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Attempts to Transmit the Virus of Acute Anterior Poliomyelitis Through Aedes aegypti.

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In experiments performed at the Army Medical School, Kelser[1] has shown that Borna disease, or epidemic equine encephalomyelitis, can be transmitted by the mosquito *Aedes aegypti.* This suggested the advisability of reconsidering the abandoned theory of insect transmission in poliomyelitis, using mosquitoes of the genus Aedes or other insects not previously considered.

Poliomyelitis presents certain epidemiological features which have suggested insect transmission: (1) it is claimed that all outbreaks, even those that extend into the winter months, begin during the summer; (2) relatively few cases can be traced to previous cases or carriers, and the incidence of infection among contacts is low; (3) the rural distribution of the disease usually equals or exceeds urban incidence; and (4) many outbreaks occur in the neighborhood of water or water systems. Arguments advanced against this possibility include: (1) the virus has not been demonstrated in the blood of human patients, and only in small amounts in the blood of monkeys; (2) large amounts of virus have been required to infect monkeys injected by the intravenous route; (3) with one exception virus has not been demonstrated in filtered saline suspensions of insects previously fed on infected human beings or monkeys; and (4) such transmission experiments as have been reported indicate that biological transmission does not occur.

In experiments based on the inoculation into normal monkeys of filtered saline extracts of insects which had previously fed on materials containing virus, it has been shown that the virus survived in *Musca domestica* as long as 48 hours (Flexner and Clark[2]; Howard and Clark[3]; that adult *Musca domestica* and *Calliphora vomitoria* developed from larvae grown on infected tissues gave negative results (Noguchi and Kudo[4]); and that virus was not demonstrable in

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1 Kelser, R. A., *J. Am. Vet. Med. Assn.*, 1933, 82, N. S. **35**, 5, 767.

2 Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **56**, 1717.

3 Howard, C. W., and Clark, P. F., *J. Exp. Med.*, 1912, **16**, 850.

4 Noguchi, H., and Kudo, R., *J. Exp. Med.*, 1917, **26**, 49.

Culex pipiens, C. sollicitans, or *C. cantator* (Howard and Clark[3]).

In similar experiments based on the inoculation of filtered saline extracts of insects which had previously fed on infected persons or animals, negative results were obtained with fleas (Kling, Pettersson and Wernstedt[5]), and with *Pediculus vestimenti* and *P. capitis* (Howard and Clark[3]). However, the latter workers reported positive results in one test out of 16 with *Cimex lectularis,* observing that in one bedbug the virus survived 7 days. In view of recent information indicating that certain viruses may be rendered innocuous by prolonged exposure to salt solution, it seems fair to question the accuracy of such negative results.

Other transmission experiments include those in which insects previously fed on materials containing virus or on infected persons or animals were subsequently tested by allowing them to feed on normal monkeys. Negative results have been obtained with lice and bedbugs (Howard and Clark[3]), and with *Culex* mosquitoes (Noguchi and Kudo[4]). Using *Stomoxys calcitrans* in large numbers, mechanical transmission of virus was reported by Rosenau and Brues[6] and by Anderson and Frost[7]; however, Anderson and Frost[8] and Howard and Clark[3] failed to confirm these findings.

From this review it appears that relatively few species of insects have been used in such experiments, either to test for mechanical or biological transmission of poliomyelitis virus. As *A. aegypti* is known to be susceptible to infection with at least 3 types of virus this mosquito was used in the following work.

The procedure used was similar to that previously followed in determining the mechanism of the transmission of dengue fever. A normal *M. rhesus* monkey was given an infective inoculation of poliomyelitis virus (Aycock strain supplied by Dr. C. Jungeblut), and on each succeeding day was exposed to a different group of normal laboratory bred *Aedes aegypti.* The mosquitoes which took blood were then kept for different periods of from 7 to 30 days, after which they were tested for infectivity by allowing them to feed on susceptible *M. rhesus* monkeys.

On April 24, 0.2 cc. amounts of virus suspension were injected intracranially and intracutaneously into a normal monkey (*M. rhesus* No. 1). This animal developed a typical case of poliomye-

[5] Report to XV International Cong. on Hygiene and Derm., Wash., 1912.

[6] Rosenau, M. J., and Brues, C. T., *Mass. State Bd. Health Monthly Bull.,* 1912, N. S. **7**, 314.

[7] Anderson, J. F., and Frost, W. H., *U. S. Pub. Health Rep.,* 1912, **27**, 1733.

[8] Anderson, J. F., and Frost, W. H., *U. S. Pub. Health Rep.,* 1917, **28**, 833.

litis, with paralysis, and was moribund when killed 8 days later The characteristic lesions of poliomyelitis were present in the spinal cord.

On each day following inoculation of the virus, monkey No. 1 was immobilized and placed in a different cage, each containing from 40 to 50 normal *A. aegypti.* All mosquitoes which failed to take blood were immediately removed and killed; while those that fed were left in their cages and were designated as lots 1C to 8C, respectively. These 8 lots of mosquitoes were kept for different periods of time, varying from 7 to 30 days and were each tested on 3 different occasions by allowing them to feed on normal monkeys.

On May 5 a total of 99 mosquitoes of the first 4 lots fed on normal monkey No. 2; 73 refed on the same monkey May 15. This animal showed no evidence of infection during almost 5 months of observation. Likewise, the mosquitoes of the remaining 4 lots were allowed to feed on a normal monkey, No. 3—a total of 65 on May 9 and a total of 45 on May 19, with negative results. The final test was made May 25 when a total of 131 mosquitoes, including insects from each of the 8 lots, fed on another normal monkey, No. 4. This animal also remained normal.

On October 10, or about 5 months later, the 3 monkeys (Nos. 2, 3 and 4) were tested for immunity by intracerebral inoculations of the original strain of virus. All 3 developed typical poliomyelitis, indicating that they were susceptible to infection by this route.

Conclusion. Under the conditions of these tests, *Aedes aegypti* failed to transmit the virus of poliomyelitis from infected to normal monkeys. However, in spite of these results and the other available negative evidence, it is believed that the general subject of insect transmission in poliomyelitis deserves further experimental investigation.