4000 years ago. Sample I-2077 from 7.3 to 7.7 feet appeared to be saltwater peat, but its age-depth relation does not conform to the general relation observed between Cape Cod and Virginia. This sample and those from greater depths have been omitted from consideration.

22. Nantucket cores were secured from the southeast extremity of Quaise Marsh on the southeast side of Nantucket Harbor. The site was at the southeast end of the marsh about 100 feet north of Polpis Road. Surface vegetation was *Spartina patens*.
23. Eastham cores were from a small marsh at the north end of Nauset Bay, immediately west of the Nauset Coast Guard Station. Surface vegetation is *Spartina patens* and dwarf *S. alterniflora*. The peat is fibrous over a substratum of dark brown clay.
24. Neponset River cores were from a salt marsh bordering the Neponset River, which is described by Johnson (*20*). Since his study, large areas have been destroyed by filling and by highway construction. Samples were secured in an undisturbed area at the southern margin of the marsh one-quarter mile west of Johnson's section A-B, in the town of Milton, Massachusetts. Surface vegetation was *Spartina patens*. The peat was underlain by 0.5 to 1.0 foot of olive-colored clay above a bottom of clay or sand and gravel.
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Virus of the California Encephalitis Complex: Isolation from Culiseta inornata

Abstract. *A virus of the California encephalitis complex was isolated from two pools of the mosquito* Culiseta inornata, *collected in mammalian burrows of Alberta during the summer of 1965. This is the first recorded isolation of California encephalitis virus from mosquitoes in Canada.*

The history of the isolation of viruses of the California encephalitis (CE) complex has been reviewed in detail by Hammon and Sather (*1*). This virus was originally isolated by Hammon and Reeves in 1943 and 1944 from wild-caught mosquitoes (*Aedes melanimon* and *Culex tarsalis*) collected in Kern County, California (*2, 3*). Small wild mammals (rabbits and squirrels) have repeatedly been shown to be infected with CE virus, and they are probably responsible for the maintenance of the virus in nature through the cycle mosquito→mammal→mosquito (*2, 4–6*). The agent was not called California encephalitis virus until 1952, when it was proved to be etiologically related to three clinical cases of human encephalitis that had occurred in California in 1945 (*5*). Almost 10 years after the first-reported human cases, interest in this virus as a human pathogen was renewed in the United States in 1963 when it was shown to be the causative agent of a case of encephalitis in Florida (*7*). Increasing evidence has been given, through isolation and serology, that CE virus may play a greater role in human pathology than was previously suspected (*8*). In Canada, only serological evidence of CE virus infection in small wild mammals was available (*9*) prior to isolation of the agent from indicator rabbits in the field in Ontario, as reported by McKiel in 1966 (*10*). We report here the isolation of two viruses of the CE complex from two pools of *Culiseta inornata* collected from mammalian burrows of Alberta.

Using a procedure described by Shemanchuk (*11*), we collected 5571 mosquitoes from mammalian burrows, divided them into 153 pools, and tested them for arboviruses. The species of mosquitoes and the number examined were: *Culex tarsalis*, 1273 (39 pools); *Culiseta inornata*, 3659 (85 pools); and *Aedes earlei*, 639 (29 pools). All mosquitoes collected for virus investigation were kept alive until identification of the species was completed. They were identified on the day of collection and divided into pools of 30 to 50 mosquitoes according to species, feeding conditions, and date and areas of collection. Each pool was placed in a 7-ml (¼-ounce) bottle which was carefully sealed; the mosquitoes were then quickly frozen on dry ice and shipped in a frozen state to the virus laboratory. The procedures employed in the isolation of arboviruses were basically those described by Chamberlain (*12*). Each mosquito pool was ground, in a cold mortar, in 2 ml of a mixture of rabbit serum and buffered water (*p*H 7.6 to 7.8) containing antibiotics and was centrifuged at 10,000 rev/min for 1 hour in a refrigerated centrifuge. The supernatant fluids were inoculated intracerebrally in 0.02 ml amounts into litters of eight suckling white Swiss mice 1 to 2 days old. The fluid remaining after mice were inoculated was kept in a freezer at −70°C for reisolation attempts. All mice were examined for 14 days and only sick mice were selected for passage. No blind passages were made. Reisolation of the strains from the original mosquito specimens was attempted in all instances. Suspensions of mosquitoes from which isolations of virus were not confirmed in reisolation attempts were regarded as negative. Isolates were identified serologically by neutralization tests in suckling mice. A crude 20 percent suspension of mouse brain was used as antigen. Three serums were used for each isolate: (i) hyperimmune-mouse serum prepared in our laboratory with the California encephalitis virus, strain Snowshoe Hare (*13*); (ii) immune mouse ascites fluid, prepared with California encephalitis virus, strain BFS-283 (*14*); and (iii) immune mouse serum prepared in our laboratory with the western encephalitis (WE) virus, strain Fleming (T.F.) (*15*). Mice 2 to 4 days old were inoculated intraperitoneally (0.05 ml per mouse) with mixtures containing constant amounts of serum but varying dilutions of virus. Results of the neutralization tests were expressed in terms of neutralization index; the LD_{50} (lethal dose, 50 percent effective) end points for this purpose were calculated by the Reed-Muench formula (*16*). Precautions were always taken to eliminate conditions of cross-contamination. Experimental work with known viruses was never carried out concurrently with the testing of field specimens. Serological work was performed in widely separated quarters of the laboratory and only after all the virus work on isolation passages and virus identification procedures were completed.

Table 1. Results of neutralization tests (NT) with mouse immune serums prepared with California encephalitis (CE) virus and western encephalitis (WE) virus. Mouse passage 3 in Swiss mice.

Immune serum (type and place prepared)	Titer of virus (log LD_{50})	NT index (log LD_{50})
Virus strain L.276 from Brooks		
CE (Atlanta)	5.8	5.8
CE (Edmonton)	6.3	4.5
WE (Edmonton)	6.3	0.5
Virus strain L.267 from Hays-Vauxhall		
CE (Atlanta)	4.22	4.22
CE (Edmonton)	4.48	4.48
WE (Edmonton)	4.32	0.5

Two viral agents were isolated from two pools of *C. inornata*, collected on 18 August. One pool, of 49 mosquitoes, came from Brooks and the other one, of 34 mosquitoes, from Hays-Vauxhall, both areas located in the south of Alberta. The mosquitoes of both pools were recently engorged, but the animal on which the mosquitoes fed was not investigated. Both viral agents caused illness and death in 5 days after inoculation of the mosquito suspension.

The isolate obtained from the pool collected in Brooks was sent to Dr. Telford Work, Communicable Disease Center, Atlanta, for identification and was found by the gel-diffusion method to be a member of BFS-283, La Crosse, Snowshoe Hare subgroup. In our laboratory, neutralization tests were performed with both isolates, and both the WE and the CE immune serums were used. No neutralization of infectivity was observed with WE immune serum. The isolates were neutralized by both CE serums—the serum prepared in our laboratory and the one obtained from Atlanta, as shown in Table 1. Two CE immune serums were used because we wanted to compare results obtained with the serums prepared in our laboratory and the one obtained from the Communicable Disease Center. California encephalitis virus was isolated from two pools of *C. inornata* collected in mammalian burrows of Alberta. This is the first isolation of CE virus from *C. inornata* in Canada.

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Sialic Acid in HeLa Cells: Effect of Hydrocortisone

Abstract. *HeLa S_3 cells grown in the presence of hydrocortisone contain more sialic acid than their corresponding untreated controls. The percentage of the total sialic acid present in the sedimentable fraction obtained from homogenates of cells treated with hydrocortisone is smaller than that of the corresponding fraction of control cells. In addition to* N-*acetylneuraminic acid, the chromatographic analysis suggests that HeLa S_3 cells probably contain trace amounts of* N-*glycolylneuraminic acid.*

Several lines of human cells undergo biochemical changes when cultured in the presence of hydrocortisone (*1*). Cell growth in this steroid-rich environment results in alterations of membrane structure (*2*). It has been postulated that modifications of the membrane structure affect membrane function and thereby the metabolism of dividing cells (*3*).

Sialic acid residues play an important role in the maintenance of membrane structure of dividing cells and are responsible for the electronegative charge of the cells as revealed by boundary electrophoresis (*4*). We now report the sialic acid content of HeLa S_3 cells growing exponentially in monolayer cultures in the presence and absence of added hydrocortisone.

HeLa S_3 cells were grown in monolayer culture with Waymouth medium supplemented by 10 percent of calf serum, 50 units per milliliter of penicillin, and 50 μg per milliliter each of streptomycin and kanamycin (*5*). These cells, found to be free of pleuropneumonia-like organisms (*6*), were cultured in Roux bottles with (treated) and without (control) hydrocortisone hemisuccinate (2 μg/ml) at 37°C for 72 hours and were harvested by gentle scraping. The cells were centrifuged, suspended in saline with tris(hydroxymethyl)-aminomethane (0.001*M*, *p*H 7.4), and a small sample was taken for cell counting. These samples were treated with an equal volume of 0.0025 percent trypsin in 0.3 m*M* ethylenediaminetetraacetate (*p*H 9 to 10 for 5 minutes at 37°C) to insure complete cell disaggregation, prior to counting in a Levi hemocytometer. The cells from each bottle were packed by centrifugation and suspended in 2 ml of deionized distilled water at 0°C. They were then disrupted by two 15-second bursts of ultrasound delivered by the microprobe of a Bronson Bio-Sonicator at 70 percent efficiency with a 14-kc output. The protein content was determined (*7*) with crystalline bovine serum albumin as the standard. The homogenates were subjected to acid hydrolysis with 0.1*N* sulfuric acid at 80°C for 1 hour to release bound sialic acid, and the sulfuric acid was then precipitated with an equivalent amount of saturated barium hydroxide. The supernatant fraction plus one water washing of the sediment were passed successively through columns (4 by 30 mm) of Dowex 50W (H^+) and Dowex I (formate). The columns were washed with distilled water, and sialic acid was eluted from the Dowex I (formate) column with

Table 1. Sialic acid and protein content (per culture) of HeLa S_3 cells grown in the presence and absence of added hydrocortisone.

Sialic acid (nmole)	Protein (mg)	Cell count $\times 10^{-7}$ (mean ± S.D.)	Sialic acid			
			Nmole/ mg protein	Nmole/ 10^8 cells	Sedimented (%)*	Non-sedimented (%)*
		Control cells (Expt. A)				
102.93	22.0		4.68	1.72		
93.66	21.0	6.00 ± 0.70	4.46	1.56	63.8	36.2
115.71	26.0		4.45	1.93	61.9	38.1
		Control cells (Expt. B)				
131.38	20.0	6.82 ± 0.40	6.57	1.93		
129.38	20.0		6.47	1.90		
		Hydrocortisone-treated cells (Expt. A)				
151.01	20.0		7.55	3.78		
146.09	18.0	4.00 ± 0.40	8.12	3.65	54.9	45.1
138.86	25.0		5.55	3.47	51.7	48.3
		Hydrocortisone-treated cells (Expt. B)				
137.17	19.2	3.64 ± 0.22	7.14	3.77		
128.95	16.8		7.68	3.54		

* Percentages of sialic acid in pellet and supernatant fraction, respectively, obtained by subjecting the sonicated cells to ultracentrifugation at 100,000*g* for 1 hour. Values are expressed with reference to the total sialic acid recovered. The overall recovery with respect to the unfractionated material was 90.6 ± 5.12 (mean ± S.D.).