laria and sniper attacks in order to obtain the basic data on man-biting rates.

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# CALIFORNIA ENCEPHALITIS VIRUS ISOLATION FROM BRITISH COLUMBIA MOSQUITOES

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During the summer of 1969, field investigations of the ecology of tick-borne Powassan (POW) virus, mosquito-borne St. Louis encephalitis (SLE) virus and California encephalitis (CE) virus were undertaken in the South Okanagan District of British Columbia around Penticton (119°30′ W, 49°30′ N). These investigations were stimulated by: (1) the detection of POW antibodies in columbian ground squirrels (*Citellus columbianus*), and CE antibodies in both snowshoe hares (*Lepus americanus*) and columbian squirrels during a preliminary serologic survey in 1967 (McLean *et al.*, 1968); (2) detection of CE neutralizing antibodies in 93 percent of snowshoe hares near Kamloops, B.C. by Newhouse and Gregson (1963); (3) demonstration of POW and CE antibodies in 1 to 3 percent of human residents of southeastern British Columbia (Kettyls and McLean, 1969) including one CE antibody conversion between 1968 and 1969.

Between April and August 1969, antibodies to group B arboviruses (POW, SLE) were detected in sera from 133 of 833 wild rodents by hemagglutination inhibition (HI) tests and 43 by neutralization tests. The major mammalian species collected, *Marmota flaviventris,* showed 94 of 422 with group B HI antibodies, but 7 of 60 *C. columbianus* also showed group B reactions. *Dermacentor andersoni* ticks were removed from 25 marmots, average 2.5 per animal (range 1 to 7), but no virus was isolated by intracerebral injection of suckling mice. No virus was isolated from nine tick pools collected by dragging between 11 April and 18 June.

Mosquitoes were collected by hand in shaded habitats at six collection sites between 8th June and 4th August. Of a total of 26 pools containing approximately 50 mosquitoes each, 16 pools were collected at the Grey Sage ranch (119°30′ W, 49° 15′ N). Principal species collected were *Aedes vexans* and *Aedes canadensis,* but no *Culex tarsalis* were collected.

An agent which induced encephalitis which terminated fatally 4 days after intracerebral injection of suckling mice aged

2 days, was recovered from pool number 69-BC-M5 comprising 50 *A. vexans* plus *A. canadensis* mosquitoes collected at Grey Sage ranch on 14th June. Upon passage of brain suspensions intracerebrally to suckling mice, encephalitis ensued after 2 days. Second passage brain suspension induced encephalitis in weaned mice 4 days after intracerebral injection of 100 $LD_{50}$, and the infectivity titer of this isolate ($10^{-5.5}$ per 0.03 ml) was identical for suckling and weaned mice. The isolate passed readily through a Millipore filter of 220 n m porosity. Sodium deoxycholate 1:1000 inactivated 100 $LD_{50}$ of virus after exposure for 30 minutes at 25° C. The isolate did not propagate in roller tube monolayer tissue cultures of primary cynomolgus monkey kidney or in a continuous line of human cells HEp 2.

Antisera to the prototype BFS-283 strain of CE virus and to the Grey Sage isolate were prepared in guinea pigs by two intraperitoneal injections, and in rabbits by one intravenous injection followed by one intraperitoneal injection. All sera were devoid of antibody to the homologous virus before immunization, but post-immunization sera neutralized 100 $LD_{50}$ or more of homologous virus. No significant differences (less than 1.0 log) were observed in the neutralization index of both sera against the homologous and heterologous virus strains, employing sera prepared both in rabbits and in guinea pigs. Antiserum to POW did not neutralize the Grey Sage isolate.

In complement fixation tests employing rabbit sera (CF antibody was not detected in guinea pig sera at any time), plus borate-saline extracts of infected suckling mouse brains as antigen, no significant antigenic differences were detected between the Grey Sage isolate and the BFS-283 prototype.

Neutralizing antibodies to the Grey Sage isolate were detected in sera from 3 of 219 mammals collected during June and 0 of 132 collected during April. Only two snowshoe hares were collected, but neither had CE antibody.

These results show that a member of the CE complex of arboviruses is endemic in the South Okanagan Region of British Columbia in addition to Kamloops, B.C. where 93 percent of snowshoe hares had antibody (Newhouse *et al.*, 1963), Rochester, Alberta where seven pools of *Aedes communis* yielded virus between 1964 and 1968 (Hoff *et al.*, 1969), Brooks, Alberta where two pools of *Culiseta inornata* yielded CF virus in 1965 (Morgante and Shemanchuk, 1967), and Hamilton, Montana where the blood of a snowshoe hare yielded CE virus in 1959 (Burgdorfer *et al.*, 1961). CE virus appears to be maintained by a natural cycle involving *Aedes* mosquitoes as vectors, and hares or squirrels as reservoirs.

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