Over a period of seven years 21 rabid bats of eight species have been found in western Montana. The epidemiology of bat rabies in this area is examined and various facets are presented.

# CHARACTERISTICS OF RABIES IN BATS IN MONTANA

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**S** INCE the isolation of rabies virus from a bat in western Montana in the late summer of 1954, bats of all species known to occur in Montana, except the rare Euderma maculata and Myotis subulatus,<sup>1</sup> have been collected and examined for rabies virus. Species examined thus far include<sup>2</sup> Eptesicus fuscus, Myotis californicus, Lasiurus cinereus, Lasionycteris noctivagans, Myotis evotis. Myotis volans, Mvotis vumanensis. Myotis lucifugus, and Corvnorhinus (Plecotus) townsendii. Most of the animals were from the Bitterroot Valley in southwestern Montana, but a few specimens were submitted from various other areas of Montana. Rabies virus was recovered by technics described in the following and the ability of bats to transmit infection by bite was demonstrated.<sup>3</sup>

Behavior of rabies-infected bats ranged from apparently normal to actively aggressive, but the majority of animals were found in circumstances that were aberrant and even suggestive of rabies. Although only two of the 21 infected bats collected bit human beings and in only one instance could the attack be considered unprovoked, belligerence was evident in several instances. In one instance a bat was "flying at" children and dogs; in another, two bats were fighting viciously and the survivor was rabid. On three occasions bats were taken away from cats and a dog but the aggressor in these cases was unknown. The dog and cats did not become ill during close observation for

six months. One apparently normal, but infected, Eptesicus kept in a captive colony for 30 days after capture did not infect four other Eptesicus kept with it.

Rabid bats are much more likely than normal bats to come to the close attention of man. This is especially true of solitary bats that normally lead rather obscure lives. Several thousand bats, most of them colonial, have been examined. Although only a few individuals of some species have been encountered, the incidence of rabies in them has been unusually high, suggesting that illness was responsible for their discovery. For example, only one Lasiurus cinereus was obtained, and it was rabid. Myotis californicus was found singly and in groups on four occasions and two bats of this species were rabid. The species had not been recorded previously in Montana. Myotis volans was found only three times and one specimen was rabid. Six Lasionycteris noctivagans were submitted one year and three the next year; the first three examined were infected.

Infrequency of encounters with normal bats of the above species does not necessarily indicate rarity, but may reflect habits and habitats that do not ordinarily bring them into contact with man.

There was usually some evidence of irritability and willingness to bite and this seemed to be an accentuation of normal disposition. The normally more surly Lasionycteris and Eptesicus were more vicious than the smaller and milder Myotis species. However, the only bat that clearly attacked unprovokedly was the little Myotis californicus. A Myotis evotis on a screen door flew, or fell, onto a man's sleeve and bit him when he tried to remove it.

In several instances there was evidence of confusion—one bat flew into the bole of a tree in daylight, another lit on the pillow of a sleeping man, a third was flying around a house in midday. In view of this confusion and a readiness to bite, the importance of provocation as a factor in human infection is questionable. Many of the bats were found under circumstances which made them actually, or potentially, accessible to children and pets, and opportunities for transmission of infection were frequent.

This paper presents the relevant data obtained in seven years of study of bats in Montana.

## Methods

In our early studies, only brain tissue was taken from bats. This was diluted with 10 per cent albumin in saline at pH 7.2 to approximately 10 per cent concentration. Weaned mice were injected intracerebrally with 0.03 ml Mice that developed early samples. signs of infection (usually paresis) were killed and brain tissue virus was then subjected to neutralization and protection tests for identification. For the neutralization test, serum was prepared from rabbits hyperimmunized with the Pasteur strain (NIH:PV-1); protection tests were done with mice previously Flury immunized with the strain (HEP)\* Later, salivary glands, brown fat, and other organs were also injected into mice.

When rabies infection was strongly suspected, attempts were made to demonstrate virus in saliva by the use of oral swabs and by inducing the bat to bite laboratory animals. Swabs were prepared by soaking cotton-tipped sticks in 10 per cent albumin-saline diluent containing penicillin and streptomycin, expressing excess diluent, swabbing the oral cavity of the bat, and agitating the swab in the diluent which was then injected into mice. Mice to be bitten by bats were selected according to the size of the bat. Two-day-old mice were used for Myotis; older mice for Eptesicus and Lasionycteris. Most of the bats would bite voluntarily when held by the nape if the thigh of a mouse were thrust at them. Others could be teased into doing so. In almost all instances discernible punctate wounds were inflicted. Only half of a litter of mice was subjected to bite. The other half was used for a control on mortality. None of the mice were given identifying marks because of possible incitation to cannibalism by the dam. Mortality from rabies was unequivocally determined in each instance tabulated, but early deaths in either bitten or control mice were undiagnosed.

Negri bodies were sought in imprints of infected bat and mouse brains stained by Seller's method. William's stain was used to demonstrate Negri bodies in sections cut from infected brains.

## Results

## Isolation of Virus by Swabbing the Oral Cavity

Every one of seven attempts to isolate rabies virus by swabbing the oral cavity of four naturally infected bats of three species has been successful (Table 1). Virus was present in saliva of bat No. B300 on at least three occasions over a period of nine days and in bat No. B548 virus was demonstrated twice in three days. We have used the same technic on 222 bats collected routinely, and on 142 captured and released, but have never isolated rabies virus (although we have isolated other viruses) from any animal that did not have the virus in

<sup>\*</sup> HEP vaccine was obtained through the courtesy of Dr. George R. Sharpless of Lederle Laboratories, Pearl River, N. Y.

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No.	Species	Sex	Circumstances Date	Br.	s.c.	B.F.	Oral Swab	Bite	Negri Bat	Body Mouse
96K	E. fuscus	۵.	In flower garden 8/20/54	+				1	+	+
08K	M. californicus	۵.	Attacked by dog 7/18/55	+	I	I	I	ł	• +	• +
080	E. fuscus	۰.	Found "sick" in unused house 8/8/55	• +	I	I	I	I	• +	• +
080	L. cinereus	۰.	Flew into bole of tree in daylight 8/18/55	+	I	I	I	I	+	• +
25K1	M. evotis	۵.	Alive on lawn 10/11/56	• +	0	I		I	- +	- 1
41B1	M. evotis	۵.	On screen door 7/17/57	+	0	1	I	I	ł	+
41G1	E. fuscus	۰.	Routine collection 8/1/57	• +	+	I	1	1	+	- +
41SI	M. volans	~•	On louvers of house 8/31/57	• +	· +	I	I	I	-	- +
A166	M. yumanensis	€0	In barn 6/19/58	• +	0	ł	I	1	I	. 0
I52	M. californicus	€O	Attacked hunter 9/21/58	• +	+	I	l	+	+	+
58 and 61 Pooled	M. lucifugus	0+ 0+	Routine collection 5/27/59	+	0	+	I	• 1	. 0	. 0
B38	E. fuscus	о+	Routine collection 5/19/59	+	+	ł	ł	I	0	0
B249	E. fuscus	€O	Alive on sidewalk 8/9/59	• +	. 0	. 0	I	·I	0	0
B256	L. noctivagans	0+	Found on pillow 8/27/59	+	+	+	+	,+	0	0
B300	L. noctivagans	0+	Found in bedroom 9/1/59	+	+	-+-	• +	+	0	0
B316	E. fuscus	0+	Cat "playing" with bat 9/11/59	+	+	+	.	. 0	0	0
B319	M. evotis	0+	On chimney in house 9/15/59	+	÷	+	I	I	I	+
B324	L. noctivagans	0+	Cat "playing" with bat 9/24/59	+	0	0	I	I	0	- +
B328	M. evotis	€0	Flying in daylight 10/6/59	+	ł	+	+	+	0	- +
A453	E. fuscus	0+	"Flying at" children and dogs 8/25/60	• +	• +	• 0	-	-	• +	- +
A548	E. fuscus	0+	Fighting with other Eptesicus 9/12/60	+	+	+	+	+	• +	- +
* Br. = brair + indicates 0 indicates - indicates	n; S.C.=salivary gland; s positive test. i negative test. i no test.	B.F. = brown	fat.		5. T					

	No. of						Number	of Mice	
Year	Bats Tested	Species Infected	Date of Bite	Date of Death of Bat	Age of Mice	In Group	Bitten	Died*	Rabies- Infected
1958	759	M. californicus	Sept. 22	Sept. 28	l-day	6	3	3	3
1959	22	L. noctivagans	Aug. 29	Sept. 8	4-day	6	3	4	2
			Aug. 31		12-day	6	3	1	1
		E. fuscus	Sept. 12	Sept. 13	12-day	6	3	1	0
		L. noctivagans	Sept. 2	Sept. 13	12-day	6	3	3	3
			Sept. 9		12-day	6	3	1	0
			Sept. 11		2-day	6	3	2	2
			Sept. 11		12-day	6	3	0	0
			Sept. 11		21-day	6	3	2	2
		M. evotis	Oct. 6	Oct. 6	2-day	6	3	3	3
1960	21	E. fuscus	Sept. 12	Sept. 18	12-day	3	3	0	0
			Sept. 12		Old adult	ts 3	3	0	0
			Sept. 15		21-day	6	3	2	2

Table 2-Transmission of Rabies to Mice by Bites of Infected Bats

\* Including nonspecific (usually traumatic) deaths.

its brain at the time of death. Thus, there is no evidence in these data that a carrier state exists.

## Transmission by Bites of Naturally Infected Bats

Eight hundred and two bats inflicted bites on mice on at least one occasion and many of them did so repeatedly (Table 2). Rabies virus was obtained only from mice bitten by bats which later died with demonstrable infection, but in two instances other infectious agents were found in mice bitten by nonrabid bats. The identities of these agents are not yet established. They may be indigenous in our mouse colony.

Results of the test of transmission efficiency cannot be compared directly with the published results of tests by others, because the species of bats involved are different and strains of mice vary greatly in susceptibility.<sup>4</sup> The tabulated data indicate that at least four species of insectivorous bats are quite capable of transmitting infection to mice by bite and that failure to transmit may be the exception. The exceptions are difficult to explain. Thus, bat No. B300 transmitted infection to three of three 12-day-old mice on September 2, 1959, and to two-day-old mice and 21-day-old mice on September 11, 1959, but failed to infect 12-day-old mice on the latter date. About one hour elapsed between the biting of the various age groups on September 11, 1959, and, therefore, it seems unlikely that virus in the mouth was greatly diminished by previous Bat No. B548 also failed to biting. transmit by bite on one occasion, September 12, 1960, when virus was demonstrated by swab, but succeeded in transmitting to two of three bitten mice three days later. Four Myotis lucifugus were bitten in the pectoral muscles by No. B548 on September 12, 1960, when transmission to mice failed. Only one of them survived the trauma for more than a week and it did not become ill in an observation period of 92 days.

## Isolation of Virus from Salivary Glands

Rabies virus was found in the salivary glands of all bats that had infectious saliva during life (Table 1), but the M. californicus that infected three of three mice by bite had barely detectable virus after death six days later. On the other hand, a Lasionycteris (No B300) had a titer of 4.4  $LD_{50}/0.03$  ml in the salivary glands 11 days after infective bite. Others had titers of virus in salivary glands that far exceeded those in central nervous tissue.

### Isolation of Virus from Brown Fat

Demonstration by Sulkin, et al.,<sup>5</sup> that rabies virus may occur in brown fat of experimentally infected bats even in the absence of virus in the brain prompted us to search for virus in this tissue of naturally infected bats. Brown fat of 11 rabid bats had been examined. All of them had virus in the brain, eight had infected salivary glands, and eight had infected brown fat. Most of the infected brown fat contained lower concentrations of virus than did the brain but no consistent pattern of relationship was evident. In some bats the concentration of virus in brown fat was so low that viremia could have accounted for its presence, but the same might be said of brain tissue in which virus was sometimes in low titer. Virus was never found in brown fat or salivary glands when absent from brain in this small series.

#### Isolation of Virus from Miscellaneous Other Organs

Heart, lung, liver, kidney, and spleen of two rabid female Eptesicus have been tested for virus. Virus in low titer was present in all the tissues except liver in one bat, and liver and spleen in the other. Presence of virus in the various tissues may imply only viremia, possibly agonal, but in that case liver and spleen should contain appreciable virus. On the other hand, specific antibody or nonspecific reactive substance<sup>6</sup> in the blood may have neutralized virus in the blood-rich organs in vitro.

#### Characteristics of Bat Isolates

All isolates obtained from bats in Montana have been compared with Pasteur fixed virus in neutralization tests and with Flury virus in protection tests to establish identity. Some of the isolates had extremely low titers and therefore posed difficulties in identification, inasmuch as the Flury-protected mice were old and more resistant to infection. Several intracerebral passages in young mice usually increased the titer to sufficiently high levels for Low titer was not characteristic test. of the isolates, however, nor was there uniformity of titer after several intracerebral passages in mice. Even isolates from different tissues of one bat varied in this respect.

Invasiveness, by way of the peritoneal route, of isolates from Montana bats was compared with isolates obtained from various carnivores and bats from other parts of the country. Webster<sup>4</sup> states that the peritoneal route is less effective than injections into tongue, cheek, thigh, or subcutaneous tissue of white mice. However, a number of weanling mice usually succumb to the injection of 0.1 ml of a 10 per cent suspension of virus, and differences in invasiveness should be readily discerni-Some representative examples are ble. presented in Table 3.

When injected intraperitoneally, the Pasteur fixed virus is much more lethal than low-passage isolates from any source. Low-passage isolates from the several bats and carnivores differ individually, but not as groups.

Incubation periods of infection in some mice injected intracerebrally with infected tissues of bats were as short as four days but in others exceeded three weeks. The average time of onset of signs of illness in mice that were injected with brain tissue was 7.4 days; after injection of oral specimens it was 10.4 days.

<u></u>			Mouse Intrac	erebral	No. Mice	No.	Mortality	
Species			State*	Passage No.	Titer	Injected	Dead	%
Pasteur (NIH	:PV-1)	101		Multiple	6.6	60	60	100
"	,,	10 <sup>2</sup>				60	56	94
"	,,	10 <sup>3</sup>				60	33	55
Skunk			Ala.	1	3.5	117	59	50
Dog			Minn.	2	2.5	167	34	20
Dog			Ala.	1	4.3	118	71	66
Fox			Ala.	1	4.5	114	13	11
Tadarida braziliensis-l	bat		N. M.	2	4.5	89 우 90 중	50 52	56 58
L. noctivagans	s bat		Mont.	2	3.4	113	16	14
E. fuscus-bat			Mont.	2	2.9	120	18	15
E. fuscus-bat			Mont.	2	2.9	261 ද 261	6 18	2 7
M. evotis-bat			Mont.	2	6.4	118	17	14
M. yumanensi	s-bat		Mont.	2	3.2	119	21	18
M. yumanensi	s-bat		Mont.	2	3.2	120	35	30
M. californicus-bat			Mont.	2	3.8	120	13	11

Table	3-Mortality	in Wean	ling Mice	Followin	g Intrap	eriton	eal	Inj	ectio	n of Mo	use
Pass	age Rabies '	Virus Isol	ates from	Various	Sources	(0.1	ml	of	<b>10</b> 1	Dilution	of
Who	Whole Brain Suspension Unless Otherwise Indicated)										

\* Isolates were obtained from Dr. Keith Sikes, Dr. R. E. Kissling, Dr. Denny Constantine, and Dr. R. Fenstermacher.

## Negri Bodies

After many years of study, and much dissertation, the exact nature of Negri bodies is still uncertain. However, there is no disagreement as to their specificity and therefore their presence or absence in bat brains is noteworthy. Hurst and Pawan<sup>7</sup> established that Negri bodies occur in bats as well as in other mammals. They have been found frequently in recent years although Burns, et al.,8 report a lower incidence than would be expected in other animals. Kissling<sup>9</sup> has noted that isolates from bats in the Southeast produce large and numerous Negri bodies in mice, whereas those from New Mexico usually produce very small and scarce Negri bodies, but there is no unequivocal correlation of the inclusions with other characteristics of host or virus in bat rabies. We have sought Negri bodies in smears and in stained sections of suspicious bats, and in mice inoculated with infectious material from each rabid bat. The inclusions were found in several of the infected bats but never in suspicious bats that were not infected. However, Stamm, et al.,<sup>10</sup> found Negri bodies in one experimentally infected bat from which virus could not be recovered.

## Discussion

A peculiarity of bat rabies in Brazil in the first decade of this century was the occurrence of much disease in cattle, but little or none in dogs or man.<sup>11</sup> However, in Trinidad, in British Guiana, and in Mexico, rabies has been transmitted from bats to man in epidemic proportions at times. The apparent failure of bats to transmit rabies to dogs in Brazil is paralleled on a smaller scale in Montana. We have isolated rabies virus from 21 bats in Montana since 1954 and we must assume that only a very small fraction of infected bats come to our attention, yet rabies in other wild animals and domestic animals is practically unknown in the state.

Biting is, of course, the established method of transmission of rabies, and observations on rabies in fruit-eating and vampire bats in South America have suggested no other method. However, other methods of transmission have been proposed occasionally. Remlinger and Bailey<sup>12</sup> demonstrated rabies virus in ixodid ticks taken from a naturally infected dog, and Reagan, et al.,<sup>13</sup> demonstrated virus in argasid ticks fed upon bats infected in the laboratory. Bell, et al.,<sup>14</sup> found no evidence of viremia in experimentally infected laboratory animals, but they infected ixodid and argasid ticks with rabies virus by artificial feeding technics. They could establish neither persistence through ecdysis nor transmission of the virus.

Épidemiological clues also suggest alternative methods of transmission. Rabies has occurred in man after intimate contact with bats but without known bite.<sup>15</sup> A capybara developed rabies when kept in a cage next to a rabid dog although there did not appear to have been any bite.<sup>7</sup> Remlinger<sup>16</sup> mentions ingestion as a proven method of contracting rabies. Inunction into scarified skin and application to mucous membranes also produce infection.<sup>7</sup>

The Fourth Report of the WHO Expert Committee on Rabies<sup>17</sup> states that "Epizootiological investigations in bat rabies have revealed that it is extremely difficult to transmit rabies by inducing known rabid bats with infectious saliva

to bite other susceptible animals in the laboratory." Our experience has been contrary to that statement since all of four rabid bats tested had infectious saliva and all of them and another bat (I52) not tested by swabbing transmitted by bite. Admittedly the infective bites were inflicted on mice which are very susceptible,<sup>4</sup> but the occurrence of human cases testifies that these were not uniquely susceptible. In view of these findings it is difficult to account for the failure of Burns, et al.,8 to demonstrate infection by bite in repeated exposures of various species to bats with infected Monkeys that not only were saliva. bitten by bats, but also killed the bats and ate them, did not become ill. Presumably both kinds of exposure of wild carnivores to rabid bats may occur in nature.\*

Comparison of efficacy of transmission of canine bites of man with bat bites of mice may not be justifiable but there can be no doubt that biting is an adequate means of inoculation. Only one infected bat, an Eptesicus fuscus (B316), tested by bite failed to transmit infection. No oral swab specimen was taken from this animal. Failures of transmission occur also in all other species adequately tested and in them, too, the reasons for failure are unknown. Dogs with infectious saliva are supposed to infect 30 to 40 per cent of persons bitten, but only about 50 per cent of infected dogs have infectious saliva, and the risk of bites from rabid dogs in general is only about 15 per cent.18

Some conception of the epizoology of rabies in chiroptera might be of help in evaluating its potentiality. There is a possibility that the disease is relatively

<sup>\*</sup> Since this manuscript was submitted for publication the occurrence of rabies in captive foxes and coyotes in bat caves under conditions that precluded transmission by bites of bats or exchange of arthropod parasites has been demonstrated by personnel of the PHS Southwest Rabies Station.

new in North American insectivorous species and that the peak of incidence has not yet been attained. Even if the disease has been well established in bats for centuries, marked fluctuation in incidence such as occurs in other species may be expected from time to time.

In order to obtain more intimate knowledge of the disease in bats it will first be necessary to learn more about the bats. With one or two exceptions we know almost nothing about their life histories in Montana during the greater part of the year. There is evidence now that lability of the thermoregulatory mechanism affects the course of disease in bats,19 and incidence of the disease may vary widely in different species and in different geographic areas, since the characteristics and habits of the species are widely variant. Even the two sexes may have extremely different habits which could affect occurrence of the disease in them.

Vaccination of people bitten by bats poses the same problems as prophylaxis after other bites. No problem exists, of course, if the inflicting animal can quickly be diagnosed as rabid by demonstration of Negri bodies or by the fluorescent antibody technic. Unprovoked attacks are considered justification for specific treatment and even provoked bites may be so suggestive as to justify treatment especially if the attacking animal can not be obtained.

A measure which might speed diagnosis and facilitate decisions is the more common use of oral swabs for animal inoculation. If for any reason an animal is to be kept for observation, an oral swab for injection may be taken immediately, thus gaining time if death should occur later. Positive or negative results will enter into decisions regarding treatment.

Unfortunately, there are always a number of cases that confront the physician with a dilemma. Although newer vaccines are not as dangerous as those made from brain or cord tissue, the full course of treatment is unpleasant to say the least and not to be given without good cause. On the other hand, consequences of delay to await the outcome of animal injection may be much more serious. A compromise decision would seem reasonable in many cases, viz., to give a primary injection immediately and to follow it in a week, or at the time of onset of illness in injected animals, by booster injections.<sup>20</sup>

From a public health standpoint there are two aspects of major significance. One is the actual and potential spread of rabies directly from bats to man. Six human cases in the United States have been attributed to bats<sup>21</sup> and frequent and widespread infections in bats that become aggressive or easily provoked constitute a danger that must be met by vaccinations of exposed persons.

The second aspect is one that is not vet demonstrated, namely, transmission from bats to other vectors. Montana has been nearly free of rabies in pets and livestock for many years in spite of a rather high incidence in bats. At this stage of our knowledge the implications of the situation can only be surmised, and even if rabies should begin to occur in wild carnivores or in domestic animals it is unlikely that we will know its source or sources. In Florida, sporadic rabies in dogs, cats, foxes, skunks, and raccoons could have its source in the numerous infected bats present, although conclusive evidence is lacking.<sup>22</sup>

Characterization of isolates from various sources offers little hope of identification of source. All rabies isolates are immunologically cross-reactive but otherwise they may vary widely in titer, incubation negrigenesis, invasiveness, characteristics. period. and other Furthermore, we have found no stigmata in our own studies or in the studies of others8 that would distinguish bat isolates.

In spite of uncertainty of transmission from bats to other vectors the conclusion has been drawn by some lay municipal authorities that the danger is real, and in at least two instances vaccination of dogs is now required because of bat rabies in communities that have never had a rabid dog. The implications and risk of opposing the measure would not be relished by any disinterested person.

#### Summary

Twenty-one rabid bats of eight species have been found in western Montana in seven years. No doubt these represent only a small fraction of the number actually present, but rabies in other animals is practically unknown in the area. Six of the infected bats were tested for ability to transmit virus by bite and five of them did so on one or more occasions. Virus was demonstrated by injection of oral swab specimens in every one of seven trials. Many of the bats showed some evidence of infection such as confusion, incoordination, or aggressiveness, and opportunities for contact with animals and man were common.

Virus was not limited in distribution to the central nervous system and salivary glands, but was also found in brown fat and in several other tissues. However, in no case was virus absent from the central nervous system when present elsewhere. Rabies virus isolates from bats possessed no demonstrable characteristics which would distinguish them from other street viruses.

No evidence was obtained to indicate recovery from rabies or the occurrence of a carrier state.

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