Dr. Albert B. Sabin  
The Children's Hospital Research Foundation  
Elland Avenue and Betheda  
Cincinnati 29, Ohio  

Dear Doctor Sabin:  

I am inclosing a rough draft which indicates our attempts to identify the virus recovered from salivary glands of bats. We believe it is evident from complement fixation and neutralization test data presented in the inclosed tables that the particular isolate is probably antigenically related to St. Louis Encephalitis virus but not identical with it.  

We would certainly appreciate a critical review of these notes.  

With kindest personal regards, I remain,  

Sincerely yours,  

KENNETH F BURNS  
Lt Col VC  
Chief, Veterinary and Virology Branches
DISEASE OF BATS ANTIGENICALLY RELATED TO ST. LOUIS ENCEPHALITIS

A bat survey was begun early in 1954 at the Brooke Army Medical Center, Fort Sam Houston, Texas, because of a malady afflicting the bats (Tadarida mexicana) that roost beneath the tile roof of the Spanish type structures. An encephalitis was apparent among these animals as manifested by deranged behavior, muscular tremors, urine incontinence, and paretic manifestations. Thousands of deaths were recorded.

This is to report that in addition to the isolation of rabies virus from brain tissue of bats, a virus antigenically related to St. Louis encephalitis was recovered, from the salivary glands of these encephalitic animals.

TOXICOLOGICAL EFFECTS OF DDT

At first the bat encephalitic manifestations were attributed to the effects of an intensified DDT program in the area. Chemical analyses of the bat omentum conducted in accordance with that outlined by Schecter et. al. (1) indicated a concentration of 184 micrograms of DDT in 3.62 grams of omentum collected from 93 bats. Hayes' (2) nomogram on the relationship between dosage of DDT and its storage in adipose tissue for several species of animal fed daily doses of the compound, indicate that the storage level in bats of 50 PPM of DDT would determine a DDT dosage level of about 0.08 mg/Kg/day. White rats can withstand daily doses of DDT at over 100 times this level. It would not be expected, on the basis of results with other species, that any harmful effects would result to bats from this amount of DDT exposure.

BACTERIOLOGICAL EXAMINATIONS

Bacteriological studies of brain, liver, and spleen employing differential mediums for the isolation of aerobic and anaerobic bacteria were essentially negative.
ISOLATION OF VIRAL AGENTS

Brain tissue

From the brain tissue of 335 necropsied bats, 9 virus isolates were obtained in white Swiss mice. (3, 4) These isolates were identified as the virus of rabies by the standard intracerebral neutralization technique. Tests with infected mouse brain tissue against known rabies immune horse serum demonstrated that the immune serum neutralized both its homologous virus and the newly isolated bat strains.

Salivary gland tissue

In addition to confirmed rabies isolations, four other viral agents were recovered from the salivary glands of encephalitic bats in white Swiss mice. The bat salivary gland virus has an incubation period of 5-6 days intracerebrally in mice. With the exception of mice and bats, host specificity studies including rabbits, hamsters, guinea pigs, and goats were negative.

The bat virus proliferates in 10-11 day developing chick embryos after inoculation of the chorioallantoic membrane. The only altered physical appearance noted is the thickening of the chorioallantoic membrane at 4-6 days of growth. It does not produce death of the embryos. Harvested 10 percent membranes are infective for 3-week-old mice when introduced intracerebrally. The incubation period is 4-6 days, resulting in paralysis and prostration.

IDENTIFICATION OF BAT SALIVARY VIRUS

Reciprocal complement-fixation tests with known neurotropic virus antigens and antisera, and with antigens and antisera to the bat salivary gland agent suggest that the bat virus to share some antigen common to St. Louis encephalitis virus (Table 1). Thus, specific SLE guinea pig antisera consistently fix complement with bat antigens. This cross fixation is of low order, and is not reciprocal since high titered bat antisera will not fix complement
No relationship to Western equine encephalomyelitis could be demonstrated by complement-fixation tests.

with SLE antigens. In addition to above data hyperimmune guinea pig sera to Eastern and Venezuelan equine encephalomyelitis, lymphocytic-choriomeningitis, rabies, Japanese encephalitis and herpes virus failed to fix complement in the presence of a\(\text{1}^\circ\text{l0-19 CF antigens.}\)

The bat virus is neutralized by potent hyperimmune St. Louis encephalitis rabbit antiserum. Cross neutralisation tests indicate this reaction is not reciprocal as high titered 1\(\text{l}^\circ\text{l0-19 antisera does not neutralize SLE virus.}\)

This agent is not neutralized by the antisera of Western Equine Encephalomyelitis, Eastern Equine Encephalomyelitis, Lymphocytic Choriomeningitis, Encephalomyocarditis, or rabies (Table 2).

By employing procedures outlined by Chanock and Sabin (5) for preparation of the hemagglutinin of St. Louis encephalitis virus, we have been successful in obtaining a potent hemagglutinin from both suckling and mature mouse brains infected with 5th-passage bat virus. This alkaline-buffered-saline-extract reacted at 4\(\text{0}^\circ\text{C.}}\) with chick erythrocytes within a zone of pH 6.3-6.6. The optimum pH for this hemagglutination reaction appears to be 6.5, with titers of 6\(\text{l}^\circ\text{0-1280.}\)

Hemagglutination-inhibition tests, performed in the same manner as that described for St. Louis encephalitis virus by Chanock and Sabin (6), showed that hyperimmune and convalescent St. Louis encephalitis sera consistently gave a hemagglutination-inhibition titer of 1:160 to 1:320 against 16 units of the hemagglutinin of the bat salivary gland virus. The human St. Louis encephalitis sera were derived from patients with clinically recognized encephalitis in California and Texas in whom the diagnosis was established by means of the complement-fixation test.
PATHOLOGIC ANATOMY

Microscopic:

Multiple sections of the cerebrum, cerebellum, and mid-brain show an intact pia-arachnoid with focal infiltration of lymphocytes. There is rather marked perivascular infiltration of polymuclear neutrophilic leukocytes. The cellular architecture of the neurons shows variable degrees of chromatolysis in focal areas. There is minimal evidence of satellitosis. Stovall-Black (?) stains fail to reveal the presence of intranuclear or intracytoplasmic inclusion bodies.

Macroscopic:

Grossly the brain has a normal appearance. In some specimens the blood vessels of the leptomeninges appeared somewhat congested, the hyperemia being for the most part in focal areas.

SUMMARY

A viral agent has been recovered from the salivary glands of encephalitic bats which possesses biologic characteristics and antigenic properties similar to those of the St. Louis encephalitis virus. In addition it is evident that the virus is distinct from Western and Eastern and Venezuelan equine encephalomyelitis, rabies, LCM and encephalomyocarditis viruses.

also similar to Dengue, West Nile, Yellow Fever
REFERENCES


### Table 1

**SEROLOGIC IDENTIFICATION OF BAT SALIVARY VIRUS (14:10-19) BY COMPLEMENT FIXATION TESTS**

<table>
<thead>
<tr>
<th>CF antigens</th>
<th>Guinep pig serum titers</th>
<th>SLE*</th>
<th>WEE*</th>
<th>Normal GP Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:10-19</td>
<td>1:256</td>
<td>1:4</td>
<td>&lt; 1:4</td>
<td>&lt; 1:4</td>
</tr>
<tr>
<td>St. Louis (Hubbard)</td>
<td>&lt; 1:4</td>
<td>1:16</td>
<td>&lt; 1:4</td>
<td>&lt; 1:4</td>
</tr>
<tr>
<td>Western Equine Enceph.</td>
<td>&lt; 1:4</td>
<td>&lt; 1:4</td>
<td>1:64</td>
<td>&lt; 1:4</td>
</tr>
<tr>
<td>Normal mouse brain</td>
<td>&lt; 1:4</td>
<td>&lt; 1:4</td>
<td>&lt; 1:4</td>
<td>-</td>
</tr>
</tbody>
</table>

*SLE: St. Louis encephalitis; WEE: Western Equine Encephalomyelitis*
## Table 2

**SEROLOGIC IDENTIFICATION OF RAT SALIVARY VIRUS (1410-19) BY CROSS NEUTRALIZATION TESTS**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Log Neut. Index vs. Homologous Virus</th>
<th>Titer 1410-1419 Virus in Respective Sera</th>
<th>Log Neut. Index of Sera vs. 1410-1419</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Rabbit Serum</td>
<td>-</td>
<td>10^-7.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1410-1419 Im. Rab.</td>
<td>&gt;4.0</td>
<td>&lt;10^-3.5</td>
<td>&gt;4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>St. Louis Im. Rab.</td>
<td>2.4</td>
<td>10^-5.7</td>
<td>1.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Western Equine Im. Rab.</td>
<td>4.1</td>
<td>&gt;10^-6.4</td>
<td>&lt;1.1</td>
<td>N.T.</td>
</tr>
<tr>
<td>Eastern Equine Im. Rab.</td>
<td>3.6</td>
<td>&gt;10^-6.4</td>
<td>&lt;1.1</td>
<td>N.T.</td>
</tr>
<tr>
<td>Rabies Im. Horse</td>
<td>Serum Titer 1/800 vs. 1000 LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>&gt;10^-6.4</td>
<td>&lt;1.1</td>
<td>N.T.</td>
</tr>
<tr>
<td>Lymphocytic chorio. Im. Rab.</td>
<td>3.0</td>
<td>&gt;10^-6.4</td>
<td>&lt;1.1</td>
<td>N.T.</td>
</tr>
<tr>
<td>Encephalomyocarditis Im. Rab.</td>
<td>&gt;4.0</td>
<td>&gt;10^-6.4</td>
<td>&lt;1.1</td>
<td>N.T.</td>
</tr>
</tbody>
</table>