Bats: Carriers of Human Disease-Producing Agents

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INTRODUCTION

For many years bats have been known to be harbingers of various agents which produce disease in man. Elsewhere in this volume certain parasites of bats are discussed including the association of these animals with *Trypanosoma cruzi* in South America and Texas (Ubelaker, 1970). Little is known concerning the association of bats with bacteria which cause disease in man. Four serotypes of *Salmonella* known to cause salmonellosis have been isolated from bats, one from bats collected in the Canal Zone (Klite & Kourany, 1965) and the other three from bats collected in southwestern Colombia, South America (Arata *et al.*, 1968). In the latter study a single isolation of *Shigella* was obtained, presumably the first from a bat. Sufficient information has not been obtained, however, to determine if these bacterial infections in bats are of public health importance. Bats are now believed to play an important role in the dissemination in nature of the fungus, *Histoplasma capsulatum.* Bats and bat caves have been associated with human cases of pulmonary histoplasmosis in various areas throughout the world (see Tesh & Schneidau, 1966) and studies with experimentally- and naturally infected bats have shown that these animals excrete *Histoplasma capsulatum* in their feces (Klite & Diercks, 1965; Tesh & Schneidau, 1966). Since this fungus is transmitted *via* the respiratory route, those individuals who frequent bat caves, such as spelunkers and persons engaged in mining guano, and also investigators involved in various studies with these animals, should be aware of the danger of contracting histoplasmosis. A plethora of other disease-producing agents have been associated with bats (Sulkin, 1962).

In this report emphasis will be placed on a discussion of bats as reservoir hosts of viruses, particularly rabiesvirus and certain of the arthropod-borne viruses (arboviruses). The ability of bats to function in the perpetuation of these viruses in nature is enhanced by the fact that, with the exception of some strains of rabiesvirus which under certain conditions may produce erratic behavior and death of bats, these animals may remain persistently infected with these viruses without exhibiting overt signs of disease. Various physiological and behavioral characteristics of bats which suggested the manner in which these animals could serve effectively as reservoir hosts for the arboviruses will be presented in connection with a discussion of an extended series of experimental and field studies which have established the role of bats in the ecology of Japanese and St. Louis encephalitis viruses.

RABIESVIRUS

The association of bats with rabiesvirus is probably the oldest and certainly the most thoroughly documented of the relationships of these animals with a human disease-producing agent (Enright, 1956; Sulkin, 1962). This association was first recognized in southern Brazil in the early 1900's, when a paralytic disease of cattle and horses was found to be caused by rabiesvirus transmitted by the bite of fruit-eating (*Phyllostoma*) and vampire (*Desmodus*) bats. The disease, or at least the recognition of it, seemed to move northward until the association of bats with rabies infection in livestock and in some instances in humans was reported from other South American countries, from Trinidad, and from Central America and Mexico. The prevalence of rabies infection in bat populations throughout the Latin American countries and the resulting human infections seemed to have little impact in the United States, possibly because at that time there was no evidence that the ranges of the bat species involved extended into the United States. No attempts had been made to isolate rabiesvirus from any of the bat species native to this country, although one group of investigators, prompted by the observations in Latin America, had reported in 1951 that two species of insectivorous bats were susceptible to experimental infection with rabiesvirus (Reagan & Brueckner). Also in 1951, a human rabies death had occurred in Texas in which the case history contained reference to the patient's having been bitten by a bat (Sulkin & Greve, 1954). Despite these suggestions that bats in the United States might be carriers of rabiesvirus, no routine investigations of this possibility were begun until after a young boy in Florida was attacked and bitten by a bat subsequently shown to be infected with rabiesvirus (Venters *et al.*, 1954) In rapid succession, reports of the isolation of rabiesvirus from naturally infected bats in Texas, Pennsylvania, California and other states appeared until at the present time rabies infected bats have been discovered in every state except Hawaii and Alaska. There have been hundreds of human exposures to rabid bats . . . at least six human deaths from rabies have occurred following association with bats.

Our interest in these animals as reservoir hosts for viruses stems from involvement in reporting the first human death in the United States believed to be due to bat bite (Sulkin & Greve, 1954). While the overall role of bats in the ecology of rabiesvirus was being investigated by public health laboratories throughout the country, we directed our efforts toward determining *how* these animals might function as reservoir hosts for this agent. We initiated a series of studies designed to characterize experimental rabiesvirus infection in bats with regard to the susceptibility of various species to different strains, whether overt or inapparent infections were produced, and the tissues which supported viral multiplication. We were particularly interested in determining if rabiesvirus administered intramuscularly would invade and multiply in the interscapular brown adipose tissue of these animals; for even then there was evidence that brown fat served an important function in hibernating animals, although its presently-known role in heat production and arousal from hibernation had not yet been defined (Smalley & Dryer, 1967; Hayward & Lyman, 1967). It seemed logical to assume, because of the level of metabolic activity attributed to this tissue and its presumed function in sustaining the hibernating bat during periods of dormancy, that it would provide an ideal site apart from the central nervous system for the replication and persistence of rabiesvirus in the inapparently infected bat. The growth of Coxsackie virus and poliovirus in the brown adipose tissue of various laboratory animals had previously been reported (Pappenheimer *et al.*, 1950; Dalldorf, 1950; Aronson & Schwartzman, 1956).

EXPERIMENTAL RABIESVIRUS INFECTION IN CHIROPTERA

Our experimental studies involved hundred of bats of two species and two strains of rabiesvirus of different origins and passage-histories. In addition to determining the susceptibility of bats maintained at room temperature to experimental infection with rabiesvirus, we took into consideration the unique thermoregulatory mechanisms of bats discussed in another section of this volume (Henshaw, 1970) and studied the influence of environmental temperature on rabies infection in these animals. Also, because of the well-known effect of pregnancy on the susceptibility of various animals to virus infections, we designed experiments to determine the influence of the gravid state on experimental rabies infection in spring-breeding Mexican free-tailed bats. Since we are now viewing this rather extensive series of experiments in retrospect we would like to briefly summarize the data and at the same time make an evaluation especially with regard to the way these experimental studies may have contributed to what is known today concerning the role of bats in the ecology of rabiesvirus.

Initial studies concerned the susceptibility of Mexican free-tailed bats

(*Tadarida brasiliensis*) and little brown bats (*Myotis l. lucifugus*) maintained at room temperature to a strain of rabiesvirus (designated Thompson) isolated from the brain of a human who contracted rabies by dog bite (Sulkin *et al.*, 1959). Virus was administered intramuscularly and tissues obtained for assay included brain, salivary glands and interscapular brown adipose tissue harvested over a period of from two to twelve weeks following inoculation from animals which had been dead less than 4 hours or were sacrificed either when showing signs of disease or in apparent good health. Evidence of infection was demonstrated in approximately 20% of the *Tadarida* and 40% of the *Myotis* included in the experiments. This strain of rabiesvirus exhibited neurotropic characteristics, and was demonstrated most frequently in the brain tissue of infected bats but was also found in salivary glands and brown adipose tissue. The levels of virus demonstrated in brown fat indicated that replication occurred in this tissue. Signs of central nervous system disease and death were observed in some bats of both species and a large proportion of the animals shown to be infected were those which were found dead or sacrificed with symptoms. Rabiesvirus was recovered, however, from the tissues of bats which showed no overt signs of illness at the time of sacrifice. Although *Myotis* appeared to be more susceptible to this strain of rabiesvirus than *Tadarida*, the overall results obtained with both species were similar. In a series of experiments with *Tadarida* inoculated intramuscularly with the Thompson strain and maintained at 24°C, tissues from 492 bats were tested for the presence of rabiesvirus and evidence of infection was obtained for 104 of these animals (21%). Table 1 shows the distribution of virus in the various tissues of 69 of the infected bats which were found dead or sacrificed in symptoms, 18 to 88 days after inoculation, as compared to tissues obtained from 35 of the infected animals which were in apparent good health when sacrificed 13 to 83 days post-inoculation. Virus was demonstrated in brain tissue of both groups with approximately equal frequency, whereas animals exhibiting signs of disease had virus present in their salivary glands more often than did the asymptomatic bats. In fact, in the latter group, rabiesvirus was recovered more often from brown fat than from salivary glands. Table 2 shows in more detail the distribution of virus in the 35 infected, asymptomatic bats. Twenty-one bats had virus in brain tissue alone; and in 6 animals brown fat was the only tissue from which rabiesvirus was recovered. Four bats had virus present in brain and salivary glands, three had virus in brain and brown fat, and in one bat sacrificed 22 days after inoculation rabiesvirus was recovered from all three of the tissues tested.

The reports concerning the role of brown adipose tissue in the pathogenesis of rabiesvirus in insectivorous bats prompted other investigators to attempt isolation of the virus from the brown fat of naturally-infected bats. In 1960 Bell & Moore reported the isolation of rabiesvirus from the pooled brown fat of two *Myotis l. lucifugus*, and from the brown fat of a single *Eptesicus f. fuscus*. Dr. Bell was kind enough to send us the *Myotis* isolate, and although this strain proved to be no more infective for *Myotis* than the Thompson canine strain, its tissue tropism was significantly different (Sulkin *et al.*, 1960a). Ninety per cent of the experimentally infected bats had virus present in interscapular brown adipose tissue, whereas virus was present in the brain tissue of only 50% of the infected animals. Thus this rabiesvirus strain isolated from the brown fat of naturally infected bats exhibited marked lipotropic characteristics in the experimentally infected animal. Of even greater interest was the observation that infected animals showed no evidence of central nervous system disease. This strain of rabiesvirus produced a completely silent infection even though the virus multiplied in many bats' brain tissue as well as in the brown fat and salivary glands.

In studies on the influence of temperature on experimental rabiesvirus infection in bats, it was found that both strains of virus used failed to multiply in the tissues of bats placed at 5°C immediately after inoculation; however, virus survived in the cold-exposed animals for many weeks, and upon transfer to a warmer environment the course of experimental rabiesvirus infection in these animals was much the same as that observed in bats maintained at room temperature from the time of inoculation (Sulkin *et al.*, 1960a). These results suggested that rabiesvirus could persist in naturally infected hibernating species of bats during the winter months, and possibly undergo increased rates of multiplication upon arousal in the spring. Rabiesvirus was subsequently isolated from the brain of an *Eptesicus f. fuscus* collected in New England in December, 1963 (Gerard *et al.*, 1965) and from the brain, brown fat and salivary glands of another big brown bat collected in Montana in December, 1965 (Bell *et al.*, 1966).

In studies on the influence of the gravid state on rabiesvirus infection in bats, we found no evidence that pregnancy increased the susceptibility of *Tadarida* to the Thompson strain of rabiesvirus (Sims *et al.*, 1963). However, during the course of these experiments, evidence of transplacental transmission of virus during late pregnancy was obtained; virus was demonstrated in the fetus of an experimentally infected bat found dead 23 days following inoculation. If this event occurs even infrequently in naturally infected bats, its influence on the persistence of rabiesvirus infection in bat population is obvious.

TABLE 1

DEMONSTRATION OF RABIESVIRUS (Thompson Strain) IN TISSUES OF EXPERIMENTALLY INFECTED BATS *(Tadarida brasiliensis)* MAINTAINED AT 24° C

CONDITION OF BATS	TIME AFTER INOCULATION (days)	NO. OF BATS	VIRUS DEMONSTRATED IN		
			BRAIN	SALIVARY GLANDS	BROWN FAT
Dead <4 hours or in Symptoms	18 - 66	69	62 (.90)	32 (.46)	13 (.19)
Apparently Healthy	13 - 83	35	29 (.83)	5 (.14)	10 (.29)
Totals	13 - 83	104	91 (.88)	37 (.36)	23 (.22)

TABLE 2

DEMONSTRATION OF RABIESVIRUS (Thompson Strain) IN VARIOUS TISSUES OF EXPERIMENTALLY INFECTED BATS *(Tadarida brasiliensis)* WITH NO OVERT SIGNS OF DISEASE

NO. OF BATS	TIME AFTER INOCULATION (days)	RABIESVIRUS DEMONSTRATED IN		
		BRAIN	SALIVARY GLANDS	BROWN FAT
21	15 - 83	+	−	−
6	13 - 75	−	−	+
4	21 - 51	+	+	−
3	20 - 25	+	−	+
1	22	+	+	+
35	13 - 83	29	5	10

This series of experimental studies demonstrated that insectivorous bats were susceptible to rabiesvirus and, more importantly, that the virus could multiply and persist for long periods of time in brain, brown fat, and salivary gland tissue of animals which showed no overt signs of disease and did not succumb. Demonstration of the lipotropic characteristic of rabiesvirus suggested that the brown adipose tissue of bats could serve as a site for rabiesvirus replication and persistence in latently infected bats, particularly during hibernation; and the low temperature studies demonstrated that the virus did persist in cold-exposed animals for prolonged periods. The repeated demonstration of rabies infection in asymptomatic bats taken from nature over the years, the many isolations of rabiesvirus from the brown fat of naturally infected bats (Bell & Moore, 1960; Sulkin *et al.*, 1960b; Girard *et al.*, 1965), and the isolations of rabiesvirus from bats collected during the winter months support the results obtained in our experimental studies, which had been designed to define how bats serve effectively as reservoir hosts for rabiesvirus.

ARTHROPOD-BORNE VIRUSES

The arthropod-borne animal viruses are, as the name implies, a family of agents transmitted between vertebrate hosts by the bites of blood-sucking arthropods, especially mosquitoes and ticks. Presently, more than 200 viruses have been tentatively classified as arboviruses, and although most of these have been further classified into some 20 to 30 antigenic groups based on cross-reactions in various serological tests, many of the agents remain ungrouped. The arboviruses present perhaps the most complex biological life cycles of any of the known viral agents. In nature, arthropods support nondetrimental cycles of virus multiplication and transmit the agent to vertebrate hosts by bite. Infection in the susceptible host, whether symptoms of disease become apparent or not, is characterized by viral proliferation in various tissues and the shedding of virus into the blood stream to establish a viremic state of variable duration which provides infective virus for feeding vectors, completing the cycle. Simply stated, an infection chain depends on the association of a viremic host, a blood-sucking vector, and a susceptible host. In reality, the multiplicity of vectors and hosts which may be involved in the natural history of any one virus is extremely difficult to determine. The arboviruses associated with illness in man and domestic animals (particularly Groups A and B) have received special attention, for the identification of specific vectors and wildlife hosts has been an invaluable aid in the control of these diseases. Various species of mosquitoes, ticks of the family *Ixodidae*, and the sand fly, *Phlebotomus papatasi*, are the blood-sucking arthropods most clearly implicated

TABLE 3

BAT COLLECTIONS IN JAPAN (1963 - 1965)

SPECIES	HABITAT	COLLECTION AREA			TOTAL
		HONSHU	KYUSHU	HOKKAIDO	
Miniopterus schreibersi fuliginosus	lava cave, quarry, shelter, water tunnel	692	475		1167
Rhinolophus cornutus cornutus	cave, quarry, mine, sea cave	195	72	70	337
Vespertillio superans	dwelling	20		90	110
Myotis macrodactylus	water tunnel, quarry, mine, cave, shelter, sea cave	2	40	67	109
Rhinolophus ferrumequinum nippon	quarry, lava cave, shelter, mine, cave	34	25	29	88
Myotis mystacinus	dwelling			42	42
Pipistrellus abramus	dwelling	21	20		41
Plecotus auritus sacrimontis	cave, lava cave	22			22
Myotis nattereri bombinus	water tunnel, mine		16		16
Murina leucogaster higendorfi	lava cave, quarry	2			2
TOTAL		988	648	298	1934

as important vectors of the arboviruses which produce disease in man. Man and domestic animals are generally considered dead-end hosts for the mosquito-borne arboviruses, since their infections are not important links in the biological cycles of multiplication which perpetuate these viruses. Birds and certain wild vertebrates are the important natural hosts. In tropical areas vertebrate-mosquito-vertebrate transmission cycles may be continuous the year round, providing constant foci of infection as sources for recurrent epidemics in man and domestic animals. In temperate zones, however, the mechanisms by which the mosquito-borne viruses, endemic in these locales, survive during the winter months when the mosquito vectors are not active to produce new infections in the spring, are not clearly understood. It has been suggested that migrating birds, wintering in the tropics where transmission cycles are continuous, serve to reintroduce virus into the temperate zones each spring or that the mosquito vectors themselves harbor the agents in a latent state during the winter; but neither of these theories has ever been proved conclusively. In recent years many animal species, particularly hibernating homeotherms and poikilotherms, including the bat (Ito & Saito, 1952; La Motte, 1958), the hedgehog (van Tongeren, 1958), the snake (Gebhardt *et al.*, 1964), the frog (Chang, 1958), and the lizard (Karstad, 1961) have been investigated as possible overwintering hosts for the arboviruses. Having developed an interest in the bat as a reservoir host for viruses through our studies of experimental rabiesviruses infection in these animals, and since the documented evidence that bats could be involved in the natural history of the arboviruses was only suggestive of such a relationship (Ito & Saito, 1952; La Motte, 1958; Laemmert *et al.*, 1946; O'Connor *et al.*, 1955; Havlik & Kolman, 1957), a series of correlated experimental and field studies designed to evaluate the participation of various species of bats in the ecology of certain arboviruses was undertaken. We shall first point up the physiological and behavioral characteristics of certain species of Chiroptera which could contribute to their effectiveness as reservoir hosts, then summarize the experimental studies which served as an indication of the feasibility of undertaking large-scale field studies, and, finally, discuss the results of surveys of bat populations for evidence of natural arbovirus infection.

Certain characteristics of bats could enable these animals to function effectively as reservoir hosts for arboviruses. Adaptation for flight in bats, a characteristic which is particularly important in an animal species involved in the ecology of an arbovirus, is discussed elsewhere in this volume (Vaughan, 1970). The long annual migrations of certain species would make these animals capable of reintroducing viruses into endemic areas each spring in the same manner as has been suggested for migratory species of birds. Bats could also disseminate virus within these areas, since they travel long distances on nightly flights in search of food and do not always return to the same roost. The phenomenon of hibernation common to many species inhabiting temperate zones would provide a mechanism for the overwintering of arboviruses. The low body-temperature and reduced metabolic rate of the hibernating bat would allow virus to persist in the tissues of infected animals during the winter months, and provide seed for recurrent viremias upon emergence from the hibernation in the spring. The interscapular brown adipose tissue, which is a well-developed, organ-like structure in hibernating species and has been shown to control entrance into and emergence from hibernation, would provide an excellent site for virus sequestration in the infected hibernating bat. The long life-span of bats could also add to their reservoiring ability, since chronic relapsing arbovirus infections in such a long-lived host would be an effective means for the persistence of these agents in nature. Finally, many bat species are insectivorous, and it is with these species that our experimental and field studies have been concerned. Consumption of mosquitoes would allow for oral infection of bats with arboviruses and the manner in which these animals catch insects, as described elsewhere in this volume (Glass, 1970), provides the opportunity for bite transmission through wing veins. In addition, many habitats of bats in nature are also occupied by mosquitoes and the quiescence of the bat, hanging motionless for the greater portion of the day, would be an advantage for the feeding mosquito. Mosquitoes are known to feed at a higher rate on relatively inactive nestling birds than on adults, and in mosquito-host preference studies using baited traps, the degree to which the bait can be restrained is an important factor (Blackmore & Dow, 1958).

EXPERIMENTAL ARBOVIRUS INFECTION IN CHIROPTERA

In the initial experimental studies, the susceptibility of several species of bats maintained at room temperature to various strains of two Group B arboviruses, Japanese B encephalitis (JBE) and St. Louis encephalitis (SLE) viruses, was determined (Sulkin *et al.*, 1963). Three bat species were used in these studies: the Mexican free-tailed bat (*Tadarida brasiliensis*), the little brown bat (*Myotis l. lucifugus*) and the big brown bat (*Eptesicus f. fuscus*). All three species were susceptible to one or more of the strains used, the intensity of the infection depending on the mouse passage level and the origin of the virus strain. Recent isolates in low mouse passage were more infective than laboratory adapted strains. Subsequent studies included experiments on the influence of the gravid state on the susceptibility of bats to SLE and JBE viruses, and transplacental transmission of these agents (Sulkin *et al.*, 1964); studies on the influence of environmental temperature on JBE and SLE virus infections in bats (Sulkin *et al.*, 1966a); and an investigation of the immune response of big brown bats to JBE virus (Sulkin *et al.*, 1966b).

The characteristics of experimental arbovirus infection in bats which, if true for natural infection, would make these animals particularly effective reservoir hosts are listed in Table 4. Viremia occurs within 48 hours following subcutaneous inoculation of a small dose of virus, and may persist for as long as 30 days in bats maintained at 24°C. This long-term viremia, together with the fact that levels of virus in the blood are sufficient to infect the vector, would certainly favor transmission. The demonstration of virus in brown adipose tissue gives meaning to the hypothesis that this tissue could serve as a depot storage site for virus in the persistently infected bat. Signs of encephalitis were never observed in experimentally infected bats, despite demonstration of virus in brain tissue, and no histopathologic alterations were discernible. The ability of a reservoir host to sustain infection without succumbing is obviously of great importance in regard to the persistence of arboviruses in nature. The demonstration of transplacental transmission of JBE and

SLE viruses with no apparent abnormalities in the fetuses would indicate that these viruses could circulate in bat populations in the absence of a vector.

In an attempt to determine if bats could maintain arbovirus infection throughout hibernation and provide infective blood meals for mosquitoes in the spring, studies were designed to characterize the course of arbovirus infection in bats as it might occur in nature during the winter months in temperate zones. Bats collected during the late summer and early fall months, at a time when the animals were physiologically prepared for entrance into hibernation in nature, were used in these studies so that cold exposure would induce a true state of hibernation rather than simply hypothermia (Menaker, 1962). It was found that an established infection is suppressed by low temperature but is sustained for several months. Virus can be detected in low levels in blood and brown adipose tissue, and when animals were finally transferred back to room temperature, viral replication occurred, increasing the levels of virus in these tissues. When bats are inoculated in an active state and placed immediately at 10°C only trace amounts of virus are detectable subsequently; but, again, the infection persists and respectable levels of virus appear in the blood following transfer to 24°C. When bats are acclimated to low temperature and inoculated while in a suppressed metabolic state no evidence of infection can be detected during the period of hibernation, yet viable virus persisted for over 3 months in such animals and produced a normal course of infection upon removal to room temperature. Thus, regardless of the stage of infection a bat might be in when entering hibernation, these studies would indicate that these animals would be capable of overwintering arboviruses.

Studies on the immune response of bats to experimental JBE virus infection provided rather interesting results. First of all we could find no clear evidence of the production of complement-fixing (CF) or hemagglutinating-inhibiting (HI) antibodies in animals known to be infected. Most bats produced antibody detectable in neutralization tests, but the quantity was low and the quality seemed poor compared to that produced by conventional laboratory animals against this agent. Perhaps the best evidence that bats do not develop a firm immunity following experimental arbovirus infection was the demonstration of spontaneous recurrent viremias, and susceptibility to reinfection in a small group of big brown bats maintained in the laboratory for 2 to 3 years.

FIELD STUDIES

Japan was chosen as the site for the initial field work because epidemics of JBE occur yearly in certain parts of that country and the need for continued studies of the ecology of the causative agent is evident. The field studies were planned in cooperation with Dr. Teiji Miura and Professor K. Toyokawa of the Department of Hygiene, University of Tokyo, and several other collaborators. Certain areas within the three main regions of Japan (Honshu, Kyushu, and Hokkaido) were selected as survey sites. The program for collection of bats required that animals be netted throughout the year in order to determine if virus was circulating in bat populations only during the months when mosquitoes and recognized vertebrate hosts could be found harboring the agent, or if infection could be demonstrated in bats netted during months when JBE virus seemed to be hidden in nature. Since our studies of experimental JBE virus infection in bats maintained at low temperature had shown that under certain circumstances virus could be recovered at intervals from the blood and brown adipose tissue of torpid bats, we were particularly interested in examining animals taken from nature during the winter months of hibernation. Collection of bats began in the summer of 1963 and continued at intervals into December 1965. A total of almost 2000 bats were obtained and, with few exceptions, significant numbers were taken from nature during each of the four seasons of the year at various sites throughout Japan. Table 3 lists the bat species collected, the habitats where these animals were found, and the number netted in each of the three main regions of Japan. Ten bat species were represented in our collections. The identification of species was made by Drs. Kiyotoshi Kaneko and Kenji Miyamoto of the Tokyo Medical and Dental University and Drs. Takeo Fukuda and Tohru Sasahara of the Miyasaki Institute of Public Health. A greater number of collections was made in Honshu than in the other two regions of Japan and larger numbers of bats were netted; eight bat species are represented in the 988 animals collected. In Kyushu, the southern region of Japan, a total of 648 bats comprising 6 species were collected, whereas in Hokkaido only 298 bats of 5 different species were obtained. The bat population as a whole is smaller in this northern region, thus making the collection of significant numbers more difficult. In addition, because of the low bat population-density in Hokkaido and the characteristic southern migration of many species as winter approaches, collectors were unable to locate bats later than October in any year. More than 50% of the total number of bats collected were *Miniopterus*, all of which were netted in Honshu and Kyushu. The second largest group was made up of *Rhinolophus cornutus*, netted in all three regions of Japan. Table 3 also lists the specific places within each prefecture where bats were collected. With the exception of a few collections made in dwellings, the remainder of the bats were obtained from caves, quarries, mines, and tunnels; and the difficulties which could be encountered in capturing these animals in such places throughout the year are evident. For this reason the total number of bats collected in Japan is a credit to those individuals who cooperated in this phase of the study.

Specimens obtained from bats included whole blood, interscapular brown adipose tissue, brain, kidney, spleen, and salivary glands for virus assay, and plasma for antibody studies. Methods used for the isolation and identification of JBE virus and for the detection of neutralizing antibodies against this agent were those in general use (Lennette & Schmidt, 1969). Since viremia is an essential characteristic of infection in animals suspect as natural hosts for the arboviruses, emphasis was placed on the assay of blood specimens from the bats collected in Japan, and only a limited number of other tissues have been tested. Because of our interest in the interscapular brown fat as a focus of infection in the persistently infected bat, pooled suspensions of this tissue from selected groups of bats were included in the assays which have been completed. A detailed account of the results of these assays appears elsewhere (Sulkin *et al.*, 1970). Thirty-three viral agents, 24 of which have been identified as strains of JBE virus, were obtained from the blood of bats collected in Honshu and Kyushu during 1963-1965. No viruses were recovered from bats netted in Hokkaido. All strains of JBE virus were recovered from either *Miniopterus* or *Rhinolophus cornutus*, probably because larger numbers of these two species were obtained for study than any of the other species included in the collections. JBE virus was recovered from males and females with approximately equal frequency. Sixteen of the strains were isolated in assays of blood specimens from 1139 *Miniopterus*, and 8 strains were recovered from the 267 *Rhinolophus cornutus* tested for viremia. JBE virus were isolated from bats during each of the four seasons of the year, the highest incidence of infection occurring in groups of *Miniopterus* netted in the fall. JBE virus was also isolated from the pooled brown fat tissue of 3 *Miniopterus* netted in October. In extensive studies of the ecology of JBE virus in Japan during 1952-1958 other investigators were unable to detect virus in the vector population or in known reservoir hosts (birds and swine) between April and late June; and although evidence of JBE virus was demonstrable in mosquitoes, birds and swine during the summer months and into September, the agent again could not be detected in nature by late September (Buescher & Scherer, 1959). Thus the results obtained in our field studies possibly represent the first evidence that JBE virus overwinters in Japan in a vertebrate host, since this agent was isolated from bats collected in the late fall (October and November), during winter months (December and January), and

TABLE **4**

DEMONSTRATION OF NATURAL ST. LOUIS ENCEPHALITIS (SLE) VIRUS INFECTION IN BATS *(Tadarida brasiliensis)* **COLLECTED IN ANGLETON, BRAZORIA COUNTY, TEXAS DURING 1964 - 1966**

COLLECTION AREA	DATE	NO. BATS COLLECTED	NO. BATS TESTED FOR VIREMIA	NO. STRAINS SLE VIRUS ISOLATED
ANGLETON (Brazoria County)	1964			
	26 August	137	137	2
	8 September	75	75	1
	15 October	45	45	0
	11 November	79	53	1
	4 December	35*		
	1965			
	20 April	90	90#	2
	5 May	52	52#	0
	12 July	50	50#	1
	28 September	36*		
	4 October	68*		
	23 November	168	168	4
	3 December	102	102	1
	1966			
	28 January	79	79	1
TOTALS		1016	851	13

*Bats tested only for neutralizing antibodies against SLE virus.
#Spleens only assayed for virus in pools of 3 to 4.

in the early spring (April and May), as well as during summer months. Plasma samples from 1734 of the bats collected in Japan were assayed for serological evidence of infection with JBE virus, and neutralizing antibodies were demonstrated in approximately 8% of the plasmas from bats netted in Honshu and Kyushu and in 3% of the plasmas from bats netted in Hokkaido (Miura *et al.*, 1969). These results support the data concerned with the isolation of the JBE virus from these animals and provide additional evidence that the bat populations in Japan are persistently infected with this viral agent.

In addition to the strains of JBE virus isolated from bats collected in Japan, several viral agents were recovered which have not as yet been fully characterized; four of these react as Group B arboviruses in the hemagglutination-inhibition test, but appear to be serologically distinct from JBE virus.

Within recent years St. Louis encephalitis (SLE) virus has become the most common cause of arboviral encephalitis in the United States, five major epidemics occurring since 1962 (Luby *et al.*, 1969). Although considerable knowledge has accumulated concerning the inter-relationships of virus, vectors and hosts since the original isolation of the agent in 1933, there are still, as with JBE virus, many missing links in our understanding of the biological life cycle of SLE virus, particularly with regard to the survival of the agent in temperate zones during the winter months.

An epidemic of SLE in Houston, Texas in the summer of 1964 encouraged the initiation of field studies in south Texas to determine if bat populations in that area were serving as virus carriers in a manner that our experimental studies had indicated them capable of doing. Although we were unsuccessful in finding colonies of bats in the Houston metropolitan area where the center of the epidemic was located, a large colony of Mexican free-tailed bats was found in the attic of a house in Angleton, Brazoria County, approximately 40 miles south of Houston, a distance within the nocturnal feeding flight range of this species (Davis *et al.*, 1962). The first collection of bats was made on 26 August 1964, which subsequently proved to be during the week when the epidemic reached a peak with regard to number of cases. In order to determine if SLE virus could be detected in the bat population subsequent to the epidemic period, additional groups of bats were netted in September, October, November, and December of 1964. Collections were resumed in April of 1965 and continued at approximately monthly intervals through January 1966, to determine if SLE virus was circulating in the bat population in what proved to be a nonepidemic year in the Houston area. Table 4 lists the specific dates of the bat collections made in Angleton, Texas during 1964-1966 and the numbers of animals obtained in each collection. A total of 1016 bats was collected, with significant numbers being taken from nature during each of the four seasons of the year. Certain groups of bats were tested only for neutralizing anitbodies against SLE virus, and we failed to obtain blood specimens from a few animals, so that only 851 bats were tested for viremia by the assay of either whole blood or spleen tissue. Two strains of SLE virus were isolated from bats obtained in the initial collection on 26 August 1964, and 11 additional strains were obtained from bats netted at intervals throughout the following 18-month period. The isolation of SLE virus from bats collected during the epidemic period in Houston was of interest since it represented the first reported isolation of this agent from a naturally infected mammal other than man; but it showed only that natural SLE virus infection could be demonstrated in bat

TABLE 5

DEMONSTRATION OF NATURAL ST. LOUIS ENCEPHALITIS (SLE) VIRUS INFECTION IN BATS (*Tadarida brasiliensis*) COLLECTED IN CORPUS CHRISTI, NUECES COUNTY, TEXAS DURING 1966 - 1967

COLLECTION AREA	DATE	NO. BATS COLLECTED	NO. BATS TESTED FOR VIREMIA	NO. STRAINS SLE VIRUS ISOLATED
CORPUS CHRISTI (Nueces County)	**1966**			
	11, 28 September	134	134	5
	12, 26 October	143	131	0
	9, 28 November	134	134	2
	22 December	66	66	1
	1967			
	1, 22 February	140	140	1
	5, 20 April	127	127	2
	26 April	60*		
	31 May	66	66	2
TOTALS		870	798	13

*Bats tested only for neutralizing antibodies against SLE virus.

populations during the time when the agent is readily demonstrable in nature in mosquitoes and avian hosts whose role in the epidemiology of SLE has long been recognized. However, the subsequent isolations of strains of SLE virus from bats netted after the epidemic period and during the following nonepidemic year indicated that populations of Mexican free-tailed bats in the Houston area are persistently infected with this agent.

In the summer of 1966 an outbreak of SLE occurred in Dallas, Texas; but we were unable to find at that time sufficient numbers of bats in Dallas or environs to enable us to determine if these animals were harboring SLE virus. However, when a second outbreak of SLE occurred in Texas during the summer of 1966, in Corpus Christi, we were fortunate in locating colonies of *Tadarida brasiliensis* roosting along beams in the ceilings of warehouses in the dock area of the city. The first collection was obtained 11 September 1966 and continued at approximately 14 day intervals through November, then monthly until June 1967 when the colonies were disrupted by officials of the city-county health department (Table 5). Davis and associates (1962), in an extensive study of the life history and ecology of Mexican free-tailed bats, found little evidence that this species overwinters in buildings in Texas, but suggested that this habit is more prevalent than observed, particularly along the Gulf coast. Our collections of bats from Angleton during the winters of 1965 and 1966 and from Corpus Christi throughout the winter months in 1966 and 1967 are evidence that at least a percentage of the free-tailed population which roosts in buildings in these locations in Texas remains throughout the winter. During the collection period in Corpus Christi, 870 bats were obtained for study and 13 strains of SLE virus were isolated from the bloods of 798 of these animals. Although collection of bats was initiated late in the epidemic period in Corpus Christi when the attack rate was declining rapidly, 4 strains of SLE virus were isolated from bats obtained in the first collection on 11 September, and additional strains were obtained from virtually all of the groups of bats collected subsequently. Although disruption of the bat colonies in Corpus Christi prevented our collecting bats from this site after May, 1967, these colonies had been adequately sampled during the fall, winter, and spring months, the seasons in which we were most interested in detecting SLE virus infection in the bats in this area. The isolation of SLE virus from bats netted in November and December of 1966 and in February, April, and May of 1967 demonstrates the persistence throughout the year of SLE virus infection in the Mexican free-tailed bat population in the Corpus Christi area. Serological evidence of SLE virus infection in *Tadarida* populations in south Texas has also been obtained; neutralizing antibodies against this agent have been demonstrated in 10 to 20 per cent of the more than 500 plasma samples tested to date. In addition to the strains of SLE virus isolated from the bats collected in south Texas, several strains of Rio Bravo virus and several as yet uncharacterized viral agents were recovered from the bloods of these bats. A detailed report concerning the field studies conducted in south Texas during 1964-1967 has been presented (Allen *et al.*, 1970).

SUMMARY

Information accumulated since the first isolation of rabiesvirus from insectivorous bats in the United States in 1953 leaves no doubt that these animals are now recognized as natural reservoir hosts for this agent, and will continue to be considered in surveys on wildlife rabies. In evaluating the role of bats in the ecology of rabiesvirus, probably the most important factor is the ability of these animals to sustain rabies infection in the absence of overt symptoms and death. Although many of the bats found to be infected with rabiesvirus have been those behaving in an abnormal manner, the virus has also been isolated from apparently normal bats, and experimental studies have shown that inoculation of massive doses of certain strains produces symptoms of rabies in only a small percentage of the bats, even though viral multiplication in brain and other tissue can be demonstrated. Thus bat populations can maintain ever shifting foci of rabiesvirus, providing a constant source of infection for wildlife predators, domestic animals and man, and offering a means of persistence of the virus in nature. In several instances bat rabies has been reported from states which have been consistently free of sylvatic rabies over a period of years, suggesting that dissemination and reintroduction of rabies infection into areas free of the disease for long periods of time, may be the greatest menace of the bats which carry rabiesvirus.

Early studies on the epidemiology of arboviruses suggested that the reservoir hosts of these agents were likely to move about rapidly over long distances, and subsequent studies proved that birds are important reservoirs. Now, almost 30 years later, information is accumulating which indicates that another animal species whose mode of locomotion is flight may also be involved in the ecology of the arboviruses. The isolation of JBE virus from bats netted in Japan during months when this agent had not been detected in vector populations, in birds, or in other known reservoir hosts seems particularly significant. The isolations of SLE virus from bats collected near Houston, Texas during an epidemic of SLE in that area and at intervals throughout a subsequent 18-month period, and the isolation of this agent from bats collected in Corpus Christi, Texas during and subsequent to an epidemic of SLE in that city, demonstrate that persistence of SLE virus infection in populations of Mexican free-tailed bats, the most abundant species in the southwestern United States. Although bats in general are most often thought of as inhabiting caves and caverns in remote places, and might be imagined as participating only in enzootic cycles of arbovirus transmission, many species of bats are known to occupy man-made structures as daytime roosting sites (Dalquest, 1970). Mexican free-tailed bats, in particular, are often found in close proximity to man. Davis *et al.* (1962) found this species roosting in buildings in 87 of 150 towns visited in south Texas, and observed that age, type of architecture, relative position in a town, or use by humans failed to exclude a building from possible habitation by these animals. This bat species could, therefore, be directly involved in the sequence of events which leads to epidemics of SLE.

In a review concerning the bat as a reservoir host of viruses in nature published in 1962 (Sulkin) we noted that, aside from surveys of bat populations for the presence of rabiesvirus, no systematic search for evidence of infection with other viral agents had been made at that time. During the intervening years, investigators have shown increasing interest in considering bats of epidemiological importance, particularly with regard to the arboviruses; and these animals are now frequently included in large scale field studies concerned with the ecology of these agents. At present there is information, based either on experimental or field studies, which suggests the association of bats with numerous arboviruses. It seems likely, then, that if sufficient evidence has not already accumulated to show that bats are important reservoir hosts for arboviruses, this proof will soon be forthcoming.

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