# Chiropteran Rabies in Montana

By J. FREDERICK BELL, M.D., W. J. HADLOW, D.V.M., and WILLIAM L. JELLISON, Ph.D.

A BAT was brought to the Rocky Mountain Laboratory of the Public Health Service by one of its employees on August 20, 1954. The bat, found in a flowerbed at the employee's home, revealed its presence by squeaking at approaching children. Suspecting a snake, the children called their father, who captured the bat without difficulty since it made no attempt to fly.

Upon cursory examination in the laboratory, it was apparent that the animal was weak. Since the bat vociferated when the children approached, it was probably irritable. After euthanasia with ether, the brain was removed and a suspension of approximately 10-percent concentration in serum-saline was prepared from one-half of the brain. Four mice were inoculated intracerebrally with 0.03-ml. portions of the suspension, and the remainder was frozen in sealed ampules. The other half of the brain was fixed in Zenker's fluid. After necropsy, the thoracic and abdominal viscera as well as the carcass were placed in 10-percent formalin.

On the 16th day after injection of brain suspension, 2 of the mice were found dead. The brains were removed, pooled, triturated in serum-saline, and dilutions of the suspension were used to inoculate other mice. The earliest signs of illness in the mice of this second passage were seen on the 11th day, and 6 mice injected with the $10^1$ dilution were dead by the 14th day. A similar incubation period was observed in third-passage mice injected intracerebrally. A fourth passage by the intracerebral route resulted in a shorter incubation period in those mice injected with 10-percent supernate, inasmuch as the first signs of illness appeared on the 7th day and all 6 mice were dead by the 11th day. Typical Negri bodies were found in the brains of the mice from the first and second passages.

Virus in the bat brain was not titrated immediately, but at a later date some of the original frozen suspension was found to contain $10^3LD_{50}$/.03 ml. The incubation period ranged from 14 to 19 days.

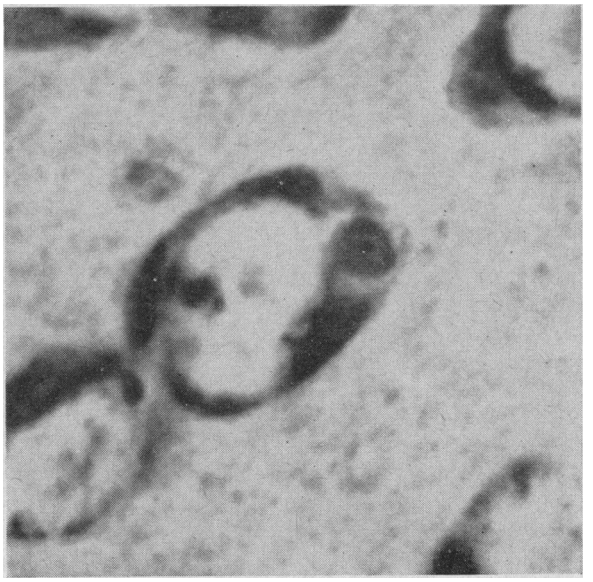
*Dr. Bell is senior surgeon, Dr. Hadlow, a pathologist, and Dr. Jellison, a parasitologist, on the staff of the Rocky Mountain Laboratory, Public Health Service, Hamilton, Mont.*

## Microscopic Findings

Histological sections of the bat brain stained by Lillie's buffered azure eosinate method revealed widespread regressive changes in the neurons as evidenced by their shrunken, distorted, and hyperchromatic appearance. The neuronal damage was most conspicuous in the thalamus and hypothalamus and in some areas of the cerebral cortex but was evident also in the hippocampal formation and in the Purkinje cells of the cerebellum. Inflammatory changes were not observed with the exception of a scanty lymphocytic accumulation around several meningeal vessels. There was moderate hyperemia of the meningeal and parenchymal vessels.

Typical round and oval Negri bodies were found in the pyramidal cells of the hippocampus and the cerebral cortex (see fig.), in the Purkinje cells of the cerebellum, and occasionally in the nerve cells elsewhere in the brain. The inclusion bodies, however, were largest and most numerous in the hippocampus. The largest measured 3.5–4.0 microns in diameter and contained distinct basophilic granules usually situated centrally in the inclusion and often surrounded by a prominent hypochromatic ring or halo. Individual neurons frequently contained several smaller, cytoplasmic inclusion bodies, measuring 1–2 microns. Some of these small inclusions appeared to be uniformly eosinophilic. The Negri bodies were found in apparently unaltered cells as well as in those showing marked regressive changes.

Microscopic sections of heart, lung, liver, spleen, kidney, and small intestine showed no noteworthy changes.



**Large Negri body in a neuron of the hippocampus in the original bat brain, stained by azure eosinate (×2700).**

### Identification of the Virus

In view of present widespread reports of rabies in Chiroptera (*1*), rabies was suspected when the first-passage mice became ill. The appearance of the sick mice and the finding of typical Negri bodies in mice and in the bat brain were evidence that the disease was rabies or a hitherto unknown lyssoid infection. Therefore, neutralization and protection tests, with known rabies serum, and cross-protection tests, using vaccines prepared from fixed rabies and from the putative virus, were done.

### Neutralization and Protection Tests

Antirabies serum was obtained from Dr. Karl Habel of the National Institutes of Health, Bethesda, Md. A serum used as a control was obtained from the blood of stock rabbits. These two serums were used in neutralization tests versus a laboratory strain of fixed rabies virus, the bat virus, and, in addition, a laboratory strain of western equine encephalitis virus which was included to establish the absence of nonspecific virucidal effects. At the time of the neutralization tests, only virus lots of low titer were available, and, therefore, the serums were tested against small doses of virus. However, the results were clear-cut: The known antirabies serum in a 1:10 dilution gave complete protection against 50 $LD_{50}$ of the bat virus and 15 $LD_{50}$ of known rabies virus (the largest doses tested) but failed to protect against even 1 $LD_{50}$ of western equine encephalitis virus. The normal rabbit serum afforded no protection against any of the viruses. Refined and concentrated antirabies serum (Lederle) injected intraperitoneally in mice also protected the animals against both the bat virus and fixed rabies virus.

### Vaccine Protection Tests

Vaccines were prepared by the method of Habel, Bell, and Wright (*2*) from the brains of mice injected with the bat virus and infected with fixed rabies virus. Sufficient numbers of mice were injected with each vaccine (*3*) to permit cross-immunization tests with groups of 12 mice per dilution. Normal mice of the same age were challenged as controls. Unfortunately, the fixed rabies challenge and control titrations were done with dilutions of suspensions of brain tissue rather than with dilutions of supernate. However, the results clearly indicate that both vaccines afforded significant protection against homologous and heterologous challenge (see table).

A few vaccinated mice were challenged via the intraperitoneal route with 0.1 ml. of a $10^1$ dilution of fixed rabies virus suspension. The results follow:

1. Normal mice (controls): 6 of 6 injected dead within 10 days.
2. Mice vaccinated with fixed rabies virus vaccine: 1 of 5 injected dead on the 2d day, and therefore apparently was not specific.
3. Mice vaccinated with bat virus vaccine: 1 of 6 injected dead on 10th day.

### Characteristics

In our limited experience with isolation of street virus, the long incubation period of this strain is unusual. Ernest Tierkel of the Communicable Disease Center, Atlanta, stated in a personal communication that a long incubation period is common in isolations from Florida bats but not in isolations from free-tailed bats (*Tadarida*) of the southwestern United States. The incubation periods in 3 rabbits injected intrathecally (cisternal puncture) with 0.2 ml. of a $10^2$ dilution of the second mouse passage were 14, 15, and 24 days, respectively. Mice inoculated intracerebrally with 0.03 ml. of the same dilution became ill on the 10th day and all succumbed by the 15th day. A pool of brain tissue of the above rabbits was tested for virus content by titration in mice ($10^2$/0.03 ml.) and 0.2 ml. was injected intrathecally into 4 rabbits. One rabbit died suddenly on the 15th day. One developed paralysis on the 30th day. The latter animal had much virus in the brain whereas another 1 of the 4, sacrificed the same day and apparently normal, had none. The 4th rabbit was alive 60 days after injection. None of the affected rabbits showed evidence of a furious syndrome but developed rather sudden paralysis.

Mice that succumbed to infection usually developed paralysis in the hind quarters from one to several days before death. Seven mice injected with the virus developed the same paretic signs as many others injected at the same time but have not yet succumbed to the infection (45 days after injection) and appear to be in good health except for the paralytic sequelae which limit their movements. These animals are being studied further to establish, if possible, that they have survived active rabies infection.

**Results of vaccine protection tests**

| Experiment No. | Vaccine | Challenge | Titer log $LD_{50}$ | Protection log $LD_{50}$ |
|---|---|---|---|---|
| 196: | | | | |
| A | Fixed rabies | Bat virus | $<2.0$ | $>2.75$ |
| B | Bat virus | do | $<2.0$ | $>2.75$ |
| C | | do | 4.75 | |
| D | Fixed rabies | Fixed rabies | $<2.00$ | $>4.00$ |
| E | Bat virus | do | 3.87 | $>2.13$ |
| F | | do | $>6.00$ | |

### Identification of the Bat

The formalin-fixed bat specimen was transferred to 70 percent alcohol and sent to the Division of Mammals, United States National Museum, Washington, D. C. There it was identified as *Eptesicus fuscus pallidus* Young by Dr. David H. Johnson and accessioned as No. 298459 in the mammal collection. While the skull had been damaged by removal of the brain, the dentition and other characters permitted specific identification. The range of this subspecies extends from the Great Plains westward nearly to the Pacific Coast and from Canada to Mexico. Upon our inquiry and reexamination of the specimen, Dr. Johnson replied that there was "no reason to doubt that it is a native of your vicinity." The question arose because of the recent prior arrival from Ohio of a canvas-covered trailer which was parked in the neighborhood where the bat was captured.

*E. f. pallidus* is a large insectivorous bat which is not known to migrate but hibernates in caves in the northern States (*4*, *5*). The known range of movement is from 33 to 61 miles in summer and winter, respectively. Engler (*6*) states that they are cannibalistic in captivity.

### Infection of Bats by Various Strains

Unfortunately, the identification of rabies in a bat came at a time when very few of the animals could be found in this area. Several reports of concentrations of bats were investigated, but in most cases only guano was present as evidence of previous habitation. A few ani-

mals, however, were recovered. Four specimens brought to the laboratory dead or which died soon afterwards were tested for rabies, but the virus was not demonstrated. Several other bats in good condition were maintained in the laboratory by feeding them on condensed milk and homogenized liver. Several of these (*E. f. pallidus*) were injected.

Bat 9194, injected intracerebrally with fixed virus, showed only mild irritability 25 days later. It was sacrificed that day and virus was detected by passage of brain tissue triturate to mice. It was notable that the incubation period of the disease in mice was several days longer than was usual for this strain. (It is possible, of course, that the bat was infected in nature before we collected it.) In the bat brain a few Negri bodies were seen in the pyramidal cells of the hippocampus but none in the Purkinje cells of the cerebellum.

Bat 9195, injected intracerebrally with bat virus (second mouse passage), showed tremors beginning on the 14th day. Weak, tremulous, ineffectual movements characterized the illness of this bat until it was sacrificed on the 25th day. Many small Negri bodies were noted in the hippocampus and multiple small Negri bodies in the Purkinje cells of the cerebellum. Inflammatory changes had occurred in the cerebral cortex and overlying meninges. The incubation period in mice injected with a suspension of brain from this bat was markedly shorter than in the case of bat 9194.

## Discussion and Summary

A virus isolated from a sick bat in western Montana has been identified as rabies virus. The virus was subjected to neutralization and protection tests with known antirabies serums, and to cross-protection tests with vaccines prepared from the bat virus and from fixed rabies virus. The results of those tests clearly indicated an antigenic relationship between the viruses. Because of small quantitative differences in cross-immunity tests, it appears that the bat virus antigen may not be fully immunizing versus fixed rabies infection. This is the converse of the conclusions of Kubes and Gallia (*7*) in regard to chiropteran and Pasteur strains.

The incubation period of the disease in mice was prolonged in first passage but became shorter in serial passages. A titer of $10^3$ $LD_{50}$/0.03 ml. was found in the original brain suspension.

Typical Negri bodies were seen in the brain of the original bat and also in two bats injected intracerebrally, one with fixed virus and the other with bat virus.

The bat from which the virus was isolated has been identified as *Eptesicus fuscus pallidus*, an insectivorous species indigenous to western United States. This is the first reported isolation from the species, and the northernmost isolation from a bat. The source of infection in the bat is conjectural. Rabies has not been reported in Montana since 1952 according to H. F. Wilkins, Montana State veterinary surgeon. Bats of the species *Eptesicus fuscus* are known to hibernate, but whether they may also migrate is not established.

### REFERENCES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