Role of Brown Fat in Pathogenesis of Rabies in Insectivorous Bats (*Tadarida b. mexicana*)* (23507)

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At least 2 human rabies deaths are believed to have resulted from exposure to bats in this country. The first was reported by Sulkin and Greve(1) and concerns a woman who died in October, 1951. The second, a member of the Entomology Section of the Texas State Department of Health, occurred in January, 1956(2). These cases, together with isolation of the rabies virus from a number of species of bats in widely scattered areas of the United States has resulted in a reappraisal of the public health significance of these animals(3,4). The initial isolation in 1953 of the rabies virus from bats in Florida(5) and the almost simultaneous isolation of the virus from other bat species by other investigators (6,7) stimulated teams of mammalogists and virologists to investigate the ecology of rabies in different geographic areas. The accumulating literature indicates that most of the studies thus far conducted have been concerned with determining which bat species are naturally infected. Only a limited number of studies have dealt with the progress of the disease in experimentally infected bats(8-11).

Recent studies by Shwartzman(12,13) demonstrating lipotropism in cortisone-treated hamsters infected with poliovirus together with previous studies concerned with the effects of environmental temperature and hormonal factors on experimental virus infections (14,15) suggested an approach to the study of the role of the bat in the biological life cycle of the rabies virus. Aronson and Shwartzman (16) demonstrated reproducible histologic lesions and high infective virus titers in brown (embryonal) fat in cortisone-treated hamsters infected with poliovirus, indicating marked lipotropism in this infection. These observations have been confirmed in part by Bodian (17) who showed high concentrations of virus in brown fat in chimpanzees after the viremic phase of the infection, and suggested that the brown fat might be regarded as a "target organ" or secondary site of poliovirus multiplication. Invasion of brown fat has also been observed in suckling mice infected with certain strains of Coxsackie virus(18,19,20) but no other examples of lipotropism in viral infection have come to our attention. The known effects of environmental temperature and hormonal factors on experimental viral infections(14,15) together with the observed sensitivity of brown fat to these and other stress mechanisms(21) suggest that this tissue might in some way be involved in the persistence of the rabies virus in symptomless carriers. Furthermore, the susceptibility of this tissue to certain virus infections is so great that it seems to persist when most other organs and tissues have become refractory (18).

The purpose of the present study has been to examine the pathogenetic sequence in experimentally infected insectivorous bats with the view to learning something of the possible mechanism by which these animals may serve as persisting reservoirs for the virus in nature. An effort has been made to study the pathogenesis of rabies in this host with the view to finding anatomical sites of viral multiplication. The results to be reported suggest that the brown (hibernating) fat provides a depot for storage of the virus. Furthermore, these studies provide another example of viral lipotropism.

Methods. Because of the tremendous populations of Mexican free-tailed bats (*T. braziliensis mexicana*) in the southwestern part of the U.S. and since a large proportion of the rabies virus isolations were made from this species, this bat was used in these studies.†

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Technics for the safe laboratory care and successful maintenance of infected experimental populations for long periods of time have been described elsewhere(22). The rabies street virus used in the present study, and designated the Thompson strain, was isolated in albino Swiss mice (CFW) from a human brain and had been through two intracerebral passages in white mice. The LD_{50} titer of the stock virus suspension for 3-week-old white Swiss mice inoculated intracerebrally was $10^{-4.8}$. Bats were inoculated intramuscularly into the heavy muscle over the breast. The inoculum consisted of approximately 8000 mouse intracerebral LD_{50} contained in 0.1 ml. The Mexican free-tailed bats (*Tadarida b. mexicana*) were netted on the day the experiment was initiated and were chilled to facilitate handling during inoculations and distribution into cages. The inoculated animals were maintained at 29 C ± 2 (relative humidity 65%) and were fed daily during the observation period of 3 months. All bats were observed daily for symptoms of rabies. Those which died during the first week were discarded. The selective affinity of various tissues of the bat for rabies virus was shown by viral assay in 3 to 4-week-old white Swiss mice (CFW). Tissues were preserved in a mechanical refrigerator at -40°C until ready to be tested. For qualitative assay of brain, salivary gland, and interscapular brown fat 3-5 mice were inoculated intracerebrally with each specimen (10% brain, 2% salivary gland, and 2-5% brown fat) and observed for a 30-day period before being discarded as negative. In the quantitative assays serial 10-fold dilutions of the respective tissues were inoculated intracerebrally into groups of 5 mice each and the LD_{50} titer was established in each instance by the method of Reed and Muench(23). Since Negri bodies are not always demonstrable in tissues of bats proven to contain virus by mouse inoculation(8,11) histologic examination of the various tissues was not made routinely.

Results. Of the 163 Mexican free-tailed bats inoculated intramuscularly with rabies virus, 26 died during the first 7 days after the virus inoculation and have been excluded from the analysis.‡ Qualitative assays for virus showed evidence of rabies infection during the observation period of 3 months in 32 or 23.3% of the remaining 137 bats. Virus was detectable in the brain in 28 (20.5%) salivary glands in 10 (7.5%), and in the interscapular brown fat in 11 (8.0%). Table I summarizes the data on 11 bats in which the brown (hibernating) fat contained detectable or significant amounts of rabies virus. It appears from these data that the virus is most widely distributed in this host between the 20th and 30th days following intramuscular inoculation. In addition there seems to be more active viral proliferation during this period of the infection. In 4 instances rabies virus was detectable in the brown fat and not in the brain or salivary gland. These animals, however, were sacrificed between the 32nd and 75th days after the virus inoculation. The remaining 21 bats which showed evidence of rabies infection all contained virus in the brain with occasional demonstration of virus in the salivary glands. A small percentage (<2%) of the bats used in these experiments may have been naturally infected with rabies virus.

In the few instances in which symptoms in-

TABLE I. Demonstration of Rabies Virus in Interscapular Brown Fat and Other Tissues of Bats (*Tadarida b. mexicana*) Inoculated Intramuscularly and Maintained at 29°C.

Duration of infection*	Rabies virus demonstrated in Brain	Salivary gland	Brown fat
22	+ (2.43)†	+ (2.33)	+ (1.66)
22	+ (2.75)	—	+
22	+ (3.5)	+ (2.75)	+ (3.0)
22	+ (2.5)	—	+ (1.5)
25	+ (3.0)	—	+ (1.5)
26	+	—	+
32	—	—	+ (2.5)
32	—	—	+
42	+	+ (2.75)	+
75	—	—	+ (1.5)
75	—	—	+ (1.5)

* Indicates day after intramusc. inoc. (0.1 ml) bat found dead or sacrificed. Virus inoculum ± 8000 mouse i.c. LD_{50}.

† Figure in parentheses indicates neg. log LD_{50} i.c. titer for mice.

‡ Studies(22) concerning the laboratory care and maintenance of this bat species have indicated that those which survive 7 or 8 days in captivity can usually be kept for several months or longer.

cluding irritability and aggressiveness occurred prior to death it was clear that the incubation period in bats following intramuscular inoculation of virus was considerably longer than in mice inoculated intracerebrally, thus confirming previous reports with other species of bats and different virus strains(8, 11). A few Mexican free-tailed bats inoculated intracerebrally with the Thompson strain of rabies virus used in these experiments also showed longer incubation periods than mice inoculated by the same route.

Whether or not animals developed symptoms of rabies and recovered could not be clearly established in these experiments because of the difficulties involved in handling these animals.

Discussion. In the present studies only 23% of Mexican free-tailed bats showed evidence of infection following intramuscular inoculation of rabies virus, and only a small proportion of these developed symptoms of rabies. This is not surprising since Enright and his associates(10) had shown that none of a limited number of the same species of bats inoculated intramuscularly with a strain of rabies virus originally isolated from *Tadarida* developed rabies symptoms. Whether these animals became infected was not established. Burns and his coworkers(8) have also shown that some *Tadarida b. mexicana* fail to develop symptoms of rabies; virus could be demonstrated in brain and salivary gland even though the animals showed no clinical evidence of illness. In earlier studies with vampire bats, Johnson(24) reported that in experimentally infected bats the incubation periods ranged from 7 to 171 days and some animals failed to develop the disease. Failure to infect a larger proportion of the bats by the intramuscular route of inoculation may have been due to the presence of neutralizing antibodies in many of these animals(8).§

The studies described in this report suggest that the rabies virus is capable of proliferation in the interscapular brown fat of the Mexican free-tailed bat following intramuscular inoculation. The brown fat may constitute a depot or "target organ" in which a hardy agent like the rabies virus is likely to persist for long periods of time. Virus has been demonstrated in this tissue in bats showing no overt signs of the disease 75 days after intramuscular inoculation of the virus. Preliminary studies to be reported later have already indicated that gravid bats‖ are more susceptible than nongravid animals, thus serving as a possible mechanism for activation and perpetuation of the agent in this spring-breeding species. While the function of brown fat is not clearly established, there is evidence that it suppresses metabolism during winter sleep (25). Since brown fat is seasonally variable in amount and most abundant during the winter months, it is likely that the decreased metabolic rate during periods of cold weather may be concerned with the latent period of the rabies virus in bats. At such times one would expect less active viral proliferation. Whether virus can be demonstrated in brown fat of bats under natural circumstances remains to be determined.

Studies designed to determine the route by which the brown fat is invaded by the rabies virus are in progress. It is hoped that temporal studies will establish the mode of progression of the virus following peripheral inoculation.

These studies in addition to including the rabies virus among those (poliovirus and certain Coxsackie viruses) which exhibit lipotropism suggest that this may prove to be a characteristic feature of infection with "hardy" viruses.

Summary. 1. Studies on the progression of peripherally inoculated rabies virus in Mexican free-tailed bats (*Tadarida b. mexicana*) suggest that the brown (hibernating) fat plays a significant role in the pathogenesis of rabies in this animal. 2. Brown fat may serve as a depot for the storage of virus in symptomless carriers of the rabies virus. 3. These studies provide another example of selective viral lipo-

§ It should be noted, however, that Schneider and his colleagues(4) failed to demonstrate neutralizing antibodies in another species of *Tadarida* (*T. cynocephala*) in another geographic area.

‖ Rabies virus has been demonstrated in the brain tissue of 2 infant bats suckling on an experimentally infected bat.

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Inhibitory Influence of a Normally Occurring Pyrimidine Precursor upon Methylcholanthrene Carcinogenesis.* (23508)

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In studies of the mechanism of action of urethane in initiating pulmonary adenomas in the lungs of mice, orotic acid, a naturally occurring pyrimidine(1) known to play a large part in nucleic acid synthesis(2) was found to be one of the most potent inhibitors of the sequential metabolic processes originally set off by the carcinogen(3). The following experiments were carried out to determine whether it might influence the carcinogenic effect of methylcholanthrene, a polynuclear hydrocarbon. The fact that polynuclear hydrocarbons exert their effect over a long period of time necessitated, however, the use of a different technic from that carried out in the studies of the short acting carcinogens (3,4).

Materials and method. Mice of the Swiss and "C" strains, raised in this laboratory and procured originally from the stock of the Rockefeller Institute for Medical Research, New York City, and mice of the "A" strain procured from Bar Harbor, Maine, were used. The Swiss and "A" strain animals develop large numbers of pulmonary tumors following administration of a variety of carcinogens. In the "C" strain the tumors are much less numerous. In each experiment the animals used were of the same sex and matched as to age and weight. They were all kept on a stock diet (Laboratory Mouse Chow, made by Dietrich and Gambrill, Frederick, Md.) This was

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