Study of Nine Rabies Street Virus Strains in the Syrian Hamster. (20493)

REGINALD L. REAGAN, MILDRED T. STEWART, AND ARTHUR L. BRUECKNER.
From the Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Md.

Specific inclusion bodies present in the central nervous systems of animals dying from rabies are called "Negri Bodies"(1,2). These are most frequently demonstrated within, but are revealed occasionally outside the nerve cells. A definite diagnosis of rabies is made upon demonstration of these bodies in stained smears. These inclusions are eosinophilic bodies, usually spherical, but may be of other shapes, and vary from 1 to 30 μ in size. The virus structure shows basophilic granules. At present the animal most frequently used for the diagnosis of rabies is the mouse. A positive diagnosis is made on the development of symptoms of rabies in the mouse and the presence of Negri bodies in the mouse brain. However, the Negri bodies are frequently small and few in number, which sometimes makes their detection difficult. In previous studies the authors found the Negri bodies present in the brains of rabies-infected hamsters to be large and quite numerous, and the authors found in one instance, that a rabies specimen was so grossly contaminated with bacteria that all hamsters and mice inoculated intracerebrally succumbed to bacterial infection within the first 24 hours, whereas those hamsters challenged rectally were unaffected by the contaminants and came down several days later with rabies symptoms, and the brains of these hamsters contained numerous "Negri bodies"(3). The present study was undertaken to compare contaminated dog brains and brain material not contaminated from the same brain specimen and the effect in hamsters after instillation of these specimens rectally.

Materials and methods. The strains of rabies virus used in this study were obtained from Dr. I. M. Moulthrop of the Live Stock Sanitary Service Branch Laboratory of Salisbury, Md. These strains are as follows: 25077 (dog brain), 25148 (dog brain), 25122 (dog brain), 25256 (dog brain), 25116 (dog brain), 25140 (dog brain), 25115 (dog brain), 25108 (dog brain), 25025 (dog brain).

These specimens had been proven positive for rabies at Dr. Moulthrop's laboratory. One half of each brain specimen was preserved in glycerin and stored in a –4°C ice box and the other half was placed in a separate jar and frozen in a –40°C deep freeze storage vault until initiation of the present study. The specimens were removed from the –40°C deep freeze and left at room temperature for several days until they ground in mortars with alundum and diluted to 20% suspensions with physiological saline. Each suspension was cultured in thioglycollate broth, and all were found to be contaminated with bacteria. The other half of each brain specimen was removed from the glycerin, ground in mortars with alundum, and also diluted to 20% suspensions with physiological saline. Each of these specimens was cultured in thioglycollate broth, and all were free from bacteria. Diagnosis of rabies in hamsters and mice was made on the following factors: (1) virus symptoms present in hamsters and mice and (2) demonstration of Negri bodies in the Ammon's horn of the hamster and mouse brain. The presence of Negri bodies was determined by staining touch preparations of the Ammon's horn with Sellers stain(4) and (3) examining the slides under an optical microscope.

Seventy-two healthy female Swiss albino mice, age 21 days, were divided into 18 groups of 4 animals each. Each group from No. 1 to No. 9 was inoculated intracerebrally with one of the contaminated rabies-bearing brain suspensions, and Group No. 10 to Group No. 18 was inoculated intracerebrally with the other half of each rabies-bearing brain suspension free of bacteria. Each mouse received 0.03 cc. Seventy-two healthy Syrian hamsters, age 21 days, were divided into 18 groups of 4 animals each. Into each group from No. 1 to No. 9 was administered one of the above contaminated virus bearing dog brain suspensions by rectal instillation, and

TABLE I. Response of Hamsters to Rabies Street Virus Administered Rectally from Contaminated Bacterial Suspensions and Bacteria Free Suspensions. Four hamsters exposed in each series. Negri bodies numerous in each series.

Group No.	Virus strain	Min to max incubation period, days	Type of rabies	
			Dumb	Furious
	Brain contaminated with bacteria			
1	25077			
2	25148	6-8	4	
3	25122	6-7	4	
4	25256	8-16	4	
5	25116			
6	25140	8-9		4
7	25115	14-16	4	
8	25108			
9	25025	14-15	3	
	Bacteria free			
10	25077	6-8	4	
11	25148	7-10	4	
12	25122	14-16	3	
13	25256	14-16	3	
14	25116	6-8	4	
15	25140	5-6		4
16	25115	5-6		4
17	25108	5-6		4
18	25025	14-16	3	

into each group from No. 10 to No. 18 was administered one of the above virus bearing dog brain suspensions free of bacteria by rectal instillation. Each hamster received 0.1 cc. For the rectal instillation, the end of an 18-gauge needle was filed off, rough ends smoothed down, and the tip lubricated with vaseline before being inserted.

Results. All Swiss albino mice inoculated intracerebrally with the bacteria contaminated specimens succumbed to bacterial infection within 24 hours. All Swiss albino mice inoculated intracerebrally with the bacteria-free suspensions showed symptoms of rabies between 6 to 8 days. The animals were sacrificed when symptoms of rabies appeared. By using the Seller stain technic numerous Negri bodies were found to be present in the brains of the infected animals.

Fifty-six hamsters which had received the virus bearing brain suspension rectally from the contaminated and bacteria free dog brain suspensions showed symptoms of rabies between the 6th and 16th days. The inoculation periods and the form of rabies (furious or dumb) for each strain from both groups are given in Table I. The animals were sacrificed when symptoms of rabies appeared. By using the Sellers stain technic numerous Negri bodies were found to be present in the brains of the infected animals. The hamsters that showed no rabies symptoms were discarded at the end of a 30 day observation period.

Summary. Nine dog brains found positive for rabies were divided into 18 groups. One half of each dog brain was preserved in glycerin, and the other half was contaminated with bacteria. The infected dog brains were instilled rectally in hamsters. All glycerinated specimens produced rabies in hamsters while only 65 of the contaminated specimens produced rabies in hamsters. The glycerinated specimens injected intracerebrally in mice produced rabies in all mice between 6 to 8 days while all mice inoculated with the contaminated specimens succumbed within 24 hours.

1. Negri, A., *Z. F. Hyg. u Infektionskr.*, 1903, v43, 507.

2. ———, *Z. F. Hyg. u Infectionskr.*, 1903, v44, 519.

3. Reagan, Reginald L., Day, William C., and Brueckner, A. L., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 654.

4. Sellers, T. F., *Am. J. Pub. Health*, 1927, v17, 1080.

Received July 8, 1953. P.S.E.B.M., 1953, v83.