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Recovery of an Adenovirus from a Feral Rodent *Peromyscus maniculatus.** (31955)

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During an intensive search for arbovirus infections in small mammals from Kern County, California, an apparently new adenovirus was recovered from the blood of a feral rodent (*Peromyscus maniculatus*, the white footed deer mouse). This report describes the circumstances surrounding the isolation and the characteristics of this virus.

From November, 1960, through April, 1963, blood samples were collected from 1,890 small wild mammals, representing 19 species. All animals were live-trapped in rural areas of Kern County, either from agriculturally developed farmlands or from desert habitats adjacent to such environments. Blood samples represented a 0.3 ml aliquot diluted in 2.0 ml of a diluent comprised of 2% heparin (1:10,000) and 20% normal rabbit serum in physiologic saline. Samples were held at —70°C in sealed glass ampoules until tested.

The blood sample from each animal was inoculated (0.01 ml) into each of 8 suckling (2- to 3-day-old) mice by the intracerebral route and 0.1 ml into each of 2 tubes of 10-day-old hamster kidney cell cultures (HKCC) (1). Presence of virus was indicated by mice becoming ill or dying within a 21-day period or by observing cytopathogenic changes in cell cultures within a ten-day period.

Primary isolation. A virus (strain E-20308) was isolated from the blood of an adult female *P. maniculatus* trapped and bled on January 18, 1963, in a desert study area approximately 45 miles west of Bakersfield, Calif. Mice and HKCC were inoculated with this blood on May 9, 1963. Six of eight mice were dead the morning following inoculation and were discarded as presumably nonspecific deaths. A second litter of mice was inoculated with the original blood sample. On the second day postinoculation, 2 mice were dead and brains were removed from 2 additional mice that appeared to be abnormal. A fifth mouse was found dead on day 4 and the other 3 remained normal. Subpassage of homogenates of the 2 brains from sacrificed mice did not produce illness in suckling mice or cytopathogenic effects (CPE) in HKCC. The cells of HKCC inoculated with the original blood

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sample were abnormal 6 days following inoculation. Cells were clumped, enlarged, and rounded, although apparently intact. This appearance was quite different from CPE produced in HKCC by western equine encephalitis (WEE) or St. Louis encephalitis (SLE) viruses(2). Subpassages in HKCC were successful and at the tenth level produced cellular abnormality within 2 days and cellular destruction or sloughing from the glass within 4 days; the tenth passage fluid titered $10^{6.0}$ $TCID_{50}$ per 0.1 ml.

No material was available from the original blood sample for reisolation attempts in HKCC.

Animal pathogenicity tests. Infant mice were inoculated by the intracerebral route with undiluted supernatant fluid from the first HKCC passage and with 10^6 $TCID_{50}$ of virus from the fifth HKCC passage. Ten-day-old embryonated chicken eggs were inoculated into the amniotic cavity with 10^6 $TCID_{50}$ of fourth passage virus. Hamsters, 8 to 9 weeks old, were inoculated with 0.5 ml of the fifth passage virus by the intraperitoneal route. Two litters of 2 to 4-day-old *P. maniculatus* (born in the laboratory) were inoculated with the undiluted tenth passage virus, one litter by the intracerebral and the other by the intraperitoneal route. None of the above experimental hosts had observable signs or symptoms of infection. No attempt was made to recover virus from any of the above hosts. The above litters of *P. maniculatus* were bled one month after inoculation for hemagglutination inhibition tests with arboviruses. No antibody was detected to the arboviruses that prevail in Kern County.

Filterability. Virus of the seventh HKCC passage was filtered through a Seitz filter (type ST, L 3 pad). Both the unfiltered and filtered materials titered 10^6 in HKCC.

Deoxycholate resistance. Both deoxycholate treated and untreated aliquots of HKCC passage level eight had the same titers ($10^{5.5}$), while there was a 4 log decrease in the infectivity titer of the control of WEE virus between the untreated ($10^{5.5}$) and treated ($10^{1.5}$) aliquots.

Serologic testing against arboviruses. The sixth passage level of virus was tested in neutralization tests in HKCC against hyperimmune rabbit sera against arboviruses that may occur in Kern County; agent E-20308 was not neutralized by antisera to: Group A, WEE, and eastern equine encephalitis; Group B, SLE, Powassan, and Modoc; Bunyamwera group, Cache Valley (3 strains Utah, U.S.A.; Ar 7272, South America; and FMS 4332, California, U.S.A.); California group, California (BFS 283), and Jerry Slough (BFS 4124); and ungrouped, Kern Canyon and Buttonwillow. The plasma sample from the original *Peromyscus* that yielded the virus was tested for HAI antibodies to arboviruses. There were no antibodies to WEE, SLE, Cache Valley, Modoc, or Buttonwillow viruses and 1:20 titers to Powassan and California viruses. Insufficient plasma was left for serologic tests against adenoviruses.

Classification as an adenovirus. The virus was classified as an adenovirus on the basis of the possession of the adenovirus group CF antigen, type of CPE, and stability.

Culture fluids from infected HKCC or BHK-21(3) cultures reacted in complement fixation (CF) tests to a titer of 1:4 or 1:8 against human adenovirus convalescent serum; paired acute and convalescent sera of 4 naval recruits with adenovirus type 4 or 7 infection were tested in CF against E-20308 antigen; all 4 showed antibody rises, the acute phase sera being negative at 1:8, and the convalescent sera being positive to titers of 1:16 or over. The sera gave no reaction with control antigen. The virus was not neutralized by rabbit antisera to adenovirus types 1 through 30 or mouse adenovirus, at 1:20 dilution, but was neutralized by homologous rabbit antiserum to a titer of 1:640 or greater.

HKCC-grown virus induced characteristic adenovirus CPE in rhesus and African green monkey kidney cultures, but like other nonsimian adenoviruses, the virus could not be carried in serial passage in these cells. Similarly, the virus produced adenovirus type CPE in HEp-2 cell cultures for 2 passages, but produced no CPE on third passage. The virus produced only minimal CPE in human embryonic kidney cultures, and none in mouse embryo or WI-38 cultures.

Chloroform-treated virus (10%, 10 min at 4°C) produced CPE as rapidly in monkey kidney cultures as did untreated virus.

Attempts to demonstrate antibody in feral Peromyscus. Ninety sera of *P. maniculatus* that were captured in the same area of Kern County as the animal that yielded E-20308, were tested at 1:10 dilution for CF antibody to the E-20308 virus and adenovirus type 2. All were negative with both antigens.

Discussion. Although the size and capsid structure of the E-20308 virus have not been determined, its serologic and biologic properties suffice to place it in the adenovirus group. It has not been compared serologically with the various adenoviruses of nonhuman species, but is sufficiently different from these in its host range in tissue culture to make it unlikely that it is a previously described adenovirus. The only other known adenovirus of rodents is that found in laboratory *Mus musculus,* which differs markedly from E-20308 in host range and antigenic composition(4).

The natural host of E-20308 virus is not established. While it seems highly probable that the virus was derived from the *Peromyscus* blood sample the absence of antibody in the *Peromyscus* population suggests that this genus may not be the basic reservoir. The ability of the virus to propagate well in cells of another genus, that is, the hamster, supports the possibility that it may be less species-specific than other adenoviruses and thus may cross species lines in nature.

The possibility must be considered that the virus derived from the HKCC used for the initial isolation; this seems very remote since both inoculated cultures developed CPE, while none of 106 additional tubes prepared from the same kidneys on this date and used as controls or inoculated with other specimens developed similar changes. The primary HKCC system has been used in the Berkeley laboratory for over five years and no other adenoviruses have appeared spontaneously; also no other adenoviruses are available at or have been isolated in that laboratory.

Summary. An adenovirus was isolated from the blood of a feral *Peromyscus maniculatus* collected in January, 1963, from Kern County, Calif. The virus was classified as an adenovirus on the basis of the possession of the adenovirus group CF antigen, type of CPE in and susceptibility of a range of cell culture systems, and insensitivity to chloroform. The virus was not neutralized by rabbit antisera to human adenovirus types 1 through 30 or mouse adenovirus. It differs from other nonhuman adenoviruses in host range in tissue culture, and is considered to represent a previously undescribed adenovirus.

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