species of fish and this fact may explain why concretions are so numerous. Nevertheless, conversion to a concreted form is not peculiar to this species: a few concretions of the gizzard shad (*Dorosoma cepedianum*) and of chicken or duck bones also are found.

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Isolation of St. Louis Encephalitis Virus from Bats (Tadarida b. mexicana) in Texas

Abstract. *A strain of St. Louis encephalitis virus has been isolated from Mexican free-tailed bats* (Tadarida b. mexicana) *collected at the time of an outbreak of encephalitis in Texas in 1964.*

An epidemic of St. Louis encephalitis in Houston, Texas, during the summer of 1964 provided us with an opportunity to explore, under field conditions, a hypothesis which has been under study in our laboratory for several years, namely, that Chiroptera may be involved in the epidemiology of certain arbovirus infections. Initial studies on experimental rabies infection in bats demonstrated viral invasion and multiplication in interscapular brown adipose tissue. This result suggested that brown fat could serve as a storage site for virus particles in the latently infected bat, thereby contributing to the ability of these animals to store rabies virus in nature (*1*). Together with reports of others on the susceptibility of bats to experimental infection with certain arboviruses (*2*), our observations (*1*) prompted studies to determine whether Chiroptera could serve as reservoir hosts for these viruses in a manner similar to that described for rabies virus. Results of studies concerned with the tissues involved in experimental Japanese B encephalitis (JBE) and St. Louis encephalitis (SLE) virus infections in bats—with gravid bats in which transplacental transmission of these agents was demonstrated, with the influence of environmental temperature on the course of experimental arbovirus infections in bats, and with the immune response of bats to experimental arbovirus infection—have indeed indicated that these animals would be ideal reservoir hosts for these agents (*3*). Furthermore, it would appear that both hibernating and migrating species, by virtue of various aspects of the physiology and ecology of the mammalian order Chiroptera, may be capable of filling certain gaps in the year-round transmission cycles of the arboviruses, and allow overwintering or reintroduction of a viral agent into a particular area.

The outbreak in Houston and environs began in the early summer of 1964 and was clearly established as St. Louis encephalitis (*4*). While efforts were being made to locate bat populations in metropolitan Houston in the vicinity of the epidemic focus, collections were made in outlying areas. Two species of bats, the Mexican free-tailed bat (*Tadarida b. mexicana*) and the evening bat (*Nycticeius humeralis*), comprised a collection made on 26 August 1964, in Angleton, Texas, 64 km (40 miles) south of Houston. Whole blood (0.1 to 0.2 ml) was obtained by cardiac puncture and placed immediately into 0.9-ml portions of chilled 10 percent rabbit serum-saline diluent. Bats were exsanguinated before tissues were removed for virus assay. Interscapular brown adipose tissue, brain, spleen, and kidneys were removed in the order indicated, with separate sets of instruments to avoid any cross-contamination, and were stored in compartmentalized containers at −76°C. Immediately prior to assay, tissues were ground in a chilled mortar with alundum and enough chilled diluent to make an approximately 10-percent suspension. All specimens were assayed by the intracerebral inoculation of 2- to 3-day old suckling white Swiss mice.

Blood specimens from 137 bats, collected at a time subsequently shown as the peak of the Houston epidemic, yielded several viral isolates. Two of these isolates, both from *Tadarida b. mexicana*, were chosen for further identification: one, designated HA-119, has been identified as a strain of Rio Bravo virus and the other, designated HA-73, proved to be a strain of SLE virus. We now report the initial isolation and identification of SLE virus from bats.

Five of nine suckling mice, each of which received 0.02 ml of a 10^{-1} dilution of blood from bat No. HA-73 intracerebrally (ic) had died or developed symptoms of central nervous system disease (CNS) 4 to 6 days after inoculation. Brains from mice which developed CNS on the 6th day were subinoculated into a litter of six suckling mice, all of which died or developed CNS 3 days later. A suspension of brain from this second suckling-mouse-brain passage (SMB_2) had a titer of $10^{-9.0}$ per 0.02 ml (ic) in suckling mice, and treatment with ether and with sodium deoxycholate reduced this titer by 3 log units. Agent HA-73 was not infective for guinea pigs or rabbits, and hyperimmune antiserum to this agent was prepared in guinea pigs. A 20-percent suspension of HA-73-SMB_3 was infective for 3-week-old weanling mice, and the second weanling-mouse-brain passage (WMB_2) had a titer of $10^{-7.3}$/0.03 ml (i.c.). Third weanling-mouse-brain passage material was infective for hamster-kidney tissue cultures, producing a cytopathic effect within 48 hours, with a titer of $10^{-7.5}$/1.0 ml. Reisolation of agent HA-73 from the original blood specimen was accomplished 2 months and, again, 6 months after the initial isolation. In addition to blood, other tissues of bat No. 73 were assayed, and virus was recovered from the interscapular brown fat and the kidneys. Prior to the isolation of agent HA-73 and the initiation of tests for its identifi-

Table 1. Results of hemagglutination-inhibition tests with the bat virus isolate (HA-73) and certain Group B arboviruses (St. Louis and Japanese B encephalitis).

Antiserum			Antigen, 4 units		
			St. Louis		Japanese B
Virus	Strain	Source	Bat isolate (HA-73)	Human isolate (Parton)	Mosquito isolate (OCT-541)
SLE	Bat HA-73	Guinea pig	160*	320	320
SLE	Human-Parton	Rabbit	320	640	160
SLE	Flicker bird-55	Rabbit	320	640	160
Rio Bravo	Bat Tm-BC-618	Guinea pig	320	320	320
Rio Bravo	Bat HA-119	Guinea pig	40	80	80
JBE	Mosquito OCT-541	Guinea pig	80	160	640

* Reciprocal of serum titer.

cation, no experiments with SLE virus had been conducted in this laboratory since the summer of 1962.

We performed hemagglutination-inhibition (HI) tests essentially according to the procedures recommended by Clarke and Casals (*5*). Serum neutralization (SN) tests were done according to the constant serum-varying virus method. Equal parts of diluted virus and unheated, undiluted serum were incubated at 37°C for 1½ hours, chilled, and inoculated (0.03 ml, ic) into groups of five weanling mice. Virus strain designations and passage levels were as follows: SLE-Parton, reference strain; SLE–flicker bird-55, 7th; SLE-TH4-9f, 3rd; Rio Bravo-Tm-BC-618, 4th; Rio Bravo-HA-119, 3rd; and the bat isolate under study, HA-73, 3rd (*6*). Results of cross HI tests with hemagglutinins and specific antiserums prepared for bat virus isolate HA-73 and certain group B arboviruses are presented in Table 1. The degree of cross reactivity of agent HA-73 with the known virus strains and their specific antiserums indicated that the bat isolate was a group B arbovirus. These results were confirmed in broader spectrum HI tests (*7*). A sample of HA-73 antiserum sent to the Yale laboratory (*7*) was unreactive against eight units of two group A antigens (Sindbis and Semliki Forest) and three antigens of the Bunyamwera group (Bunyamwera, Guaroa, and Maguari), whereas positive results were obtained with five group B antigens (SLE, 1:640; West Nile, 1:80; JBE, 1:160; Dengue II, 1:80; and Russian spring-summer encephalitis, 1:80).

Viral isolate HA-73 obtained from the blood of a Mexican free-tailed bat is a strain of SLE virus (Table 2). A comparison of the log neutralization indices (LNI) obtained by checkerboard SN tests shows that bat isolate HA-73 was neutralized by antiserums prepared against the Parton reference strain and the flicker bird-55 strain of SLE virus and by human convalescent serum (*6*) obtained during the Houston epidemic to the same extent as by its homologous antiserum. On the other hand, HA-73 was not neutralized by antiserum prepared against two strains of Rio Bravo virus (Tm-BC-618 and HA-119). The antiserum prepared against HA-73 neutralized all three known strains of SLE virus (flicker bird-55, Parton, and TH4-9f) by three or more log units and, in addition, demonstrated significant protection against both strains of Rio Bravo virus. This one-way cross between the SLE and Rio Bravo viruses is characteristic of the relationship between these two agents (*8*). The isolation of both Rio Bravo virus (HA-119) and SLE virus (HA-73) from a single collection of bats points up the fact that the persistence of Rio Bravo virus in bat populations in the southwestern United States must be recognized and considered in the specific identification of group B arboviruses isolated from these animals (*9*). The degree to which persistent infection with Rio Bravo virus would influence the susceptibility of bats to SLE virus infection or the isolation of SLE virus from a dually infected animal is currently under study.

Whether or not the incidence of SLE virus infection in bat populations in south Texas approaches that recorded for known reservoir hosts of the arboviruses in the United States remains to be determined. Even a single isolation of virus from Chiroptera seems significant, however, in view of the characteristics of experimental arbovirus infection in these animals (*3*). Since the initiation of field studies in the Houston area, bat populations have been sampled at intervals in an attempt to isolate SLE virus from bats during nonepidemic periods. Several additional viral isolates are now being characterized. In correlated studies concerned with providing evidence that mosquitoes feed on bats in nature, engorged mosquitoes collected in and around bat habitats are being tested for the presence of bat blood in stomach contents. Mosquitoes were observed in close proximity to the bat population inhabitating the site from which the collection on 26 August 1964 was made.

Table 2. Results of cross-neutralization tests with the bat virus isolate (HA-73) and with selected strains of St. Louis encephalitis (SLE) and Rio Bravo (RB) virus. GP, guinea pig; R, rabbit; H, human.

Antiserum			Virus strains					
			St. Louis encephalitis				Rio Bravo	
Virus	Strain	Source	Bat (HA-73)	Bird (flicker-55)	Human (Parton)*	Mosquito (TH4-9f)	Bat (Tm-BC-618)	Bat (HA-119)
SLE	Bat HA-73	GP	3.0†	3.4	3.0	3.3	3.4	3.2
SLE	Flicker bird-55	R	3.2	3.0	≥2.5	ND	1.8	1.8
SLE	Human-Parton	R	3.2	ND	2.2	ND	ND	2.4
SLE	Houston convalescent	H	2.7	ND	3.2	3.9	4.0	ND
RB	Bat Tm-BC-618	GP	0.5	0.4	ND	1.0	6.0	6.0
RB	Bat HA-119	GP	1.2	1.6	0.8	ND	5.0	6.0

* Reference strain. † Log neutralization index; ND, not done.

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d-Tubocurarine Chloride: Effect on Insects

Abstract. *Injection of* d-*tubocurarine chloride into certain insects produces complete flaccid paralysis. The site of injection is closely related to the region of primary paralysis. The effect depends on concentration, with distinct differences in the optimum concentrations for various species so far tested. A dose-response curve has been prepared for* Calliphora erythrocephala.

While recordings were being made from the chemosensory hairs on the labellum of *Sarcophaga bullata*, undetermined amounts of *d*-tubocurarine chloride were injected into the living adult fly in an attempt to reduce or eliminate random muscular movement that was making it difficult to obtain normal records. The curare was injected by one of us who was not familiar with the copious literature (for example, *1*, *2*) reporting that curare has no effect on insects. We were surprised to find a complete, vertebrate-type, curarine response in the injected fly.

Additional studies have shown that *d*-tubocurarine chloride does indeed affect various different species of insects; injection produces a recognizable syndrome and the affected insect is completely immobilized. In some instances the proboscis is extended. After a specific time period, which varies with the species, spasmodic twitching of the legs begins and there is some fluttering of the wings. Sometime thereafter the insect is able to stand when prodded but moves very little; still later it begins to walk slowly, but the hind legs remain completely paralyzed for extended periods. The insect begins normal walking movements but remains somewhat sluggish; finally comes complete recovery.

The site of injection is important and seems to be very closely related to the ensuing response. If the curare is injected into the anterior end of the thorax or pronotum, the front legs are the first part of the body to show the symptoms and to become paralyzed. If the injection is into the midthorax or mesonotum, the middle pair of legs shows paralysis first; if into the posterior part of the thorax or metanotum, the hind legs are first. Injection into the abdomen affects all legs at about the same time, but the time between injection and response is much longer than when the curare is injected into the thorax. After the insect has shown response to curare (complete body paralysis), the hind legs are always the last part to recover.

The effects described depend on concentration, with distinct differences in optimum concentrations for the species tested. Table 1 lists dosages required to effect complete flaccid paralysis in various insects; in most instances these figures probably represent an overdose.

Most of the species tested were represented by at least two wild-caught specimens. Each insect was fastened to the end of a syringe and injected with curare until there was a noticeable effect; it was then removed and allowed to recover. Insects that were dosed so heavily that they did not fully recover within 3 to 4 hours invariably died. Along with each injection, a control of the same species was injected with a comparable volume of saline with never any observable effect. All injections were made with a microliter syringe (C. H. Stoelting Co.) calibrated at 0.43 μl per turn. The curare used was isotonic *d*-tubocurarine chloride (USP)

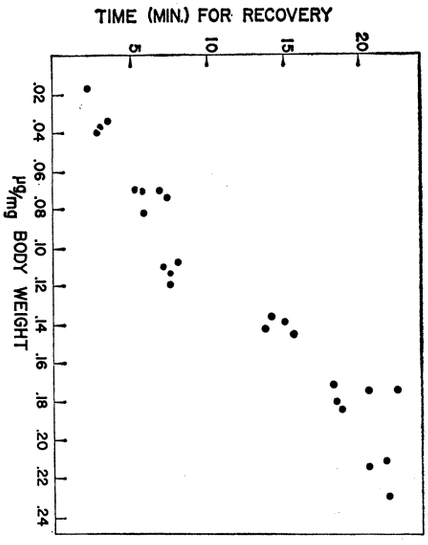

Fig. 1. Dose-response curve for injection of *d*-tubocurarine chloride into *Calliphora erythrocephala.* The amount of curare is plotted against time required for full recovery from the paralysis.

supplied in concentrations of 3 and 15 mg/ml and in pure crystalline form (Abbott Drug Co., No. SM 69997).

We attempted to establish a dose-response curve. Figure 1 is a plot of the amount injected versus recovery time for *Calliphora erythrocephala*; each point represents one fly. Injected with 0.02 to 0.12 μg/mg (here and throughout this report, dosage is per milligram of total body weight), the flies were only partially paralyzed and the recovery period was relatively short; with 0.12 to 0.24 μg the flies were com-

Table 1. Effects on various insects of injection with *d*-tubocurarine chloride. PI, partial to intermediate; CP, complete paralysis.

Genus	Body weight (mg)	Effect of dosage (μg/mg)	
		PI	CP
	Orthoptera		
Nemobius	46	0.702	
Acheta	234	.279	
Tryxalis	247	.261	
Tryxalis	100		1.161
Periplaneta	270	1.110	
	Hemiptera		
Phymata	23.1		1.12
Oncopeltus	36.1		0.895
Metapodius	82.0		1.02
	Coleoptera		
Tetraopes	84		0.461
Diabrotica	6.2		1.04
Epicauta	283.1		0.069
Tenebrio	100.2	0.320	
	Hymenoptera		
Camponotus	11.5		1.12
Bombus	184		0.595
	Diptera		
Calliphora	37.6		.137
Sarcophaga	94.2		.129