ST. LOUIS ENCEPHALITIS IN PANAMA

I. ISOLATION OF THE VIRUS FROM FOREST MOSQUITOES AND HUMAN BLOOD*

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In the present publication we wish to report isolation of St. Louis encephalitis virus in Panama from human blood and mosquitoes infected in nature. Various isolations were made over a period of eight months in Paya, Darien Province indicating that this region may constitute an important endemic center. The virus was recovered in the course of a long-term study of the local arthropod-borne viruses with special reference to the ecology of yellow fever. In the conduct of this project mosquito collecting stations were set up on either a permanent or a temporary basis in various locations. Those stations from which St. Louis virus was recovered (see map) are briefly described below.

Buenavista Station. An area of partly cleared forest on the Atlantic slopes of the Continental Divide about five miles east of the small village of Buenavista located on the Transisthmian Highway. Collections were made here from July 11 to July 23, 1957.

Payas Station. An area located in the forested slopes at the junction of the Paya and Tuira Rivers in the Province of Darién, near the Colombian border, where collections were begun in February 1958 and have been continued on a long-term basis. In the present report, however, there are included only the results of collections made before January 1959.

Tucacorna Station. This station is located in the Province of Darién of the slopes of Mt. Tucacorna near the headwaters of the Puerto River, a tributary of the Tuira, at an elevation of 3,000 feet. It is some 25 kilometers from the mouth of the Paya River and the Paya Station. Collections were made there from August 22 to December 9, 1958.

MATERIALS AND METHODS

Mosquitoes were collected in glass vials as they alighted to feed on men stationed on platforms in the forest canopy. The construction and operation of such stations were described by Galindo, Trapido and Carpenter. All mosquitoes were left from 24 to 72 hours at environmental temperature in the field before being packed in Thermos jugs filled with ice. These jugs were shipped by car to the laboratory. After identification they were stored in sealed glass tubes in dry CO₂ until tested. The mosquito pools were then ground with inactivated, filtered, ten percent normal serum saline containing 1000 units of penicillin and 1000 micromgrams of streptomycin per cubic millimeter, centrifuged at 5° C, and the supernatant injected into white Swiss mice. Adult mice were used for the Buenavista collection, 0.03 ml being delivered intracerebrally. For all later collections 2-day-old suckling mice were available and were inoculated intracerebrally and subcutaneously with 0.02 ml of the inoculum by each route. Mice were kept under observation for a period of 28 days.

RESULTS

Pertinent details concerning the St. Louis encephalitis virus isolates are given in Table 1. The first recovery of this agent (Buenavista strain) was made in mid-July from a pool of 95 Sabethes chloropeterus captured in Buenavista. Five of 6 adult mice inoculated intracerebrally with a suspension of these mosquitoes developed marked nervous symptoms in 6 days and were sacrificed or died. A total of 516 Sabethes chloropeterus was obtained during the 12-day collection period but no further St. Louis isolations were made. It is of interest that yellow fever virus was active simultaneously in this area and was recovered 4 times from Haemagogus lucifer and H. spegazzinnii falco.

Seven months later, in February 1958, a 34-year-old male Panamanian employee (P.M.) of the Gorgas Memorial Laboratory engaged in collecting mosquitoes at our newly established
station in Paya, Darien developed a transient moderate fever with severe headache. Blood was drawn and forwarded to the laboratory where it was injected into 8 each 2-day-old mice, one of which sickened and was sacrificed for subinoculation 10 days later. By the third passage the virus produced severe nervous symptoms in all infant and adult mice injected intracerebrally after an incubation period of 4 days. This individual showed a marked rise in antibodies during convalescence. In acute phase blood the neutralization index was 0.5 log as contrasted with 3.3 logs in blood drawn 40 days later. It is perhaps of interest to note that no work with the Buena Vista strain of virus, which was later identified as St. Louis, had been carried on in the laboratory for a period of several months prior to this isolation.

Mosquito collections were continued in Paya where the virus was recovered from 3 of a total of 169 mosquito pools processed during the year, including 37 of S. chloropterus and 28 of Sabethes spp. It was isolated once in early June 1958 from a pool of 133 S. chloropterus, again in mid-July from a pool of 139 Sabethes spp., including S. cyaneus, S. tarsopus (and a related new species), S. undosus and S. fabricii, and again in the third week of August from a pool of 153 S. chloropterus. Total catches of S. chloropterus were 5340 and of Sabethes spp. 3607. No further isolations from mosquitoes were made during the remainder of the year, but virus was probably recovered from

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**Fig. 1.** Localities in Panama where St. Louis virus has been isolated

**TABLE I**

Isolations of St. Louis virus from mosquitoes and human blood, 1957-1959

<table>
<thead>
<tr>
<th>Source of virus strain</th>
<th>Place of origin</th>
<th>Date of collection</th>
<th>No. of mosquitoes in pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabethes chloropterus</td>
<td>Buena Vista</td>
<td>July 1957</td>
<td>95</td>
</tr>
<tr>
<td>Human blood</td>
<td>Paya</td>
<td>January 1958</td>
<td>—</td>
</tr>
<tr>
<td>Sabethes chloropterus</td>
<td>Paya</td>
<td>June 1958</td>
<td>133</td>
</tr>
<tr>
<td>Sabethes spp.*</td>
<td>Paya</td>
<td>July 1958</td>
<td>130</td>
</tr>
<tr>
<td>Sabethes chloropterus</td>
<td>Paya</td>
<td>August 1958</td>
<td>153</td>
</tr>
<tr>
<td>Sabethes chloropterus</td>
<td>Tocaruna</td>
<td>September 1958</td>
<td>114</td>
</tr>
<tr>
<td>Human blood</td>
<td>Paya</td>
<td>September 1958</td>
<td>—</td>
</tr>
</tbody>
</table>

* Pool consisting of Sabethes cyaneus, S. tarsopus, (and a related new species), S. undosus, and S. fabricii.
human blood in September 1958. This individual (P.G.), a 40-year-old Panamanian male was engaged in field studies and had fed on himself a number of mosquitoes belonging to the species *S. chloropterus*, *H. spegazzini falco* and *Aedes leucoceraeus clarki*. He became ill 6 days later with symptoms similar to those of P. M. viz., fever, headache, and retroorbital pain. Blood was obtained on the second day of fever for mouse inoculation. Only 1 infant mouse in the group of 7 which were injected with this serum sickened, indicating a low titer of virus in the blood. No significant rise in circulating neutralizing antibodies was found in this individual during convalescence. Further serological studies will be carried out.

From the Tacareuma Station, 1 mosquito pool out of a total of 47 including 15 of *S. chloropterus* and 6 of *Sabethes* spp. proved to contain St. Louis virus. It was a suspension of 114 *S. chloropterus* collected in mid-September 1958. No further isolations were made from material obtained at this station. During the year a total of 1686 *S. chloropterus* and 634 *Sabethes* spp. respectively from Tacareuma were tested.

All virus strains showed considerable uniformity once fully established in mice. Intracerebral injection in infant or adult mice produced nervous symptoms such as tremors, ataxia, convulsions, paralyses, and prostration after an incubation period of 3 to 4 days, with a fatal termination in 5 to 7 days. Infant mice were quite susceptible also by peripheral routes.

### Identification of the Virus

The virus strains were readily identified as belonging to Group B according to the classification of Casals and Brown. Cross-challenge and mouse protection tests showed them to be distinct from yellow fever and Ilheus. As no other known Group B viruses, antigens, or antisera were available here for comparison, a mouse hyperimmune serum against the Buena Vista strain was forwarded to Drs. Theiler and Casals at the Rockefeller Foundation Virus Laboratories. They performed block complement-fixation tests using the following viral antigens: St. Louis encephalitis, Ilheus, yellow fever (Asibi), Japanese B, West Nile, dengue Tr. 1751 and An. 4073. Highest titers were found against St. Louis antigen leading them to the conclusion that the virus was "either St. Louis or a virus much closer to St. Louis" than any of the other agents tested.

Later, opportunity was afforded to have studies made with the Buena Vista virus strain in the laboratories of Dr. W. C. Reeves of the University of California and Dr. W. L. Pond of the National Institutes of Allergy and Infectious Diseases. Dr. Reeves in the tissue culture neutralization tests obtained complete neutralization with St. Louis serum and negative results with antisera to Japanese B, western equine encephalomyelitis, California and Rio Bravo bat salivary virus. Dr. Pond, using the mouse protection test, obtained a high neutralization index with St. Louis antiserum and negative or low titer reactions with Japanese B, Ilheus, yellow fever, eastern and western equine encephalomyelitis and West Nile viruses.

In order to complete the identification of this virus cross-challenge experiments were carried out. For this purpose in January 1959 the Hubbard strain of St. Louis encephalitis virus was obtained from the American Type Culture Collection. Thirty mice previously immunized against the local virus were challenged by the intracerebral injection of 10⁻⁴ LD₅₀ of the Hubbard strain. All survived whereas all controls were dead within 5 days. Similarly 30 mice previously immunized against the Hubbard strain resisted intracerebral challenge with 10⁻⁵ LD₅₀ of the local Buena Vista strain of virus, a dose fatal to all controls within 7 days.

The other six strains of virus here described were identified by immunological comparison with the Buena Vista strain. Mouse hyperimmune sera were prepared against each of the strains and used in intracerebral mouse protection experiments. Hyperimmune serum against the Buena Vista virus was tested for capacity to neutralize the other strains and conversely, hyperimmune serum prepared against the other strains was tested for its content of antibodies against the Buena Vista virus. In cross-challenge experiments mice previously immunized against the Buena Vista strain were challenged by the intracerebral injection of a minimum of 10,000 LD₅₀ of the test virus and vice versa. Several other strains of virus immunologically related to St. Louis, but showing some differences from it also were recovered from mosquitoes during the course of this investigation. They are not described here pending further analysis.

### Discussion

The finding of St. Louis virus in Panama is of interest for several reasons. First, it represents
the first recovery of this agent from Middle America. Repeated isolations of St. Louis virus from mosquitoes and one recovery from a fledgling dove have been reported from Trinidad.\textsuperscript{2, 3, 5} but otherwise we have found no reference to isolations outside the United States. Second, this is the first report of natural infection with this agent in mosquitoes of the tribe Sabethini. Previous isolations were from culicines such as Culex tarsalis, Culex pipiens and Aedes dorsalis,\textsuperscript{6} and from C. coronator, C. couvelli and Psorophora ferox in Trinidad.\textsuperscript{5} Sabethes chloropterus is of special interest because it was repeatedly found infected with St. Louis encephalitis virus here and also because it has been shown to harbor two other Group B viruses, Ilheus,\textsuperscript{2} and yellow fever,\textsuperscript{3, 5} which latter virus it is capable of transmitting by bite.\textsuperscript{8, 9} The simultaneous activity of St. Louis and yellow fever viruses in Buena Vista and of Ilheus and yellow fever in Guatemala\textsuperscript{5} may explain confusing serological reactions in human and animal serum obtained at times in surveys.

St. Louis virus has been isolated rarely from human blood. Blattner and Heys\textsuperscript{10} recovered it from the blood of an 8-year-old boy in which it persisted for approximately 4 days after the onset of symptoms. In the present study virus was probably recovered from the blood of two men suffering a mild clinical infection. Isolation from the first of these is corroborated by a rise in neutralizing antibodies during convalescence. No significant rise was found in the other. Virus titers in the blood of both were low as neither produced infection in all infant mice of the litters inoculated. Both had been vaccinated against yellow fever and had been exposed in their work to the attacks of thousands of mosquitoes, some of which probably harbored other Group B agents. It is possible that those previous immunizations to a related virus or viruses may have influenced the clinical picture of the infection. The recovery of St. Louis virus over a period of at least 8 months in Paya, Darien suggests that this may be an important center. Further epidemiological studies are now in progress.

SUMMARY

Isolation of St. Louis encephalitis virus in Panama is reported. The virus was recovered five times from Sabethes chloropterus. It was also recovered from a pool of S. cyaneus, S. tarsanus (and a related new species), S. metalurus, S. falci-