by examination of the terminalia, 116 were *pilosus* (Dyar and Knab); 49 were *opisthopus* Komp; 2 were *atratus* Theob.; 2, were *iolambdis* Dyar; 2 were *erraticus* Dyar and Knab; and 1 was probably *mulrennani* Basham (damaged specimen).

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7. We gratefully acknowledge the cooperation and assistance of the Everglades National Park supervisors and rangers, without which this study could not have been carried out.

20 May 1964

Venezuelan Equine Encephalitis Virus in Veracruz, Mexico, and the Use of Hamsters as Sentinels

Abstract. *Venezuelan equine encephalitis virus was recovered in the state of Veracruz, Mexico, during July and August 1963 from young, weaned hamsters, and from baby mice used as sentinel animals, and from* Culex *mosquitoes. Hamsters of 5 to 10 weeks of age became infected in nature and were nearly as susceptible as suckling mice to subcutaneous inoculation of VEE virus.*

Venezuelan equine encephalitis (VEE) virus is an arthropod-borne virus (arbovirus) pathogenic for man and horses which has hitherto been known to occur only in northern South America and Panama. As recently as 1961 and 1962, several thousand persons in the Guajira peninsula of Venezuela and Colombia and a few hundred people in Panama have been infected (*1*). The disease is characterized mainly by fever, myalgia, and respiratory and gastrointestinal symptoms, the central nervous system being affected only rarely in man. In equine epizootics, encephalitis and death have been more common manifestations than in human epidemics (*2*).

During July and August 1963, VEE virus was discovered on the tropical eastern coast of Mexico in the state of Veracruz, less than 1600 km from Texas, Louisiana, and Florida, thus establishing its presence in North America and exposing evidence of a potential danger to the health of man and certain domestic animals in Mexico and possibly in the United States.

The initial isolations of VEE virus in Mexico were from hamsters used as bait in mosquito traps. On 23 July 1963, two hamsters, 6 weeks of age, were placed in a small trap made of metal and wire screen. On 29 July, one hamster was dead and the other ill. Suspensions of pooled heart, lung, and kidney tissues from both hamsters yielded agents which upon intracranial inoculation to suckling mice killed them in 30 to 48 hours. The agent from the sick hamster (designated 63U2 virus) could be passed through a 450-mμ Millipore filter. After three passages of this virus in mice a complement-fixing antigen was obtained from the brains and this antigen reacted with VEE mouse antiserum. Antigen-antibody titers with 63U2 antigen and VEE antiserum were 1:64/1:512; the titer of this VEE antiserum against VEE virus was also 1:512. To a lesser extent, 63U2 complement-fixing antigen also reacted with mouse antiserums to the Mucambo and Pixuna viruses which are both related to VEE virus. However, the complement-fixing antigen did not react with antiserums that were specific for eastern encephalitis virus, western encephalitis virus, Oriboca virus, or a Bunyamwera group virus from Mexico.

After three passages in mice the infectivity of 63U2 virus for suckling mice inoculated intracranially was neutralized by antiserums made against VEE viruses from Trinidad, Colombia, and Panama and TC-83 vaccine; the neutralization indices ($\log_{10}$) against 63U2 (and against the homologous VEE virus) were 3.2 (>5.7), 2.0 (3.2), 2.4 (4.6), and >3.9 (not tested). That the 63U2 virus represents a new strain of VEE virus is supported by the fact that it was isolated and cultured in laboratories where VEE virus had never previously been used. The procedures used for the complement-fixation and neutralization tests are described elsewhere (*3*).

Other strains of VEE virus were also detected in 17 out of 19 additional hamsters, 5 to 10 weeks of age, which were used as sentinels during August 1963. The hamsters died or were found ill 3 to 14 days (usually 3 to 7 days) after being placed in mosquito traps or in wire cages accessible to mosquitoes. Heart, lung, and kidney tissues were removed, pooled, and frozen on dry ice in the field for subsequent examination. Each strain was successfully isolated from tissue suspensions which were injected intracranially and subcutaneously into suckling mice; the strains were identified by complement-fixation tests with mouse antiserums made from the 63U2 strain and a serum from a convalescent patient who contracted a laboratory infection with 63U2 virus. One to 2 months later each strain was again isolated by inoculation of frozen tissue suspensions into primary cultures of chicken embryo cells in fluid medium and identified by neutralization in cell cultures with rabbit antiserum to 63U2 virus. The sensitivity of hamsters to the 63U2 strain of VEE virus inoculated subcutaneously was shown in simultaneous titrations in mice and hamsters; subcutaneous LD_{50} (50 percent lethal dose) titers per 0.01 ml for the virus after the third passage in suckling mice were $10^{-9.7}$ in 1- to 3-day-old mice, $10^{-8.5}$ in hamsters 45 days of age, and $10^{-8.3}$ in hamsters 100 days of age, and after the fifth passage in suckling mice, $10^{-9.8}$, $10^{-9.0}$, and $10^{-8.5}$, respectively. This susceptibility of hamsters to 63U2 virus plus the ease with which they could be housed and cared for in cages exposed in nature over periods of days to weeks made them effective and convenient sentinels for the Mexican strains of VEE virus.

Although some of the materials collected in 1963 have not yet been tested for virus, 17 additional strains of VEE virus have been recovered in chicken embryonic cell cultures from brains of baby mice used as sentinels in the endemic area (the mice were exposed 20 to 24 hours in coarse wire-mesh cages 1 to 2 m above ground). One strain has been isolated from a suspension of 53 *Culex iolambdis* and *coronator* inoculated into suckling mice. This recovery

from mosquitoes represents one positive pool of 47 pools tested; over 400 mosquito pools from the endemic area remain to be examined.

The habitats in Mexico where these strains of VEE virus were recovered include secondary forest, mangrove, and a small patch of rain forest dominated by canopy-forming trees, all bordering a lagoon off the Gulf of Mexico coast at the village of Sontecomapan, Veracruz. These areas are in a narrow strip of coastal lowland immediately north of a small range of volcanic mountains surrounding Lake Catemaco, approximately 500 km southeast of Mexico City and 160 km southeast of the city of Veracruz. Whether VEE virus has a wider geographic distribution extending into more populated areas of the eastern coastal lowlands of Mexico and whether the virus has only recently moved to Mexico or has been endemic there for many years must be determined by future studies. Nevertheless, its presence constitutes a potential threat to the health of man and certain domestic animals in Mexico, and if carried northward, perhaps by migrating birds, it could also become a threat to inhabitants of the United States.

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4. These investigations were performed by collaboration among persons from Cornell University Medical College, the Pan American Health Organization, the Government of the United States of Mexico, the Instituto de Virologia de la Secretaria de Salubridad y Asistencia and the Instituto de Salubridad y Enfermedades Tropicales of Mexico, and were supported in part by USPHS training grant No. 5-T1-AI-231 from the NIAID and in part by the Surgeon General, Department of the Army, under sponsorship of the Commission on Viral Infections of the Armed Forces Epidemiological Board. VEE, Mucambo, and Pixuna virus antiserums and their VEE antibody titers were kindly supplied by J. Casals, R. Shope, and R. McKinney.

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"Chromaffin" Cell: Electron Microscopic Identification in the Human Dermis

Abstract. *A granulated cell in human skin has an ultrastructure similar to that of chromaffin cells of the adrenal medulla. This cell is closely associated with unmyelinated axons and may be the site of peripheral store of noradrenaline.*

The presence of chromaffin cells (those cells containing adrenaline or noradrenaline) in the human dermis has been a matter of controversy. Adams-Ray and Nordenstam (*1*) and Burch and Phillips (*2*) treated chromated skin with the Sevki stain (*3*) and concluded that chromaffin cells are present. These cells, in their opinion, are distinguishable from mast cells and have staining characteristics similar to those of the medullary cells of the adrenal. In contrast, Mercantini (*4*) and Matz and Skinner (*5*) state that the staining characteristics attendant on the use of the Sevki stain are not only dissimilar to those described by Adams-Ray *et al.* but also do not allow differentiation of mast cells, the commonly present granulated cell in the dermis, and chromaffin cells. Thus far electron microscopic observations (*6*) have failed to demonstrate conclusive differences between the granules of the so-called chromaffin cells of the skin and mast cells.

The data from physiological observations (*7*) on the cutaneous vasculature suggest that peripheral stores of noradrenaline are present and that they are controlled by cholengeric, postganglionic sympathetic fibers.

The cell shown in Fig. 1, from the dermis of the axillary skin of an 11-month-old white boy, was found incidentally during a study of the ultrastructure of a reactive inflammatory skin lesion, juvenile xanthogranuloma. The lesion is formed of histologically dissimilar cells, histiocytes, and proliferating fibroblasts, and it does not contain granulated cells. This cell contains membrane-limited, electron-opaque granules that are 1000 ± 200 Å in diameter.

These granules are similar to those in the medullary cells in the human adrenal. They are smaller than the granules of human mast cells which average 5000 ± 2000 Å in diameter. The granules are concentrated at one pole of the cell, and adjacent to this pole there are three unmyelinated axons within a Schwann cell. The limiting membrane of two of these axons are in appositional contact with the cell membrane of the granulated cell.

The ultrastructure alone does not identify the pharmacological nature of the content of the granules. However, the similarity in structure to the noradrenaline-containing cells of the

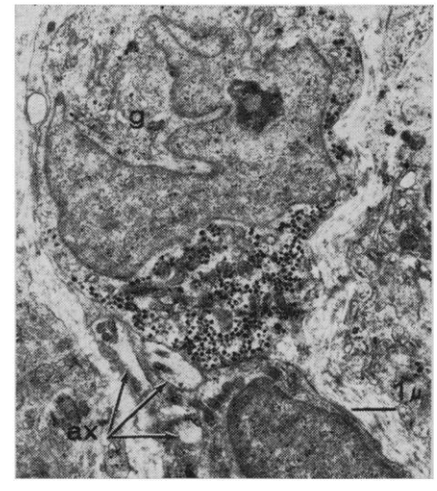

Fig. 1. The round electron-opaque secretory granules are concentrated amidst the mitochondria, at the lower pole of the cell. The arms of the irregularly shaped nucleus enclose the Golgi zone (*g*) wherein a few granules are seen. The three unmyelinated axons (*ax*) are within the adjacent Schwann cell.

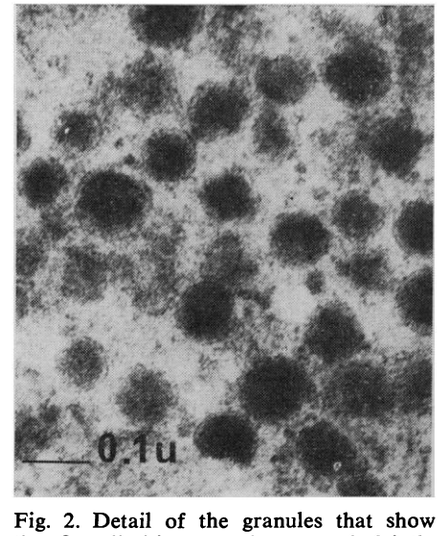

Fig. 2. Detail of the granules that show the fine limiting membrane and faintly granular matrix.