ever, they have the same coloring as the rest of the plants in the culture which have 72 (±1 or 2) chromosomes. The anthers were shrunken, transparent and practically empty. Among immature anthers a few gigantic, misshapen pollen grains were found, these apparently consisting of the entire pollen-mother-cells which had developed an exine, and a few compound small grains, the results of only partial cytokinesis. A dividing tapetal cell nucleus of one of these plants showed 38 chromosomes, including one very small one. The other plant had 36 to 38 chromosomes, as indicated by an examination of the diakinesis and metaphase I stages in pollen-mother-cells. In both plants only a few bivalents were found at metaphase I. The univalents, of greater length than when associated as bivalents, were scattered somewhat along the axis of the spindle. The walls of the pollen-mother-cells were extremely thin. It was concluded that although these two dwarf plants also belong to the 36-chromosome class, they are to be considered as haploids of the 72-chromosome population in which they occurred.

The contrast in morphological appearance and in chromosome behavior between the 36-chromosome plants from Durango and those from Texas emphasizes the fact that more than mere chromosome number is needed for an understanding of the appearance and breeding behavior of guayule plants. Judging by the plants obtained from seed collected in Mexico and in Texas, the 36-chromosome Durango plants seem to be the only type in certain locations and to comprise an appreciable part of the population in other locations where 54-chromosome plants also occur; whereas the 36-chromosome plants from the O2 Ranch in Texas are among the off-types found in a population that seems to consist almost entirely of 72 (±1 or 2) chromosome plants.

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## FATAL ENCEPHALITIS IN MAN DUE TO THE VENEZUELAN VIRUS OF EQUINE ENCEPHALOMYELITIS IN TRINIDAD

BY the early part of October, 1943, approximately seventy cases of fatal encephalitis had occurred among the horses and mules in Trinidad, British West Indies. The epizootic was tentatively diagnosed as equine encephalomyelitis by the local authorities from the clinical symptoms presented. Accordingly, the sector veterinarian of Trinidad, Major R. T. Gilyard, Army of the United States, sent representative portions of brain tissue from two donkeys, two horses and a mule that had died of the disease to the laboratories of the Army Veterinary School, Medical Department Professional Service Schools, Army Medical Center, Washington, D. C., for diagnosis. The first material, that from a donkey, was received on October 11, 1943. Later he submitted brain tissue from a human case of encephalitis that had died on August 22, 1943. Brain tissue was shipped in buffered glycerine by airmail express and arrived within two days after shipment.

A ten per cent. suspension of brain tissue was prepared from each of the six cases and injected intracerebrally in 0.1 cc amounts into two guinea pigs, and in 0.03 cc amounts into three Swiss mice. The inoculated animals presented typical symptoms of equine encephalomyelitis in periods varying from three to eight days. They were sacrificed while in a moribund condition and their brains removed for further study.

The laboratory animals inoculated with brain suspensions from the donkeys, horses and mule became prostrate within 96 hours. Those inoculated with the human brain tissue became prostrate by the eighth day. This may be explained by the fact that brain tissue from the animals was received within two to three days after autopsy, whereas the human brain tissue had been held in Trinidad for six weeks in buffered glycerine solution under refrigeration before it was shipped to this laboratory.

Laboratory animals infected with virus from each of the six cases were sacrificed when prostrate, their brains removed, and when found to be bacteriologically sterile were prepared for typing of the viruses.

These six strains of virus when isolated were injected intracerebrally into each of three groups of guinea pigs; one group immunized against Western type virus, the second group against the Eastern type and a third group of normal animals. The groups of Western immune and normal guinea pigs died within 96 hours and in the Eastern immune group the death period extended to the fifth or sixth day, a condition that has previously been noted in Eastern type immune animals injected with the Venezuelan equine encephalomyelitis virus.[1] The results on the animal brains were reported to Major Gilyard on October 21, 1943.

By this time we had obtained Venezuelan equine encephalomyelitis vaccine and immunized a group of guinea pigs. Two weeks after completion of vaccination this group exhibited no illness following intracerebral injections of the six isolated viruses, indicating that the virus was the Venezuelan type in all six instances. Guinea pigs immunized against the Eastern and Western viruses and normal animals served as controls.

[1] C. E. Beck and R. W. G. Wyckoff, SCIENCE, 88, 530, 1938.

Of the three immunologically different strains of equine encephalomyelitis viruses known to be present in the Western Hemisphere, fatal human cases due to the Eastern type virus were established by Fothergill, Dingle, Faber and Connerly[2] in 1938, and somewhat later in the same year Howitt[3] reported the isolation of the Western type virus from a fatal human case.

Although Venezuelan type equine encephalomyelitis in man may have been suspected,[4] there is record of only two cases having been reported. These were of a very mild type and occurred in the United States in laboratory personnel working with the virus.[5]

The present report records the first instance in which the Venezuelan strain of equine encephalomyelitis virus has been proven to occur naturally in man, producing a fatal infection. Further, it establishes the fact that all three strains of equine encephalomyelitis viruses known to be present in the Western Hemisphere are capable of producing a fatal encephalitis in man.

From available information, this is the first outbreak of equine encephalomyelitis in Trinidad, B. W. I., and immunity tests have proven it to have been caused by the Venezuelan strain of virus.[6]

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## MULTIPLE ELECTRODE HOLDER FOR THE HORSLEY-CLARKE INSTRUMENT

FOR electroencephalographic study of the interior of the animal brain an apparatus to hold several electrodes in place simultaneously is essential. Specifications were submitted to Mr. R. Kittel, Chicago, the maker of the Horsley-Clarke instrument, who designed and constructed a satisfactory attachment. Three bakelite rectangles, each 46 × 57 mm and about 1 mm thick, were drilled with holes to fit No. 11 bead needles coated with insulating varnish. The holes are in 41 rows 1 mm apart, and each row has 31 holes 1 mm apart. The three plates were then fastened together with screws at the corners, using metal sleeves on the screws to hold the plates 5 mm apart. The complete assembly is equivalent to a block 13.5 mm thick. It is mounted on a brass frame attached by screws at four points to the upper lateral bars of the Horsley-Clarke instrument. When in place, needles in the holes are vertical with respect to the brain. An adjustment on the frame allows the multiple holder to be moved laterally, so that the center row of holes can be placed exactly over the midline.

Insulated bead needles inserted through the holes in the holder into the brain keep their positions without fastening. The depth of a needle point in the brain is controlled by measuring the length of needle exposed above the upper surface of the multiple holder. Since this upper surface is 46 mm above the interaural plane, and a needle is 53 mm in length, the needle point is at the interaural plane if 7 mm of needle remains exposed. Connections to the amplifier and stimulating device are made by inserting fine copper wires through the eyes of the needles. As many needles can be used at one time as there are points on the selector switches.

The holes in the multiple carrier now in use will do for 26-gauge hypodermic needle tubing if it is desired to use shielded electrodes. It was not necessary to order the drilling of so many holes. The attachment would be just as useful, and less expensive, if it had about 800 holes, properly placed, instead of 1,271.

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[2] L. D. Fothergill, J. H. Dingle, S. Faber and M. L. Connerly, *New England Jour. Med.*, September 22, 1938.
[3] B. Howitt, SCIENCE, 88, 455, 1938.
[4] Mentioned in: A. S. Lleras and L. Figueroa, Biol. Inst. Nac. Hig. Semper Martinez, 1942, 8, 3.
[5] J. Casals, E. C. Curren and L. Thomas, *Jour. Exp. Med.*, 77, 521, 1943.
[6] Since this manuscript was submitted an additional eight non-fatal cases of infection acquired in laboratory workers were reported by E. H. Lennette and H. Koprowski, *Jour. Am. Med. Asn.*, 123: 1088, 1943.

## BOOKS RECEIVED

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