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PROLONGED INCUBATION PERIOD OF RABIES IN A NATURALLY INFECTED INSECTIVOROUS BAT,

Eptesicus fuscus (BEAUVOIS)

Rabies is notorious among infectious diseases for length and variability of incubation period as well as for its malignant nature. In man, and in some domestic animals, the extremes of incubation period are rather well established and data are also available for experimental infections in many species, including wild animals. (Regamey, R. H., Hennessen, H., Ikic, D., and Ungar, J., Eds. 1966. Internat. Symp. Rabies Vol. I., S. Karger, Basel / New York). However, opportunities to determine incubation periods in naturally infected wild animals under controlled circumstances are uncommon. This report describes the delayed occurrence of rabies in a naturally infected insectivorous bat.

The infected bat was one of five Eptesicus fuscus collected at a sawmill in Missoula, Montana, on September 19, 1968. Because of frequent occurrence of rabid bats in western Montana (Bell, J. F., Lodmell, D. L., Moore, G. J. and Raymond, G. H., 1966, Public Health Reports 81: 761), the animals were considered undesirable on the premises. The method of collection was not stated, but two of the bats had broken wings and died within a few days of capture. Of the three remaining bats, one was given to a colleague at the Rocky Mountain Laboratory, one (the infected animal) died on April 20, 1969, 213 days after capture and at the time of this writing (March 2, 1970, 529 days) the fifth bat is still alive. Virus was not demonstrable in the saliva of this bat on September 19, 1969.

On September 20, 1968, the day of delivery to the laboratory, saliva swabs were taken from all five bats and eluates of the swabs were injected into 21-day-old mice by the intracerebral (IC) route. No infectious agents were isolated. A band was placed on the right wing of each bat, they were placed in individual

jars and fed a mixture of meat, oatmeal and vitamins previously determined to be satisfactory for growth and maintenance of *Myotis and Eptesicus*. Water was available at all times. The jars were kept on shelves in a room that occasionally housed a few other bats, none of them with known rabies infection. There was no possibility of contact between the bats. The ambient temperature in the room was usually about 23°C. Relative humidity was variable but usually low.

After 209 days in captivity, the infected bat became unusually sensitive to stimuli and reacted to disturbances, including entry of a person into the room, by agitated movement and by squeaking. Close observation revealed the occurrence of a generalized tremor.

Saliva swabs were taken from this bat on days 209, 210, 211 and 212 and eluates of the swabs were inoculated into groups of six 21-day-old mice by the IC route. Rabies virus was recovered from at least one mouse in each group. The bat was induced to bite the thighs of three 12-day-old mice on day 210 and all of these mice died of rabies. Three mice bitten on day 211 did not die, perhaps due to poor penetration of the skin.

The bat died on day 213. Rabies virus was isolated from brain, salivary glands and brown fat by IC inoculation of suspensions of these tissues into 21day-old mice. Identity of the virus as rabies was confirmed by the fluorescent antibody technique and by neutralization with known specific antiserum (Table 1). The incubation periods varied from 11 to 15 days.

The time of exposure and the exact time of first occurrence of signs of illness in this bat are not known, but a minimal incubation period of 209 days is established in this case.

TABLE 1. Identification of rabies virus with specific antiserum.

Tissue	Titer of virus LD₅₀/0.03 ml	Serum protection index
Brain	105.0	10 ^{3.0}
Salivary gland	10 ^{8.8}	>102.0
Brown fat	102.7	>101.4

The phenomenon of the very long incubation period often seen in man and in various species of animals after both natural and artificial exposure is fascinating but little understood. Baer and Bales (J. Inf. Dis. 117: 82, 1967) reported an incubation period of 177 days in a Freetail bat infected by IC injection of virus. Sulkin et al. (International

Symposium on Rabies, Talloires 1: 81, Karger, Basel/New York, 1966) have demonstrated the ability of brown fat of bats to support the replication of rabies virus, but incubation periods may also be very long in animals without brown fat, e.g. dogs. The stimulus that activates the infection is as poorly understood as the status of the virus during dormancy. Soave et al. (Am. J. Vet. Res. 25: 268, 1964) have shown that stress will activate infection in guinea pigs. In the present case, the stress of long captivity could have been responsible for activation of the infection.

Unfortunately, no samples of saliva from the bat were tested for infectivity in the weeks or months preceding signs of illness and, therefore, the question of whether a symptom-free carrier state could ocur in an insectivorous species remains unanswered.

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