depression, stupor, anorexia, myopalmus, dehydration with rapid loss of flesh, splanchnic ridge, progressive weakness, paralysis and final collapse in recumbency) developed in both these animals. The normal animal (No. 1100) was sacrificed when completely prostrate on the third day after inoculation, September 27. The western-immune (No. 1011—vaccinated 5/12 and 5/19/38 with formolized western type encephalomyelitis chick embryo tissue vaccine and exposed intracerebrally to western [Iowa-1937-1] virus 8/25/38—survived, after showing only a slight, transitory, febrile reaction and mild indisposition) was dead on the morning of the fourth day after inoculation, September 28. The eastern-immune (No. 1042—exposed intralingually 6/28/38 to eastern virus [South Carolina-1937-1]—developed typical, severe encephalomyelitis, recovering without treatment) remained normal and continues to be so at this writing.

All the guinea pigs injected with the suspension of mouse brain likewise developed symptoms of typical encephalomyelitis (eastern type virus infection) and died, or were destroyed for harvesting of virus when prostrate, on the third to fourth days following injection. A subsequent, bacteriologically sterile passage of the virus from two representative guinea pigs in the group was effected. Suspensions of brain tissue from the two horses (1100 and 1011) also produced typical, bacteriologically sterile encephalomyelitis in guinea pigs when inoculated intracerebrally.

The symptoms in the inoculated animals, the comparatively brief incubation period, the acute course of the disease, the immunity against the injected virus in the eastern-type-encephalomyelitis-immune horse and the equal susceptibility of the normal and the western-type-encephalomyelitis-immune horses add conclusive evidence that the virus recovered by Dr. Fothergill from a human case is indistinguishable from eastern-type equine-encephalomyelitis virus. It may be further noted now that all five of the strains of equine-encephalomyelitis virus which have been recovered by the Bureau from Massachusetts horses during the August–September, 1938, epizootic have been definitely determined to be of eastern type through exposure of guinea pigs immunized against eastern and others immunized against western type equine vira.[7]

The addition of man to the list of species susceptible to equine-encephalomyelitis virus again brings to the fore the problems of epizootiology in connection with the equine disease and justifies further consideration and investigation of the role which the many other known susceptible species[8, 9] might have in the spread of the disease amongst horses, as well as to or from the human family.

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[7] Unpublished work.

[8] L. T. Giltner and M. S. Shahan, SCIENCE, 78: 2012, 63–64, 1933.

[9] L. T. Giltner and M. S. Shahan, *Jour. Am. Vet. Med. Asn.*, 88: n.s. 41, 3, 363–374, 1936.

THE BEHAVIOR OF THE VIRUS OF YELLOW FEVER IN THE MOSQUITO, AËDES TRISERIATUS[1]

THE extrinsic incubation period of yellow fever was described by Corré, and the time limits of this period were determined with a very considerable degree of accuracy by Carter[2] in 1898 before this disease was known to be transmitted by the mosquito, *Aëdes aegypti*. The conclusions of Carter were based on data obtained from his observations of epidemics of yellow fever under natural circumstances. Within the past decade methods have been developed for the study of yellow fever under experimental conditions, more particularly by the infection of monkeys and mice.

In 1904, Dr. Smith,[3] of New Jersey, raised the question whether the mosquito *Aëdes triseriatus* (Say, 1823) prevalent in that state might conceivably serve as a vector of yellow fever. This mosquito is neoarctic in its distribution, and it has been described as occurring from Maine as far southward as Florida and westward to Montana, an area which lies outside the endemic zone of yellow fever. Our experiments were conducted with mosquitoes (*A. triseriatus*) from the region of Ithaca, N. Y. One of us (Baker) succeeded in establishing a colony of this species in a greenhouse insectary at Cornell University. The first experiment in transmission was conducted at the Harvard Medical School in the autumn of 1935, using the technique which we have ordinarily employed with *A. aegypti*.[4] About 35 mosquitoes (*A. triseriatus*) were given an infective feeding on a monkey (*Macacus rhesus*) dying of yellow fever. After an incubation period of 17 days, at about 28° C., 7 of these mosquitoes were living and 6 fed on a monkey. Six days later there was an indefinite febrile reaction (104.1° F.) on one day only, and the monkey remained in excellent condition. One month after the mosquitoes had fed arrangements were being made to inject yellow fever virus for an immunity test. Fortunately a delay of a few days occurred

[1] From the Department of Tropical Medicine, Harvard Medical School, Boston, Mass., and the Department of Entomology, Cornell University, Ithaca, N. Y.

[2] H. R. Carter, *New Orleans Med. and Surg. Jour.*, 52: 617–636, 1900.

[3] J. B. Smith, "Report of the New Jersey State Agricultural Experiment Station, Trenton, New Jersey." MacCrellish and Dingley, State Printers: Trenton, N. J., 1904.

[4] A. W. Sellards, *Am. Jour. Trop. Med.*, 12: 79–92, 1932.

and no injection was made; this monkey died of yellow fever 36 days after being bitten by mosquitoes. In the meantime, the colony of mosquitoes at Cornell had been discontinued.

It was not feasible to resume this work until the present year. In March, larvae (*A. triseriatus*) were collected from tree-holes at Ithaca and reared in the laboratory of the Department of Entomology at Cornell University. Mosquitoes which had previously taken an infective blood meal were allowed to feed on four monkeys. These animals showed no febrile reaction, but two of them died of yellow fever after intervals of 10 and 13 days. Blood taken from the two surviving monkeys failed to protect mice against yellow fever. The two monkeys which survived were bitten by mosquitoes which were kept for 14 to 15 days at about 28° C., whereas the two monkeys which died were infected by mosquitoes which were incubated at 37° C.

Ten mosquitoes which had ingested infective blood of dying monkeys were tested for virus after incubation periods of 13 to 16 days. Each mosquito was ground in a mortar with a little serum-saline, and injections were made intracerebrally in white mice. The virus was recovered from 6 of these 10 mosquitoes.

Briefly, the virus of yellow fever in its ordinary form was transmitted to monkeys (*Macacus rhesus*) by *Aëdes triseriatus,* and there was some evidence of attenuation of the virus in this mosquito.

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CORRELATION BETWEEN SELF-BREAKING AND BLUE NUCLEI AMONG CERTAIN COMMERCIAL TULIP VARIETIES[1]

IT has been pointed out in "The Antithetic Virus Theory of Tulip Breaking"[2] that while the virus content of the plant determines the type of breaking in all pink and nearly all red tulips some dark red varieties always self, regardless of their virus content. In such tulips the dark red anthocyanin of the flower epidermis is darkened in certain areas and left unchanged in others; the ground color is not exposed. Likewise the black tulip, La Tulipe Noire, when infected with a virus, merely darkens. Several years ago the writer made the unpublished and then unrelated observation that nuclei apparent in mounts of the flower epidermis of La Tulipe Noire are sometimes blue. A study of virus effects on the new race of tulips known as Mendel tulips has shown that without exception every red Mendel variety bearing flowers with a white ground and blue base, selfs or darkens when inoculated with inoculum containing tulip virus I, the color-removing virus. Of the 49 red varieties studied, 21 evidence a blue pigment in the epidermis of the basal portion, which occurs (1) free in the cytolymph of epidermal cells, (2) as prismatic crystalline masses, (3) *within the nuclei of the cells* or (4) in combinations of these three conditions. Frequently, the nuclei are so blue that no structure can be seen within them. The pigmentation of the nuclei is greatest in cells that are beginning to degenerate but is also evident in cells that are apparently healthy. There is no correlation whatever between the presence of a virus and the occurrence of these blue nuclei. The pigment is present in both healthy and diseased individuals, and blue nuclei are evidenced wherever it is abundant. A further study of La Tulipe Noire shows that its approach to blackness is due to the presence of intensely blue cells scattered among the dark red cells of the flower—the nucleus-staining pigment is not confined to the base of the flower. The blue pigment reacts positively to the qualitative reactions for an anthocyanin, changes to a rose color at pH 4.5 and to a yellow-green between pH 7 and 8. Solubility tests tend to differentiate it from the conspicuous red anthocyanin of the tulip flower. Blue nuclei are found in the bases of red Darwin tulips that likewise self in the presence of the color-removing virus. It is not claimed that all red tulips having this pigment will always self, but no exception has been found for dark red Mendels.

Freshly made mounts of tulip flower epidermis are beautiful microscopical objects. The clarity of the self-staining of the pigmented nuclei offers an exceptional opportunity for nuclear study, especially since the pigment seems confined to the karyolymph. Moreover, the blue pigment acts as a selective intravital stain and exhibits vacuolation phenomena with astounding clearness.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE COMBUSTION TYPE OF CARBON MONOXIDE ESTIMATOR

THIS method depends upon the conversion of carbon monoxide into carbon dioxide by combustion and the reaction of this carbon dioxide with strontium hydroxide (or other alkali-earth hydroxide), using phenolphthalein as an indicator. Obviously any carbon dioxide or hydrocarbon vapor which may be present in

[1] Published as Technical Paper No. 289 with the approval of the director of the Oregon Experiment Station. Contribution of the Department of Botany, Oregon Experiment Station, in cooperation with the Bureau of Plant Industry, U. S. D. A.

[2] *Annals of Appl. Biology,* 25: 254–270, 1938.