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Source: Journal of Wildlife Diseases, 15(3) : 455-466

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-15.3.455

VIROLOGIC AND SEROLOGIC SURVEY FOR EASTERN EQUINE ENCEPHALOMYELITIS AND CERTAIN OTHER VIRUSES IN COLONIAL BATS OF NEW ENGLAND [1]

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Abstract: To test the hypothesis that hibernating colonial bats serve as an overwintering reservoir host of eastern equine encephalomyelitis (EEE) and certain other arthropod-borne viruses in southern New England, 1128 bats of 4 species were collected from 1966 through 1976. Blood and tissue samples and ectoparasites from these bats were tested in suckling mice, wet chicks, and/or chick-embryo tissue cultures for virus. Rabies, the only virus isolated, was recovered from the brain, salivary glands, and brown fat of an apparently healthy adult male *Myotis keenii* found hibernating in western Massachusetts.

EEE neutralizing antibody was detected in 1.3% of the adult bats tested. A significant difference was noted between prevalence of antibody in hibernating (0.3%) and nonhibernating (3.4%) bats tested during the first 4 years of this study. One of 26 (3.8%) sera neutralized California encephalitis virus and no neutralizing or hemagglutination-inhibition antibody was detected with western equine encephalomyelitis virus.

INTRODUCTION

One of the most important, but least understood, aspects of the ecology of arthropod-borne viruses (arboviruses) in New England is virus survival during interepidemic periods. Eastern equine encephalomyelitis (EEE) virus has been detected in avian or culicine hosts from late July through mid-October in southern New England.[9,21] While the presence of EEE virus is an annual occurrence in this area, the mechanism by which it overwinters is unknown.

Bats have been implicated, either by the detection of natural infections or by inoculation studies in the laboratory, as reservoir hosts in the maintenance cycles of several viruses including arboviruses.[2,20] EEE virus was recovered from 10 naturally-infected bats collected during 1968 and 1969 in New Jersey.[8] These included 7 *Eptesicus fuscus*, 2 *Myotis lucifugus*, and 1 *Lasiurus cinereus*. In addition, EEE neutralizing (NT) antibody was detected in 5 of 21 bats (3 *Tadarida braziliensis*, 1 *L. cinereus*, 1 unidentified species) from Georgia,[14] 1 *(E. fuscus)* of 6 bats from New Jersey,[7] and 3 of 207 bats (2 *M. lucifugus*, 1 *E. fuscus)* from Massachusetts.[4,12] The objective of this study was to test the hypothesis that colonial bats serve as reservoir hosts of EEE virus in southern New England. The present paper presents results of surveillance for natural infections of EEE and certain other arboviruses by attempted isolation from blood, organs, and ectoparasites and by the demonstration of antibody.

MATERIALS AND METHODS

Nonhibernating bats were collected by hand with gloves or forceps from maternal colonies or with mist nets after dusk;

[1] This study was supported in part by National Institutes of Health Grant Number 1 R01 A1 11927.

hibernating bats were collected by gloved hand from the walls of abandoned emery mines in western Massachusetts. Blood samples were taken by cardiac puncture from lightly etherized bats and diluted 1:10 with heparin (10 units/ml) in phosphate-buffered saline (PBS). Bats collected from hibernacula during 1973 through 1976 were held at 27C for at least one week prior to blood sampling. Organ samples, including brain, interscapular adipose tissue, submaxillary salivary glands, spleen, liver, heart, lungs, kidneys, and mammary glands, were removed aseptically from selected bats. Approximately 10% suspensions were prepared in 10% fetal calf serum or 0.75% bovine albumin in PBS (pH 7.2) with penicillin and streptomycin. Ectoparasites were identified with the aid of a binocular microscope, pooled by species, location, and host, and triturated in the same type of diluent as the organ samples. Pool size averaged 144 but ranged from 2 to 880 specimens.

Virus isolation was attempted in chick-embryo tissue culture (CETC) or in wet chicks (WC) in the early stages of this study and in suckling mice (SM) in the later years. Brain suspensions from selected sick or dead mice or chicks were serially passed until a clear mortality pattern was apparent. The virus isolate was identified by complement-fixation (CF) tests.

Sera were screened for NT antibody by plaque-reduction (PRNT) tests in CETC[4] prior to 1970 or by suckling mouse neutralization test (SMNT) after 1970.[16] Low suckling mouse passage strains of EEE and the eastern subtype of western equine encephalomyelitis (WEE) virus originally isolated from *Culiseta melanura* mosquitoes in southern New England were used in NT tests. Higher passages (17 and 8, respectively) of California encephalitis (CE) (strain BFS 283) and Chobar Gorge (CG) (701700-8) viruses were also employed. CF and hemagglutination-inhibition (HI) tests were done on microtiter plates[16] with the initial dilutions of sera at 1:10. Again, local strains of EEE and WEE and prototype strains of other viruses were used.

RESULTS

No arboviruses were recovered from the blood and/or organs from 1128 colonial bats of 4 species collected in southern New England (Table 1). In addition, no virus was isolated in SM from 203 mites *(Spinturnix* spp.), 2 ticks *(Ornithodoros kelleyi),* 107 bedbugs *(Cimex pilosellus),* or 1369 fleas *(Myodopsyllus insignis)* collected from

TABLE 1. Virus isolation attempts from bats collected in southern New England 1966 throuth 1976

	Assay System			Total
	CETC	SM	WC	Tested*
Little Brown Myotis *(Myotis lucifugus)* (810 bats)				
blood	339	287	5	623
brain	1	246	172	409
brown fat		253	173	413
mammary glands		90		90
salivary glands		246	173	409
lung		258	173	421
heart		248		248
liver		247		247

TABLE 1. (continued)

spleen		247		247
kidney		174	173	337
Keen's Myotis *(Myotis keenii)* (222 bats)				
blood	128	54	2	182
brain		40	60	100
brown fat		40	60	100
salivary glands		40	60	100
lung		40	60	100
heart		40		40
liver		40		40
spleen		40		40
kidney			60	60
Big Brown Bat *(Eptesicus fuscus)* (89 bats)				
blood	48	40		88
brain		10	25	35
brown fat		9	25	34
mammary glands		2		2
salivary glands		9	25	34
lung		9	25	34
heart		9		9
liver		9		9
spleen		9		9
kidney		1	25	26
Eastern Pipistrelle *(Pipistrellus subflavus)* (7 bats)				
blood	5	2		7
brain			2	2
brown fat			2	2
salivary glands			2	2
lung			2	2
kidney			2	2
All Bats (1128 bats)				
blood	520	383	7	900
brain	1	296	259	546
brown fat		302	260	552
mammary glands		92		92
salivary glands		295	260	545
lung		307	260	557
heart		297		297
liver		296		296
spleen		296		296
kidney		175	260	425

*Some samples tested in more than one assay system.

CETC = primary chick embryo tissue culture.
SM = intracerebral inoculation of suckling mice.
WC = subcutaneous inoculation of wet chicks.

these bats. A virus, serologically identified as rabies, was recovered from the interscapular adipose tissue, submaxillary salivary glands, and brain, but not the heart, lung, liver, or spleen, of an apparently healthy adult male *Myotis keenii* collected from a mine in western Massachusetts on 19 November 1973 (Table 2).

Sera from 11 of 876 (1.3%) bats neutralized EEE virus (Table 3). NT antibody was demonstrated in adults, but not immatures, of 3 of the 4 bat species collected in this survey. HI antibody was detected in only 2 of 336 sera (both samples had NT antibody) and none of 159 samples was positive by CF tests with EEE antigens (Tables 3, 4).

In the early stages of this study (1966-1969), a significant difference ($P<0.01$) was noted in EEE antibody rates between hibernating (0.3%) and nonhibernating (2.9%) bats (Table 5). A significant difference ($P<0.01$) was noted also in NT antibody rates between male (0.3%) and female (2.4%) bats in the early years.

Western equine encephalomyelitis antibody was not detected by NT, HI, and/or CF tests in 894 bats tested in this study (Table 3). Group B antibody was not demonstrated in 337 sera tested by HI and/or CF with St. Louis encephalitis, Rio Bravo, or Montana *Myotis* leucoencephalitis viral antigens (Table 3). One of 26 serum samples neutralized CE virus in PRNT tests; however, the quantity of serum was insufficient to confirm this observation. No HI antibody was detected in 237 additional samples using CE antigen nor was snowshow hare antibody demonstrated in 159 samples tested by CF (Table 3). In addition to the alphaviruses, flaviviruses, and bunyaviruses already mentioned, CF antibody was not detected in 159 sera tested with Cache Valley, mouse hepatitis, rabies, or Whitney's *Clethrionomys gapperi* virus. Chobar Gorge virus was not neutralized by sera from 64 *M. lucifugus* or 36 *E. fuscus* (Table 3).

TABLE 2. Serologic identification by complement-fixation of virus strains An-192-73b, An-192-73d, and An-192-73e isolated from an adult male, Keen's Myotis *(Myotis keenii)* collected in western Massachusetts on 19 November 1973.

	An-192-73b	An-192-73d	An-192-73e	Rabies	NHD	DUV	LB	MOK	KOT	OBOD
An-192-73b	256/64*	512/64	256/64	128/32	8/8	0	0	16/16	0	0
An-192-73d	256/64	512/128	256/128	128/64	16/8	0	0	16/8	0	0
An-192-73e	512/64	512/128	256/128	128/64	16/16	0	0	16/32	0	0
Rabies	512/64	512/512	256/256	256/512	32/256	ND	0	ND	ND	ND
DUV	16/8	32/16	16/16	ND	ND	32/64	ND	ND	ND	ND
KOT	0	0	0	ND	ND	ND	ND	ND	16/8	ND
OBOD	0	0	0	ND	ND	ND	ND	ND	ND	64/16

*Reciprocal of serum titer/Reciprocal of antigen titer

Rabies = CVS-27
NHD = Nigerian horse disease virus
DUV = Duvenhage virus
LB = Lagos bat virus
MOK = Mokola virus (IbAn 27377)
KOT = Kotonkan virus (IbAn 23380)
OBOD = Obodhiang (UgAr 1275)
ND = Not done

TABLE 3. Serologic results of colonial bats from southern New England, 1966-1975.

Virus	Test	*Myotis lucifugus*	*Myotis keenii*	*Eptesicus fuscus*	*Pipistrellus subflavus*	Total
Eastern Equine Encephalomyelitis	PRNT	1/340 (0.3)*	4/142 (2.8)	1/49 (2.0)	0/5	6/536 (1.1)
	SMNT	3/258 (1.2)	0/42	2/38 (5.3)	0/2	5/340 (1.5)
	HI	1/255 (0.4)	0/42	1/37 (2.7)	0/2	2/336 (0.6)
Western Equine Encephalomyelitis	PRNT	0/359	0/144	0/49	0/5	0/557
	HI	0/255	0/42	0/38	0/2	0/337
Rio Bravo	HI	0/255	0/42	0/38	0/2	0/337
California Encephalitis	PRNT	0/12	1/11 (9.1)	0/4	0/0	1/26 (3.8)
	HI	0/196	0/3	0/38	0/0	0/237
Chobar Gorge	SMNT	0/64	0/0	0/36	0/0	0/100

*Number positive/number tested (percent positive)

PRNT = plaque-reduction neutralization test (1966-1969)
SMNT = suckling mouse neutralization test (1970-1975)
HI = hemagglutination-inhibition test (1970-1975)

TABLE 4. Eastern equine encephalomyelitis seropositive bats collected in southern New England, 1966-1974.

Number	Species	Age	Sex	Area	Date	Serology		
						NT	HI	CF
MM-208	*Myotis keenii*	Adult	M	Chester (Hampden Co.) Mass.	17.IX.67	+	ND	ND
M-1720	*Eptesicus fuscus*	Adult	F	Easton (Bristol Co.) Mass.	3.VIII.67	+	ND	ND
M-2040	*Myotis lucifugus*	Adult	F	Middleboro (Plymouth Co.) Mass.	17.VI.68	+	ND	ND
M-2095	*Myotis keenii*	Adult	F	Middleboro (Plymouth Co.) Mass.	16.VII.68	+	ND	ND
M-2111	*Myotis keenii*	Adult	F	Middleboro (Plymouth Co.) Mass.	16.VII.68	+	ND	ND
MM-901	*Myotis keenii*	Adult	F	Chester (Hampden Co.) Mass.	17.I.69	+	ND	ND
An-82-73	*Myotis lucifugus*	Adult	M	Morris (Litchfield Co.) Conn.	11.VII.73	+	1:40	<1:4
An-125-73	*Eptesicus fuscus*	Adult	F	Hebron (Tolland Co.) Conn.	3.VIII.73	+	1:10	<1:4
An-143-73	*Myotis lucifugus*	Adult	M	Hebron (Tolland Co.) Conn.	3.VIII.73	+	<1:10	<1:4
An-226-74	*Myotis lucifugus*	Adult	M	Chester (Hampden Co.) Mass.	4.XII.74	+	<1:10	ND
An-304-74	*Myotis lucifugus*	Adult	F	Chester (Hampden Co.) Mass.	4.XII.74	+	<1:10	ND

ND = Not Done
F = Female
M = Male
NT = Neutralization Test
HI = Hemagglutination-Inhibition
CF = Complement-Fixation

TABLE 5. Eastern equine encephalomyelitis neutralizing antibody in hibernating and nonhibernating adult bats collected in southern New England, 1966-1974.

	Nonhibernating		Hibernating		
	1966-69	1973-74	1966-69	1973-74*	Total
Little Brown Myotis *(Myotis lucifugus)*	1/85 (1.2)**	1/184 (0.5)	0/255	2/74 (2.7)	4/598 (0.7)
Keen's Myotis *(Myotis keenii)*	3/40 (0.8)	—	1/102 (1.0)	0/42	4/184 (2.2)
Big Brown Bat *(Eptesicus fuscus)*	1/47 (2.1)	2/37 (5.4)	0/2	0/1	3/87 (3.4)
Eastern Pipistrelle *(Pipistrellus subflavus)*	—	—	0/5	0/2	0/7
TOTAL	5/172 (2.9)	3/221 (1.4)	1/364 (0.3)	2/119 (1.7)	11/876 (1.3)

*Hibernating bats collected during 1973 and 1974 were held at approximately 27°C for more than a week prior to blood sampling.
**Number positive/number tested (percent positive).

DISCUSSION

Several factors might influence the difference in EEE neutralization rates between hibernating and nonhibernating bats. The physiological state of an animal is known to influence antibody production; reduced body temperature and metabolic rates in hibernating vertebrates have been associated with suppressed synthesis of immune globulin.[18] Hibernating bats inoculated with EEE virus failed to develop demonstrable NT antibody;[15] similar results have been reported in bats infected with Japanese B encephalitis virus.[15,19] In the later years of the present study (1973-1974), bats collected from hibernacula were held at 27C for at least one week prior to blood sampling. The NT antibody rates in these bats (1.7%) approached that of the active bats (1.4%) (Table 5).

The difference in EEE antibody rates between male and female bats was attributed to differences in the sex ratios of hibernating and nonhibernating bats. Nearly three-quarters of the bats sampled in the winter were males

TABLE 6. Eastern equine encephalomyelitis antibody in hibernating and nonhibernating male and female bats collected in southern New England, 1966-1974.

Nonhibernating	Male	Female	Total
Myotis lucifugus	1/46 (2.2)*	1/223 (0.4)	2/269 (0.7)
Myotis keenii	1/11 (9.1)	2/29 (6.9)	3/40 (7.5)
Eptesicus fuscus	1/33 (3.0)	2/51 (3.9)	3/84 (3.6)
Pipistrellus subflavus			
TOTAL	3/90 (3.3)	5/303 (1.7)	8/393 (2.0)
Hibernating			
Myotis lucifugus	1/236 (0.4)	1/93 (1.1)	2/239 (0.6)
Myotis keenii	0/100	1/44 (2.3)	1/44 (0.7)
Eptesicus fuscus	0/1	0/2	0/3
Pipistrellus subflavus	0/6	0/1	0/7
TOTAL	1/343 (0.3)	2/140 (1.4)	3/483 (0.6)
TOTAL			
Myotis lucifugus	2/282 (0.7)	2/316 (0.6)	4/598 (0.7)
Myotis keenii	1/111 (0.9)	3/73 (4.1)	4/184 (2.2)
Eptesicus fuscus	1/34 (2.9)	2/53 (3.8)	3/87 (3.4)
Pipistrellus subflavus	0/6	0/1	0/7
TOTAL	4/433 (0.9)	7/443 (1.6)	11/876 (1.3)

*Number positive/number tested (percent positive).

TABLE 7. Eastern equine encephalomyelitis neutralizing antibody in nonhibernating adult colonial bats collected in southern New England, by year, 1966-1974.

	1966	1967	1968	1969	1973	1974
M. lucifugus	0/3*	0/31	1/42 (2.4)	—	1/118 (0.8)	2/140 (1.4)
M. keenii	—	1/13 (7.7)	2/23 (8.7)	—	0/3	0/39
E. fuscus	—	1/4 (25.0)	0/22	0/1	2/37 (5.4)	0/1
P. subflavus	—	—	—	—	—	0/2
TOTAL	0/3	2/48 (4.2)	3/87 (3.4)	0/1	3/158 (1.9)	2/182 (1.1)

*Number positive/number tested (percent positive).

TABLE 8. Eastern equine encephalomyelitis neutralizing antibody in colonial bats collected in southern New England, 1966-1974, by counties.

	Massachusetts					
	Plymouth	Bristol	Norfolk	Hampden		Hampshire
				hibernating**	nonhibernating	
Myotis lucifugus	1/51 (2.0)*	0/5	—	0/255	2/105 (1.9)	0/2
Myotis keenii	2/22 (9.1)	0/1	—	1/102 (1.0)	1/53 (1.9)	0/6
Eptesicus fuscus	0/42	1/6 (16.7)	0/8	0/2	—	—
Pipistrellus subflavus	—	—	—	0/5	0/2	—
TOTAL	3/115 (2.6)	1/12 (8.3)	0/8	1/364 (0.3)	3/160 (1.9)	0/8

	Connecticut		
	New Haven	Litchfield	Tolland
Myotis lucifugus	—	1/180 (0.6)	—
Myotis keenii	—	—	—
Eptesicus fuscus	0/1	—	2/28 (7.2)
Pipistrellus subflavus	—	—	—
TOTAL	0/1	1/180 (0.6)	2/28 (7.2)

*Number positive/number tested (percent positive).
**All other bats nonhibernating

whereas only about one-third of those collected during the summer were males. The difference in antibody rates was less pronounced (0.9% for males versus 1.6% for females for the entire study) when winter bats were maintained at higher temperatures prior to sampling (Table 6).

Annual NT antibody rates varied from 1.1% in 1974 to 4.2% in 1967 (Table 7). Antibody rates were highest in Plymouth (2.6%) and Bristol (8.3%) Counties in Massachusetts where enzootic and epidemic EEE virus activity has been reported[9] and in Tolland County, Connecticut where 2 of 28 *E. fuscus* were positive (Table 8). The summer distribution, and thus the area where the bats probably acquired infections, of bats collected from hibernacula in Hampden County, Massachusetts is unknown. Long flights (250-300 km) of *Myotis* and *Pipistrellus* between summer feeding grounds and winter caves have been reported in the northeastern states. *Myotis* bats banded at the Hampden County mines have been recaptured in southeastern Massachusetts (185 km SE), central and western Connecticut (30-90 km S and SW), western New York (100 km WSW), and western Vermont (110-180 km N).[6,10] Bats banded in southwestern Vermont (110 km N), northeastern Connecticut (85 km SE), and southwestern Massachusetts (30 km SW) were recovered in these mines.[5,10] The limited evidence available indicates that the summer dispersal of *Myotis* species is to the south. Davis and Hitchcock[5] suggest that *M. lucifugus* summering in the enzootic areas in southeastern New England overwinter in a cave on Mt. Aeolus in Vermont. *E. fuscus* do not migrate far from their summer range.[10]

The lack of WEE antibody in this study suggests that bats may be refractory to infection with this virus or that infection may be fatal or disabling to the bats. Colonial bats may not be ecologically associated with WEE virus cycles. However, EEE and WEE viruses are isolated from the same mosquito species *(Cs. melanura)* and from passerine birds in the same ecosystems (wooded swamps) in southern New England. WEE virus is common in avian and culicine hosts from July to October in this area.[11] This virus was isolated from the salivary glands from 1 *E. fuscus* of 2425 bats that were submitted for rabies examinations in New Jersey from 1962 to 1969.[8]

Flaviviruses, reported from bats in many areas throughout the world,[1] were not detected by virus isolation or serology in bats from New England (Table 3).

CF antibody was not detected in 159 sera (118 *M. lucifugus*, 2 *M. keenii*, 38 *E. fuscus)* tested with EEE and 9 other viral antigens. Complement-fixing antibody was not demonstrated in bats inoculated with EEE[15] or with Whitney's *Clethrionomys gapperi* virus[17] in this laboratory nor in bats inoculated with Japanese encephalitis virus by Sulkin and his coworkers.[19,20] Sulkin attributes the lack of CF antibody to quantitative rather than qualitative deficiencies in the immunoglobulins produced by bats.[20]

CONCLUSIONS

Serologic evidence confirms that natural infections of EEE but not WEE virus do occur in colonial bats of the genera *Myotis* and *Eptesicus* in southern New England.

LITERATURE CITED

1. BERGE, T.O. (Ed.) 1975. International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates. DHEW Publ. No. (CDC) 75-8301.
2. CONSTANTINE, D.G. 1970. Bats in relation to the health, welfare and economy of man. In: *Biology of Bats,* Volume II. W.A. Wimsatt, ed. Academic Press, New York.

3. DANIELS, J.B., J.J. RATNER and S.R. BROWN. 1961. Plaque reduction, a sensitive test for eastern encephalitis antibody. Science 133: 640-641.
4. ——, G. STUART, R.E. WHEELER, C. GIFFORD, J.P. AHEARN, R. PHILBROOK, R.O. HAYES and R.A. MACCREADY. 1960. A search for encephalitis and rabies in bats of eastern Massachusetts. New England J. Med. 263: 516-520.
5. DAVIS, W.H. and H.B. HITCHCOCK. 1965. Biology and migration of the bat, *Myotis lucifugus,* in New England. J. Mammal. 46: 296-313.
6. GIFFORD, C.E. and D.R. GRIFFIN. 1960. Notes on homing and migratory behavior of bats. Ecology 41: 378-381.
7. GOLDFIELD, M. and O. SUSSMAN. 1967. EE and WE in New Jersey's non-avian vertebrates. Mtg. Wildl. Dis. Ass., Urbana, Illinois.
8. —— and ——. 1970. Eastern encephalitis in New Jersey during 1969. Proc. Ann. Mtg. N.J. Mosq. Exterm. Assoc. 57: 11-15.
9. GRADY, G.F., H.K. MAXFIELD, S.W. HILDRETH, R.J. TIMPERI, R.F. GILFILLAN, B.J. ROSENAU, D.B. FRANCY, C.H. CALISHER, L.C. MARCUS and M.A. MADOFF. 1978. Eastern equine encephalitis in Massachusetts, 1957-1976. A prospective study centered upon analyses of mosquitoes. Amer. J. Epidemiol. 107: 170-178.
10. GRIFFIN, D.R. 1940. Migrations of New England bats. Bull. Mus. Comp. Zool., Harvard 86: 217-246.
11. HAYES, C.G. and R.C. WALLIS. 1977. Ecology of western equine encephalomyelitis in the eastern United States. Adv. Virus Res. 21: 37-83.
12. HAYES, R.O., J.B. DANIELS, H.K. MAXFIELD and R.E. WHEELER. 1964. Field and laboratory studies on eastern encephalitis in warm- and cold-blooded vertebrates. Amer. J. Trop. Med. Hyg. 13: 595-606.
13. ITO, T. and S. SAITO. 1952. Susceptibility of bats to Japanese B encephalitis virus. Jap. J. Bact. 7: 617-622.
14. KARSTAD, L.H. and R.P. HANSON. 1958. Infections in wildlife with the viruses of vesicular stomatitis and eastern equine encephalomyelitis. Trans. N. Amer. Wildl. Conf. 23: 175-186.
15. MAIN, A.J. Eastern equine encephalomyelitis virus in experimentally infected bats. J. Wildl. Dis. 15: 467-477.
16. ——, W.G. DOWNS, R.E. SHOPE and R.C. WALLIS. 1976. Avian arboviruses of the Witless Bay Seabird Sanctuary, Newfoundland, Canada. J. Wildl. Dis. 12: 182-194.
17. ——, R.E. SHOPE and R.C. WALLIS. 1976. Characterization of Whitney's *Clethrionomys gapperi* virus isolates from Massachusetts. J. Wildl. Dis. 12: 154-164.
18. SCHMIDT, J.P. 1965. Response of hibernating mammals to physical, parasitic, and infectious agents. Proc. Intern. Symp. Nat. Mamm. Hibern. 33: 421-438.
19. SULKIN, S.E., R. ALLEN, R. SIMS and K.V. SINGH. 1966. Studies of arthropod-borne virus infections in Chiroptera. IV. The immune response of the big brown bats *(Eptesicus fuscus)* maintained at various environmental temperatures to experimental Japanese B encephalitis virus infections. Amer. J. Trop. Med. Hyg. 15: 418-427.
20. —— and ——. 1974. Virus infections in bats. Monographs in Virology. 8: 1-103.

21. WALLIS, R.C. and A.J. MAIN. 1974. Eastern equine encephalitis in Connecticut: Progress and problems. Mem. Conn. Entomol. Soc. pp. 117-144.

Received for publication 2 October 1978