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Isolation of Powassan Virus from a Spotted Skunk in California

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An apparently healthy adult male spotted skunk, *Spilogale putorius* (No. M2724) was captured in a live-trap at a farmhouse near Windsor, Sonoma County, California on 1 April 1969. The surrounding area was mostly pasture land for cattle. There was a creek nearby with large oak trees and brush along the water course. In this coastal mountain region there have been sporadic cases of rabies in skunks and cattle for the past 30 yr. The experimental design was to test a throat swab specimen for virus by the intracranial inoculation method using 1- to 2-day-old mice. On 22 April, M2724 was anaesthetized, bled out, and a pool of salivary glands, trachea and lung, and a separate specimen of kidney tissue were harvested for direct test in mice and for cell culture. The brain tissue was tested for rabies virus by the rabies fluorescent antibody (RFA) test and for virus by the mouse inoculation test (Johnson, 1979, *In* Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, Lennette and Schmidt (eds.), Am. Pub. Health Assoc., pp. 843–877). The tissue specimens were prepared by grinding the tissue in a mortar and preparing a 10% suspension in phosphate-buffered saline solution containing 0.75% bovalbumin fraction V and antibiotics. For tissue culture the tissues were minced with curved scissors, rinsed in physiological saline solution, trypsinized, and the washed cells were planted in one bottle and several test tubes for each specimen. Hanks' solution with 0.5% lactalbumin hydrolysate, antibiotics and 15% inactivated fetal bovine serum was used for the outgrowth medium. When the cell sheet had formed, the outgrowth medium was removed for testing and storage and replaced with Eagle's maintenance medium containing antibiotics and 2% inactivated fetal bovine serum. Both Hanks' and Eagle's medium were adjusted to pH 6.9 to 7 with sodium bicarbonate solution. The medium was changed as necessary and tested for virus by the mouse inoculation test. The serum-virus neutralization test (Shope and Sather, 1979, *In* Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, Lennette and Schmidt (eds.), Am. Pub. Health Assoc., pp. 767–814) was done using 1- to 2-day-old mice. The serum-virus mixture was given by the intraperitoneal route. The undiluted Powassan antiserum was tested against selected 10-fold dilutions of the Canadian strain of Powassan virus and the California isolate.

The throat swab specimen, and the tissue suspension of the brain, the salivary glands, trachea and lung pool, and the separate specimen of kidney tissue were negative for rabies virus and other viruses pathogenic for mice. The cell cultures of the salivary gland, trachea and lung pool grew well and showed no cytopathic change. The medium, removed from these cultures and tested through the 37-day holding period, was negative for virus. The

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outgrowith medium, removed from the kidney cultures at 1 wk, was negative for virus by the mouse test. On day 13, the cells in the kidney cultures began to show cytopathic change and detach from the glass. Mice inoculated with the medium removed from the cultures at this time, developed spasticity and paralysis of the limbs, beginning on the 5th day and all were dead by the 8th day. The maintenance medium was changed on days 13, 17, 21 and 37. Each harvest was positive for virus. The virus was pathogenic for infant and adult mice when inoculated by either the intracerebral or intraperitoneal route. The virus was identified as Powassan virus by the serum-virus neutralization test. The Powassan antiserum prepared at the California laboratory gave a log neutralization index (LNI) of >5.0 when tested against the M2724 isolate. The virus isolate was sent to Dr. Robert E. Shope of the Yale Arbovirus Research Unit. Their ascitic fluid antibody (AB) for Powassan virus gave an LNI of >4.8 when tested against the M2724 isolate. Their ascitic fluid AB pools for St. Louis virus, Modoc virus and Rio Bravo virus gave an LNI of <1.8 with a homologous AB having an LNI of >6.0. The serum of skunk M2724 had an LNI of >3.0 when tested against the homologous virus isolate. We have found virus neutralizing antibodies to Powassan virus in the serum of three golden-mantled ground squirrels, *Spermophilus lateralis*, two from Hackamore Station, Modoc National Forest, Modoc County, California and one from Klamath County, Oregon. Four striped skunks (*Mephitis mephitis*) and five spotted skunks were tested and in addition to M2724, one of the spotted skunks from Alameda County, California had antibodies to Powassan virus with an LNI of >2.0.

Cell culture studies of kidney tissue of carnivores such as skunks and weasels and serological studies should be conducted to determine the geographical distribution of arthropod-borne viruses such as Powassan virus. This is the only reported isolation of Powassan virus in the United States from west of the Rocky Mountains (Karabatsos (ed.), 1985, International Catalogue of Arboviruses, Including Certain other Viruses of Vertebrates, 3rd Ed., Am. Soc. Trop. Med. Hyg., pp. 827–828).