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The First Isolation of St. Louis Encephalitis Virus from Mosquitoes in Florida. (29421)

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The virus of St. Louis encephalitis (SLE) has been isolated from man in Florida during 1952(1,2). Since 1959, three recognized outbreaks of SLE which have occurred in the Tampa Bay area of the state have been studied with progressively more detail(3). During 1961, the first intensive collection of mosquitoes was made for virological examination. In this report are presented the details of the initial isolation and identification of SLE virus from Florida mosquitoes. This strain, recovered one year prior to the large number of SLE virus isolations from mosquitoes during the widespread 1962 epidemic(4, 5), was used to prepare hemagglutinating (HA) and complement-fixing (CF) antigens for the study of that outbreak.

Materials and methods. Mosquitoes which were collected in standard battery-operated New Jersey (N.J.) light traps were placed in vials, sealed and transported to the laboratory on dry ice. Pools of 50 mosquitoes or less were made by species, trap locations and collection dates, triturated in 4 ml of 10% rabbit serum-saline diluent, pH 7.4-7.6, and 0.02 ml inoculated intracerebrally (i.c.) into 2-4 day old mice.

Sensitivity of the virus to the action of sodium desoxycholate was determined by the method described by Theiler(6).

The techniques utilized for preparing HA and CF antigens from the isolate and for carrying out the hemagglutination-inhibition (HI) test were essentially those described by Clarke and Casals(7).

Complement-fixation tests were performed with 2 units of antigen and 2 exact units of complement; total volume 0.6 ml. Titers were based on the highest dilution of serum showing 3 or 4+ fixation of complement.

Serum-neutralization (SN) tests were performed by the constant serum, varying-virus technique. Mixtures of serum and virus were incubated at 37°C for one hour and 0.03 ml inoculated i.c. into groups of 6 weanlings Swiss white mice, Webster strain.

Isolation. Of 961 mosquitoes collected during a 2-week period, 2 pools designated P15 and P16 yielded a viral agent. These were from a single collection trapped on November 8, 1961 near Lake Maggiore in the southern part of St. Petersburg, Pinellas Co., Fla. This area is predominantly a hardwood swamp, a portion of which is utilized as a zoo and botanical garden.

Mosquitoes in the 2 virus-containing pools were identified as *Culex species*. Further classification was impossible because the scale pattern of the mosquitoes had been disrupted by the turbulence of the trap-collecting mechanism.

On the 9th day following inoculation, suckling mice injected with suspensions of 2 pools of *Culex sp* were either ill, moribund or dead. A 10% brain suspension prepared from sick and moribund animals was passaged to additional suckling mice. Illness

TABLE I. HI and CF Test Results with P15 Virus and Selected Arboviral Hyperimmune Sera.

Antisera		HI titer*		CF titer*	
		SLE P15 antigen	Homologous antigen	SLE P15 antigen	Homologous antigen
Group A	EEE (NJO)	<10	640	<10	80
	WEE (Flemming)	<10	2560	<10	80
Group B	Dengue 2	10	20	<10	ND†
	Ilheus	160	640	10	ND
	MVE	160	640	10	ND
	SLE (Hubbard)	640	640	40	80
	SLE (P15)	80		20	

* Reciprocal of titer. † Not done.

was observed in the latter group at approximately 90 hours after inoculation. Subsequent passages of 10^{-2} suspensions of brain material resulted uniformly in death of the inoculated animals after 70-74 hours by the i.c. route and at 90-96 hours by the i.p. route.

Prior to these isolations, no SLE virus had been present in the laboratory since May 1961. Attempts to reisolate the agent from stored, frozen —65°C, mosquito pool suspensions P15 and P16 were successful. Additional study of the isolates was confined to P15.

Identification. The agent designated P15 was found to be sensitive to sodium desoxycholate. The titer of the virus in the control titration was $10^{-7.5}$ LD_{50}/0.03 ml while the titer after exposure was $<10^{-2}$ LD_{50}/0.03 ml in weanling mice.

A hemagglutinating antigen was prepared from third suckling mouse passage (SM_4) brain material, titered 1:5120 and the optimal pH range was 6.2-6.6 at 37°C. Characterization of the agent by means of HI and CF tests indicated that it belonged to the group B arboviruses as shown in Table I.

Utilizing the S-N test for additional serologic study of the isolate, SLE (Hubbard) immune serum neutralized 2 $logs_{10}$ of the virus.

Confirmation of our findings was obtained by 2 reference laboratories as presented in Table II. Cross neutralization tests performed by Miss Gladys Sather in the Graduate School of Public Health, Univ. of Pittsburgh, further confirmed the identification of the isolate P15 as a strain of St. Louis virus.

Response of other laboratory hosts to infection with P15 was studied. SM_4 brain material titered $10^{-9.1}$ LD_{50}/0.03 ml i.c. and 10^{-3} LD_{50}/0.1 ml i.p. in 3-week-old mice. Average survival time of 3-week-old mice inoculated i.c. was 6.1 days. Rabbits inoculated subcutaneously and guinea pigs intraperitoneally showed no evidence of illness.

TABLE II. Comparison of Neutralization Indices* of P15 Virus (SLE) with Selected SLE Strains.

Virus	Antiserum	Neut index
SLE 569 (Colorado)	SLE (BFS 968)	1.7
	SLE (P15)	>2.5
SLE (Webster)	SLE (Webster)	2.5
SLE (P15)	SLE (BFS 968)	3.1
	SLE (Webster)	3.0
	SLE (P15)	3.0
SLE (P15)	EEE	<1.6
	WEE	<1.6

* Tests performed in laboratories at Univ. of Pittsburgh (Dr. William McD. Hammon) and/or USPHS, Communicable Disease Center (Dr. P. H. Coleman), Atlanta.

Summary. A summary of data relating to the initial isolation and identification of St. Louis encephalitis (SLE) virus from *Culex* mosquitoes in Florida has been presented. The agent P15 was isolated from mosquitoes collected in November 1961 and was used to prepare HA and CF antigens for serological studies of the 1962 epidemic in Florida.

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Experimental Production of Colloid Goiter in the Cebus Monkey.* (29422)

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It has been demonstrated in rats that dietary uracil augments the hypercholesteremia and cardiovascular sudanophilia incident to cholesterol feeding. These effects are associated with a distinct hyperplasia of the thyroid(1,2). It was also shown that orotic acid counteracts these uracil effects, especially in females. In an attempt to reproduce these findings in Cebus monkeys, striking thyroid changes were seen, quite different from those in rats.

Materials and methods. Four *Cebus albifrons* monkeys, 2 males and 2 females, were placed on a purified basal diet of the following composition: casein, 15%; corn oil, 15%; sucrose, 65.5%; salt mixture (Hegsted IV, Nutritional Biochemicals Corp.), 4%; and choline, 0.5%. To each kg of this diet were added 10 mg of thiamine, 10 mg of riboflavin, 10 mg of pyridoxine, 49 mg of niacin, 30 mg of calcium pantothenate, 1 mg of folic acid, 0.2 mg of biotin, and 20 drops of oleum percomorph (Mead Johnson & Co.). Ascorbic acid (50 mg) was added daily to each monkey's ration.

Cholesterol (0.5%), uracil (0.5% or 0.75%), and orotic acid (0.5% or 1%) were added to this basal diet as seen in Fig. 1, these ingredients being included at the expense of sucrose. Fig. 1 also illustrates the serum total cholesterol(3) responses, the animals being bled at biweekly intervals.

After 72 weeks of uracil ingestion, the animals were killed and complete autopsies performed. Tissues were fixed in 10% neutral formalin and alcian blue, hematoxylin, and eosin (AB, H & E) were used as a general stain. Special techniques used will be mentioned as well as biochemical determinations performed on blood and liver. Control thyroid data to be used for comparison were derived from 27 additional Cebus monkeys, of both sexes, which were comparable to the present animals in age and duration of cholesterol feeding. In addition 7 of these had orotic acid feeding trials identical to those above.

Results. Addition of cholesterol to the basal diet resulted in a moderate increase in serum cholesterol levels, which was augmented by addition of dietary uracil (Fig. 1). No differences in this latter response could be seen between the two levels of uracil used. Elevations in total lipids(4) and beta lipoproteins(5) were observed, as expected (6). In regard to other serum components, the serum urea level(7,8) was elevated in all 4 monkeys; the highest level was 78 mg/100 ml for monkey No. 3; the lowest was 47 mg/100 ml for monkey No. 2; control values ranged from 33 to 41 mg/100 ml. Similar observations have been noted in rats(2).

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