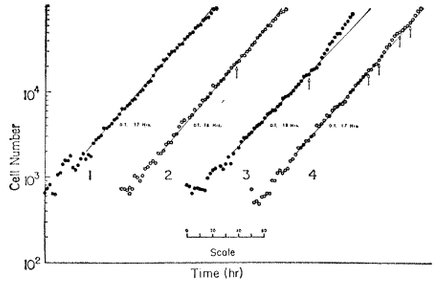

Fig. 2. Growth, plotted as cell number per milliliter against time, of four subcultures of *Euglena gracilis*, strain Z, maintained in separate light and temperature controlled growth chambers, sampled simultaneously every 2 hours by individual sampling systems operated by the same timer. Dark lines through the circles have a slope which gives the doubling times (D.T.), shown at the right of each curve, for the cultures. Arrows indicate the points at which light intensities were changed using six 40-watt General Electric cool white fluorescent bulbs instead of the usual three. In culture No. 4, the first arrow indicates an increase, and the second arrow a decrease (and so forth) in light intensity.

which samples are to be taken will depend upon the design of the experiment. These features can be varied and the desired program obtained by simple adjustments of the timing mechanism, the turntable head, the speed of the synchronous driving motor, and the pipette speed. Also, more than one fraction collector can be operated by the same timing mechanism.

Figure 2 is from an experiment illustrating a "bioassay" of the sampling system. Four subcultures of *Euglena*

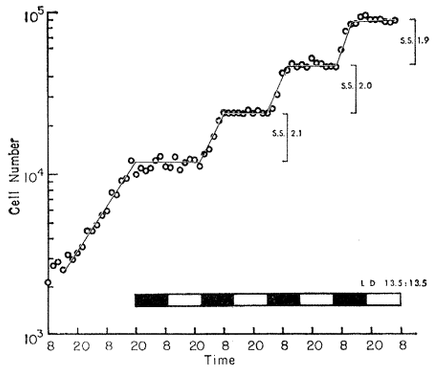

Fig. 3. Growth, plotted as in Fig. 2, of *Euglena* maintained a light cycle of 13.5 hours light and 13.5 hours dark. Alternating blocks of light and dark along the abscissa represent the cycle. In the step portion of the curve, figures are given for step size (S.S.), defined as the average cell number in the plateau after a burst divided by the average cell number in the plateau before division.

gracilis, strain Z (*3*) were grown in 1500 ml of inorganic medium (*4*) in constant light (*5*) at 25°C. Each subculture was placed in a separate temperature-controlled growth chamber equipped with a sampling device regulated by the same timing mechanism. The figure shows that the doubling times (D.T.) for the four cultures ranged from 16 hours for culture No. 2 to 18 hours for culture No. 3. In culture No. 3, which had the longest doubling time, an increase in light intensity (initiated at the time indicated by the arrow) caused an increased division rate, whereas in culture No. 2, which had the shortest doubling time, a similar increase in light intensity had no effect on the rate of division. Light conditions in the cabinets housing cultures No. 2 and No. 3 were, therefore, different before the changes in intensity. Thus, culture No. 2 was at saturating light intensities prior to the increase in intensity and the doubling time was unaffected, whereas culture No. 3 was at subsaturating intensities and the increase in intensity brought about an increased division rate. Culture No. 4 was exposed to a cycle of two differing intensities, namely, 8 hours of higher intensity followed by 16 hours of lower intensity. The initial effect of the higher intensity was to inhibit division slightly.

Figure 3 shows synchronously dividing *Euglena gracilis* in a light cycle (LD 13.5 hours light, 13.5 hours dark). After an initial 2 days in continuous light, the culture was placed on this light schedule. Division ceased during the first cycle and began in the dark period of the second cycle. Thereafter, divisions were confined to the first 10 hours of the dark periods. In any division period there was an approximate doubling in cell number—a twofold increase in cell number giving a step size (S.S.) of 2.

The sampling system has been efficiently used as a monitoring device for studies of biochemical events during the cell cycle (*6*) and for studies of ultraviolet-induced bleaching in synchronized *Euglena* (*7*).

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8. I thank Drs. C. S. Pittendrigh and V. G. Bruce for their advice and criticism on this manuscript.
9. Supported in part by a predoctoral fellowship from NIH and partially by funds from grants to Dr. C. S. Pittendrigh from the Fannie E. Ripple Foundation and the Air Force Office of Scientific Research.

24 April 1964

Serological Evidence of Arbovirus Infection in the Seminole Indians of Southern Florida

Abstract. *A serological survey of the Seminole Indians in south Florida for evidence of exposure to arbovirus infection indicated not only past infection with the recognized North American encephalitis viruses—a high incidence for St. Louis encephalitis—but exposure to a Bunyamwera group agent and to Venezuelan equine encephalitis virus, activity of which had not been previously recognized north of Panama.*

Exotic arboviruses may be defined as those which were originally isolated abroad or are otherwise epidemiologically related to a geographical area outside the continental United States. Indigenous arboviruses are those originally isolated and described within the United States.

While working with R. M. Taylor in the Nile Delta of Egypt in 1954, we isolated a number of virus strains from *Argas* ticks which were intimately associated with breeding colonies of the cattle egret, *Bubulcus ibis* (*1*). In 1958, we initiated a search in the U.S. for exotic *Argas* tick viruses that might be linked with rookeries of this Old World egret species which had recently become established in southern Florida. Although this investigation produced no evidence of a similar tick-egret-virus ecology, it brought us into contact with the indigenous Seminole Indians of the Brighton and Big Cypress reservations (Fig. 1).

These Indians are lifelong residents of palmetto, sawgrass, hammock, swamp, and everglade, which are habitats characteristic of south Florida, the area of most extensive and variable tropical attributes in the continental United States. Just as military sentries are posted to signal unusual or sig-

nificant enemy activity, indigenous residents such as the Seminole Indians may serve as sentinels for the detection of arbovirus infections which occur in particular habitats. Such infections may be disclosed by signs of overt disease, by isolation of the virus, or by the development of antibodies which can be identified by retrospective serological study.

The Indians were previously remote from comprehensive medical care, and any clinical disease they may contract as a result of arbovirus infection is as yet undefined. On the other hand, serological study can be expected to provide definitive information on exposure of these Indian sentinels to infection by arboviruses which may be periodically active or are permanently established in these natural habitats of south Florida. The assessment of arbovirus activity in these regions is of particular importance since they are rapidly becoming popular recreational and residential areas for populations of nonimmune immigrants from more temperate environments.

Serological studies of indigenous residents of Brighton and Big Cypress Seminole Indian reservations were undertaken in April 1960 (*2*). By antecubital vein puncture, blood was collected in 20-ml vacutainers from 166 Indian residents representing about two-fifths of the total population. In the New York laboratories of the Rockefeller Foundation, selected sera were screened in dilutions 1/10, 1/20, and 1/40 by hemagglutination-inhibition (HI) tests against a number of hemagglutinating (HA) arbovirus antigens, according to the technique described by Clarke and Casals (*3*). The antigens selected for use in these tests were prepared from the infected brains of suckling mice or from viremic mouse sera extracted by the sucrose-acetone or acetone-ether procedures devised by Clarke and Casals (*3*).

The arboviruses selected (Table 1) were those that were isolated elsewhere in the Western Hemisphere and that we thought might be active in the tropical environment of Florida, or viruses from other continents whose antigens showed broad group reactivity and might therefore reveal the presence of related virus antibodies. HI antibodies which develop after arbovirus infection generally persist for long periods, often for life. Therefore, sera selected from the older age group were tested first against the largest number of antigens to telescope in time the tribal exposure to arboviruses. Results were negative for most hemagglutinins and further tests for such viruses were not carried out. Those sera that were positive by preliminary screening were tested again in eight dilutions to establish an endpoint titer.

Table 1. Hemagglutinating arboviruses tested for inhibition by sera from Florida Seminole Indians.

Group A	Group B	Group C	Bunyamwera group	Other arboviruses
Eastern equine encephalitis	Yellow fever	Marituba (AN15)	Bunyamwera (Africa)	California
Western equine encephalitis	Dengue II	Oriboca (AN17)	Cache Valley (BeAr 7272)	
Mayaro	St. Louis encephalitis	Caraparu (AN3994)	Guaroa	
Venezuelan equine encephalitis	Ilheus		Chittoor (India)	
Chikungunya (East Africa)*	Bussuquara		Ilesha (Africa)	
Sindbis (Afro-Asia)	Powassan			
AMM 2021 (Malaya)	Modoc			
AMM 2354 (Malaya)	Russian spring summer encephalitis (Asia)			
Aura	Murray Valley encephalitis (Australia)			

* Only those viruses with parenthetical geographical terms are not of Western Hemisphere origin.

As a representative of Cache Valley virus, the BeAr 7272 strain from Belem, Brazil (*4*), was used because no hemagglutinin from the Holden (6V-633) Utah strain had been obtained. None of the sera from the Seminole Indians have yet been tested against the recently isolated strains of Bunyamwera group arboviruses from Big Cypress.

Series of neutralization tests for certain specific viruses, such as Venezuelan equine encephalitis (VEE) were undertaken later in the Arbovirus Laboratory of the Communicable Disease Center in Atlanta. Parallel titrations were carried out intracerebrally in adult mice against three different VEE virus strains from Trinidad, Colombia, and Panama. The neutralization indices ($\log_{10}$) for all of these sera ranged from 2.5 to 4.0 against VEE virus, indicating close antigenic character of the original infecting virus.

Although tested at random, the serological results were analyzed according to which reservation the serum donor had resided in since birth or for the longest period, if marriage or other change in family relationship had transferred residence from one locality to the other. That this affected the final analysis reflects the distinctly different environmental features of each

Table 2. Incidence of arbovirus HI antibodies in sera of Seminole Indians.

Age groups (years)	Group A						Group B SLE		Bunyamwera group CV	
	EEE		WEE		VEE					
	+	%	+	%	+	%	+	%	+	%
Brighton reservation										
1-15	1/44	2	1/44	2	0/44	0	1/44	2	1/4	25
16+	4/57	7	2/57	4	7/57	12	12/57	21	5/28	18
All	5/101	5	3/101	3	7/101	7	13/101	13	6/32	19
Youngest donor*	6/M		9/M		21/F		8/F		13/M	
Big Cypress reservation										
1-15	0/22	0	0/22	0	2/22	9	2/22	9	1/1	100
16+	2/43	5	0/43	0	36/43	84	16/43	37	7/32	22
All	2/65	3	0/65	0	38/65	58	18/65	28	8/33	24
Youngest donor*	33/M		None		8/F		6/M		11/M	
Total										
1-15	1/66	2	1/66	2	2/66	3	3/66	5	2/5	40
16+	6/100	6	2/100	2	43/100	43	28/100	28	12/60	20
All	7/166	4	3/166	2	45/166	27	31/166	19	14/65	21
Youngest donor*	6/M		9/M		8/F		6/M		11/M	

* The age and sex of the youngest resident yielding a positive result is shown.

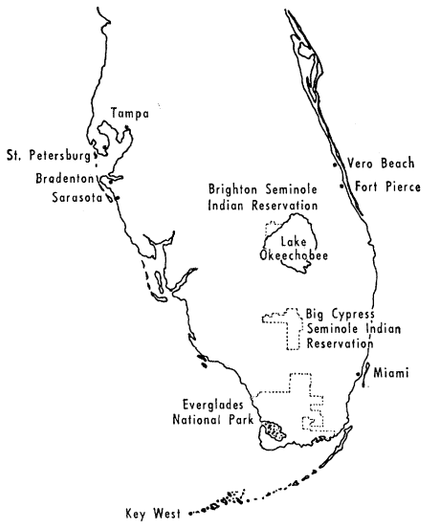

Fig. 1. Location of Seminole Indian Reservations and Everglades National Park in southern Florida.

reservation. A summary of these results is given in Table 2.

The low incidence of antibodies to several viruses, such as the eastern and western equine encephalitis viruses of Casals' group A, which are known to have widely scattered nonhuman activity in Florida, was unexpected. The higher percentage of antibodies to St. Louis encephalitis virus (SLE), VEE, and Bunyamwera group viruses indicates intensive and extensive exposure of the Seminole Indians to infection by mosquito-transmitted arboviruses, ecologically associated with the local habitat (biotope) in which they live. While exposure to SLE virus appears to have been substantial in both reservations—in the palmetto, grass, and cattle land of Brighton, as well as in the Big Cypress swamps and Everglades which extend southward continuously to the mangrove shores of the Caribbean Sea—the high incidence of immunity to VEE virus was concentrated in the Big Cypress habitat.

Where significant titers (1/20 to 1/160) for SLE virus appeared, certain other group B hemagglutinins were inhibited, but only to an extent indicative of group reactivity. Also listed in Table 2 are the minimum ages of donors of sera positive for different viruses. This information indicates that exposure to most of the viruses for which there is evidence of infection has been relatively recent and, for certain viruses, extensive.

These results indicate that SLE virus was frequently active and that it might be maintained in a natural cycle in the subtropical sylvan area of south-central Florida. On the other hand, the localization of VEE antibodies in proximity to the Everglades is evidence of a more selective association of the Big Cypress Indians with the Everglades habitat. Here, because of the striking difference between adults and children in the incidence of VEE virus, this virus appears to have been sporadic in appearance, possibly expanding under favorable conditions from a maintenance focus of infection deeper in the Everglades or introduced by some unknown mechanism of dissemination from its known endemic-epidemic foci in the southern Caribbean littoral and northern South America. Prior to this observation of VEE antibodies in the Big Cypress Seminole Indians, there had been no indication of human infection with this type of arbovirus in the United States, or, in fact, north of Panama.

The incidence of inhibitory sera for Cache Valley virus is substantial. Simultaneously with the demonstration of Bunyamwera group antibodies in the Seminole Indians, indicating that, as in Africa and South America, Bunyamwera group viruses do infect man, Casals and Whitman (*4*) demonstrated that the Cache Valley virus belongs antigenically in the Bunyamwera group.

The significance of finding Bunyamwera group antibodies in the Indian population was obvious (*5*) and it appeared imperative to follow up the serological surveys with actual searches for virus in accordance with principles defined by the WHO Study Group (*6*). A field station was therefore established at the Big Cypress Reservation headquarters in March 1961 (*7*) and a search for viruses in mosquitoes, birds, and other animals was begun on a systematic basis. Numerous strains of Bunyamwera group viruses were isolated from mosquitoes, providing a clearer definition of the significance of the Bunyamwera group antibodies in the Indian population. No further evidence of VEE virus activity was obtained.

The recent occurrence of central nervous system disease of SLE virus etiology in the Tampa Bay area (*8*) has reinforced our apprehensions concerning the high incidence of SLE antibodies in the indigenous population of the contiguous area of rural south-central Florida; evidently, mosquito-borne arbovirus activity in rural habitats of south Florida may be associated with subsequent epidemic occurrence of arbovirus disease in man.

The results of this work stimulated the extension of our investigations southward, deeper into a variety of habitats in the Everglades. What was initiated as an arbovirus exploration in 1960 has now emerged as a continuing study of substantial public health significance.

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20 May 1964

Venezuelan Equine Encephalitis Virus from South Florida

Abstract. *Venezuelan equine encephalitis virus was isolated three times from the* Culex (Melanoconion) *species of mosquitoes collected in south Florida in June, July, and October, 1963. Specific antibody was present in 16 of 28* Peromyscus gossypinus *(cotton mice) and 3 of 16* Sigmodon hispidus *(cotton rats) sampled from one of the infected sites in January 1964.*

Studies by Work in 1960 (*1*) revealed a significant incidence of Venezuelan equine encephalitis (VEE) antibodies in Seminole Indians of the Big Cypress reservation, located about 50 km south of Clewiston, Florida, on the northern edge of the Everglades. This finding stimulated attempts to