were removed aseptically and pooled for each group, ground in a mortar with alundum and diluted to a 20% suspension with physiological saline. Each of the 10 pooled groups, including the control Group #10, were injected (0.03 ml each mouse) intracerebrally in each of 6 Swiss albino mice (Table II) in order to confirm the presence or absence of the virus in sucklings showing no rabies symptoms.

Summary. From this observation it appears that rabies may be transmitted from lactating hamsters to suckling hamsters. Seventy-seven percent of the mothers showed rabies symptoms between 14 to 20 days.

TABLE I. Response of Lactating Hamsters Injected in Flank with 0.1 cc Street Virus Rabies Strain, V308. Suckling removed after 6 exposures. 9 groups.

Appearance of symptoms (days)	Negri bodies
14	+
16	+
16	+
16	+
17	+
20	+
20	+
—	—
—	—

TABLE II. Response of Suckling Hamster after Weaning from Lactating Parent (Infected with Rabies Street Virus V308).

Group No.	No. of suckling	Onset of rabies (days)	Negri bodies (sucklings)	Mouse inoculation	Negri bodies
1	5	8	+	+	+
		8	+	+	+
		9	+	+	+
		9	+	+	+
		9	+	+	+
2	5	9	+	+	+
		9	+	+	+
		9	+	+	+
		9	+	+	+
		—*	—	—	—
3	5	8	+	+	+
		8	+	+	+
		8	+	+	+
		10	+	+	+
		12	+	+	+
4	4	—	—	—	—
		—	—	—	—
		—	—	—	—
		—	—	—	—
		—	—	—	—
5	4	22	+	+	+
		—	—	—	—
		—	—	—	—
		—	—	—	—
		—	—	—	—
6 to 9	—	—	Negative		
10	6	—	" (uninoculated controls)		

* Showed no clinical symptoms.

31% of the suckling hamsters developed rabies symptoms between 8 to 22 days. Confirmation of rabies was made on all positive animals by Negri body examination. The possibility exists that rabies virus was present in the saliva of the mothers before the suckling animals were weaned, and consequently infection could have occurred by some route other than the milk. This could occur even without biting. Direct infection of mucous surfaces as the result of licking is possible, although this seems remote due to the high percentage of suckling hamsters showing rabies symptoms.

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