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THE RELATION OF MOSQUITOES AND FLIES TO THE EPIDEMIOLOGY OF ACUTE POLIOMYELITIS.

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Of several hypotheses concerning the mode of conveyance of poliomyelitis, that of direct contact, supported by Flexner, appears to be the only one which stands on a substantial experimental basis.[1,2] Although in all epidemics instances have been reported in which more than two members of a family were victims of the disease, as a rule only one out of several children in a family is attacked. This peculiar feature of the epidemiology of poliomyelitis seemed to support the theory that the disease may be inoculated by the bite of an infected insect rather than transmitted directly from one individual to another.

Typical examples of insect transmission of disease are found in yellow fever, malaria, spirochetoses, trypanosomiases, and other tropical fevers. Rosenau and Brues[3] in 1912 announced their success in transmitting poliomyelitis from an infected to a normal *Macacus* monkey by means of the bites of stable-flies (*Stomoxys calcitrans*). Their experiment consisted in allowing many thousands of the flies to feed first on an infected monkey and immediately afterwards on a normal monkey. The transmission was mechanical, but not in the sense of an intermediary host. Anderson and Frost[4] confirmed Rosenau's finding in a

[1] Flexner, S., The mode of infection and etiology of epidemic poliomyelitis, *Am. J. Dis. Child.*, 1915, ix, 353.

[2] Flexner, The nature, manner of conveyance and means of prevention of infantile paralysis, *J. Am. Med. Assn.*, 1916, lxvii, 279.

[3] Rosenau, M. J., and Brues, C. T., Some experimental observations upon monkeys concerning the transmission of poliomyelitis through the agency of *Stomoxys calcitrans*, *Tr. XVth Internat. Cong. Hyg. and Demography*, Washington, 1913, i, 616.

[4] Anderson, J. F., and Frost, W. H., Transmission of poliomyelitis by means of the stable fly (*Stomoxys calcitrans*), *Pub. Health Rep., U. S. Mar. Hosp. Serv.*, 1912, xxvii, 332.

limited number of instances, and then later failed to obtain further positive results. Howard and Clark[5] were unable to transmit the infection from an infected to a normal monkey by the bites of *Stomoxys calcitrans*. The peculiarity of conveyance of the infection is far from being solved by the theory of transmission by these blood-sucking flies.

The most plausible hypothesis so far advanced seems to be that offered by Amoss and Taylor,[6] who, having experimentally demonstrated the existence of a protective principle in the secretion of the nasal mucous membranes of normal individuals, consider that the incidence of infection may to a considerable extent depend upon the condition of these mucous membranes; that is, as long as they remain intact, the virus will be destroyed before it can become established in the individual. The disturbance of this natural protective mechanism exposes to infection, but not the presence or absence of the virus alone. They were able to show that the protective substance in question was more generally and abundantly present in adults than in young children.

The sudden disappearance of poliomyelitic cases with the return of cold weather is often used as an argument in favor of insect transmission of the disease. Flies and mosquitoes have constantly been under suspicion. While the elimination of these insects from our households is desirable as a matter of routine cleanliness, yet without a systematic experimental investigation they should not be accepted as a factor in the epidemiology of poliomyelitis. Indeed, it would be unwise if, through a misapprehension of the facts, the attention of health officers and laity alike were to be diverted from the real source of danger.

Scope and Mode of Experiments.

The question whether a given variety of insect plays a part in the spread of poliomyelitis is within reach of exact determination, since these insects are amenable to artificial propagation through many generations. During the past several months, we have been able to collect and propagate in tanks in the laboratories a quantity of *Culex pipiens* from sewer waters near Jersey City[7] and in

[5] Howard, C. W., and Clark, P. F., Experiments on insect transmission of the virus of poliomyelitis, *J. Exp. Med.*, 1912, xvi, 850.

[6] Amoss, H. L., and Taylor, E., Neutralization of the virus of poliomyelitis by nasal washings, *J. Exp. Med.*, 1917, xxv, 507.

[7] We are greatly indebted to Dr. Headlee, chief entomologist of the New Jersey Agricultural Experiment Station, at New Brunswick, and to Dr. Chidester

Queens County, Long Island. It was our intention to study several well known genera of mosquitoes at the same time, but owing to the lateness of the season, none of the *Aedes* or *Anopheles* species were available. The only variety which we were able to obtain was *Culex pipiens*. Experiments were also performed to determine whether the common house-fly or the bluebottle fly is able to take up the poliomyelitic virus and make it increase within its body.

There are at least two totally different ways of attacking the problem. The first method is to feed young growing larvæ with the active poliomyelitic virus by putting it in the polluted water in which they are being grown. When the adult mosquitoes have hatched out, they may be allowed to feed on a normal *Macacus* monkey. The second procedure is that ordinarily practised by investigators and consists in allowing the mosquitoes to feed first on an infected animal and then, within a certain length of time, on a normal animal.

The experiments with non-biting flies had to be carried out by still another method. They were allowed to feed on the nervous tissues containing an enormous amount of the poliomyelitic virus until they pupated. Part of the pupæ were allowed to hatch. Both the pupæ and the imagos were then crushed together and the emulsion was filtered through a Berkefeld filter. The clear filtrate was introduced intracerebrally and subsequent events were observed.

Experiments with Mosquitoes Hatched in Polluted Water Experimentally Contaminated with the Virus of Poliomyelitis.

To twelve jars, covered with a wire net cage and containing mosquito larvæ of various ages in ordinary stagnant water, were added varying amounts of the brain or cord emulsions or their filtrates derived from two monkeys that died of experimental poliomyelitis. The virus was introduced every 24 hours for a period of 10 days. The temperature of the laboratory was kept at about 70°F. and the

of Rutgers College, New Brunswick, for their assistance and instruction as to the propagation of mosquitoes in the laboratory. We wish to express our gratitude also to Mr. Winchell of Rutgers College, who assisted us in collecting the specimens in the field.

relative humidity at about 40 per cent. Numbers of adult mosquitoes, both male and female, hatched out from the jars every day. They were carefully collected and used for transmission experiments on normal monkeys. The rate of hatching from day to day is shown in Table I.

TABLE I.

Date.	Female.	Male.
1916		
Oct. 31	21	20
Nov. 1	12	9
" 2	11	2
" 3	17	8
" 5	10	12
" 6	15	8
" 7	30	18
" 8	25	9
" 9	13	18
Total.....	154	104

The first transmission experiment was performed with mosquitoes on two young *Macacus rhesus* monkeys. As the mosquitoes attack the monkeys only during the night, the experiment was begun at dusk and discontinued the next morning. The results were as follows: Of 150 females and 60 males put into the cage, 114 females were fully engorged with the blood, 23 females were apparently not engorged, and 52 males were still living. Some of the mosquitoes had been killed by the monkeys.

Close observation was maintained on the monkeys for 21 days, but no sign of poliomyelitis developed nor has any been noticed up to the present time.

Some of the engorged females sooner or later laid eggs and perished, while some died without oviposition. A number of female mosquitoes hatched out of the egg boats and also from the virus-containing water were kept alive for about 3 weeks and then allowed to bite two young *Macacus* monkeys. There were about 40 well engorged specimens when examined the following morning. The monkeys showed no symptoms of poliomyelitis.

These experiments demonstrate that the mosquitoes raised in water containing the virus of poliomyelitis in large quantities cannot transmit the infection by their bites to normal *Macacus* monkeys. There is no indication that the virus enters and multiplies in the body of mosquitoes, even if 3 weeks are allowed to elapse between the time of hatching and that of the biting experiment. The offspring of these females are not capable of transmitting the disease to the monkey.

Experiments to Determine Whether the Females Well Engorged with the Blood of Infected Monkeys Will Ever Be Capable of Transmitting the Infection by Their Bites to Normal Macacus Monkeys.

In order to decide this point, eight groups of mosquitoes, some obtained from the vicinity of Jersey City, and some from Queens County, Long Island, were allowed to feed on several *Macacus* monkeys inoculated for this purpose. To insure ourselves of including different stages of the infection, feedings were made every 24 hours after the inoculation of the virus into the monkeys and were extended into the paralytic stage, which of course varied in different animals from 6 to 9 days, according to the activity of the virus and the quantity injected. The protocols of these feeding experiments show the time which elapsed between the feedings and the transmission experiments. The term "feeding mosquitoes" is used merely to denote that these mosquitoes have sucked blood from an infected monkey.

Four out of the eight groups were tested on November 20; that is, 1 to 2 weeks after the time of feeding. The remaining four groups were allowed to bite after having been kept from 2 to 3 weeks. It must be mentioned that the engorged females do not survive for any length of time after oviposition, and comparatively few specimens remained alive in the second series of groups.

For the purpose of transmission two young *Macacus* monkeys were employed for each of the two experiments (Groups 1 to 4 and 5 to 8). We also inoculated 2 cc. of sterile normal horse serum into the intrathecal space in order to derange the protective mechanism which the meninges provide against the penetration of the virus

into the central nervous system in experimental poliomyelitis in monkeys. It may be recalled that Flexner and Amoss[8] demonstrated that a fraction of the virus, intravenously introduced, will readily localize in the central nervous system when the meninges are previously disturbed by the inoculation of various substances, although the monkey may bear a multiple of such doses with impunity if there has been no previous meningeal injury.

Group 1. Feeding Mosquitoes.—Nov. 13, 1916. Adult mosquitoes hatched in the laboratory from the larvæ collected in Queens were allowed to feed on *Macacus rhesus* 1 on the 4th day of experimental poliomyelitis. 30 mosquitoes were well engorged when examined the next morning. Used for experiments on Nov. 20, or in 7 days.

Group 2. Feeding Mosquitoes.—Nov. 15, 1916. Another lot of adult mosquitoes hatched in the laboratory from the same material were fed on the same monkey (No. 1) on the 6th day of the disease. The animal showed partial paralysis of the limbs. 23 well engorged females were obtained. Used for experiments on Nov. 20, or in 5 days.

Group 3. Feeding Mosquitoes.—Nov. 16, 1916. Another lot of adults from a similar source were fed on the same monkey (No 1) on the 7th day of the disease. The animal was completely paralyzed. 17 engorged females were collected. Used for experiments on Nov. 20, or in 4 days.

Group 4. Feeding Mosquitoes.—Nov. 7, 1916. A lot of adults hatched in the laboratory from the larvæ from Queens were fed on *Macacus rhesus* 2 on the 9th day of the disease. 25 engorged females were obtained. Used for experiments on Nov. 20, or in 13 days.

Group 5. Feeding Mosquitoes.—Dec. 4, 1916. A lot of adult mosquitoes hatched in the laboratory from the larvæ secured from a Jersey City suburb were fed on two *Macacus rhesus* monkeys, Nos. 3 and 4, on the 7th day of experimental poliomyelitis. 13 engorged females were obtained. Used for experiments on Dec. 19, or in 15 days.

Group 6. Feeding Mosquitoes.—Dec. 1, 1916. A lot of adult mosquitoes hatched in the laboratory from the materials gathered in the neighborhood of Jersey City were fed on *Macacus rhesus* 5 on the 3rd day of experimental poliomyelitis. 34 engorged females were obtained. Used for experiments on Dec. 15, or in 14 days.

Group 7. Feeding Mosquitoes.—Nov. 24, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 6 on the 2nd day of the disease. 45 engorged females were obtained. Used for experiments on Dec. 15, or in 21 days.

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[8] Flexner, S., and Amoss, H. L., The relation of the meninges and choroid plexus to poliomyelitic infection, *J. Exp. Med.*, 1917, xxv, 525.

Group 8. Feeding Mosquitoes.—Nov. 25, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 7 on the 5th day of the disease. The animal had not yet become paralyzed. 20 engorged females were collected. Used for experiments on Dec. 15, or in 20 days.

The results of the experiments were negative, in spite of the fact that many females (about fifty) attacked the monkeys and became fully engorged with the blood sucked from them.

As in the earlier series of experiments, many of these engorged females oviposited and gave rise to a new generation of larvæ. It was easy to propagate them in the laboratory for an indefinite period. These offspring were in turn allowed to bite a normal monkey, but so far no positive transmission of the infection by their bites has resulted.

Experiments with Non-Biting Flies Reared in the Laboratory with an Abundant Quantity of Poliomyelitic Virus.

Non-biting adult flies may mechanically carry the virus of poliomyelitis just as they do various pathogenic microorganisms.[9] But no experimental evidence has been adduced to prove whether the virus can penetrate into the interior of the fly larvæ when the latter are fed on the poliomyelitic material, or, if it does, whether it is capable of multiplication within the body cavity of the larvæ. The question here is not the same as that of blood-sucking flies or mosquitoes, which may be the intermediary hosts as well as the direct transmitters of the virus by their bites. But in the case of non-biting flies, though the virus may multiply in their bodies, its distribution must depend upon their well known habits of gathering about food or persons while they regurgitate, resuck, and excrete. In this way the question of the non-biting flies as intermediary hosts of the virus would be quite as important as that of the biting varieties.

In the present experiment, a number of young larvæ of house-flies (*Musca domestica*) and of bluebottle flies (*Calliphora vomitoria*) were fed with the brain tissue of a *Macacus rhesus*, which had died of ex-

[9] Flexner, S., and Clark, P. F., Contamination of the fly with poliomyelitis virus. Tenth note, *J. Am. Med. Assn.*, 1911, lvi, 1717.

perimental poliomyelitis with typical symptoms and pathological findings. The activity of the material used was established in a subsequent experiment.

Group 1.—Nov. 23, 1916. About 50 house-fly larvæ were put on several slices of the fresh poliomyelitic monkey brain (No. 1). The larvæ eagerly penetrated into the nervous tissue as if trying to shield themselves from the light. At room temperature the tissue underwent a putrefactive decomposition within 24 hours, so the larvæ had to be placed on a fresh lot of the virus-containing brain. When left in the decomposed mass of tissue, many larvæ died. After being fed on the poliomyelitic brain for about 6 days, the larvæ became pupæ. After pupation, they were left with the brain tissue for several days longer and then washed from the tissue and removed to the refrigerator.

Group 2.—Nov. 28, 1916. Another lot of house-fly larvæ were similarly fed with poliomyelitic brain emulsion (No. 1) for 6 days. Pupation took place on Dec. 4. The pupæ were washed and removed to the refrigerator on Dec. 9.

Group 3.—Nov. 23, 1916. 25 larvæ of bluebottle flies were fed on several slices of brain tissue from a *Macacus rhesus* which had died of poliomyelitis. The larvæ attacked the tissue vigorously and within 24 hours seemed to have consumed a great deal of it. Unlike the house-fly larvæ, they feed on dead flesh or meat during their larval stage, but it was necessary to put them on fresh material every 24 hours in order to protect them from the deadly effect of the decomposed nervous tissue. Pupation occurred on Dec. 7. After several days, the pupæ were washed and removed to the refrigerator.

For the purpose of obtaining adult flies from the infected pupæ, a dozen specimens from each lot were put in a Petri dish and left at 80° F. Imagos hatched within about 12 days. These and the pupæ were used for the experiment described below.

Preparation of Filtrate of the Infected Pupæ and Imagos of the House-Fly and the Bluebottle Fly.

70 house-fly pupæ, Groups 1 and 2. 12 " imagos, " 1 " 2.	Weight 1.36 gm.
15 bluebottle fly pupæ, Group 3. 6 " " imagos, " 3.	" 0.97 "

These were mixed together and thoroughly ground up in a mortar, 22 cc. of 0.9 per cent saline solution being added. The milky emulsion thus obtained was briefly centrifuged at a low speed to separate the coarse particles from the fluid. The latter was then passed through a sterile Berkefeld filter V, the result being a clear and sterile filtrate.

Animal Inoculation.—A *Macacus rhesus* was inoculated intracerebrally with 3 cc. of the above filtrate on Dec. 16, 1916. The result was negative, no symptoms suggestive of poliomyelitis having been observed.

The foregoing experiment indicates that it is improbable that the virus of poliomyelitis is taken up by fly larvæ and multiplies therein. Not only does the virus fail to multiply, but probably it is rapidly destroyed within the body of the insects. The notion that these non-biting flies may act as intermediary hosts or a virus reservoir is not justified by the evidence brought out in the present study.

SUMMARY.

1. *Culex pipiens* raised from the larval stage in water experimentally contaminated with an abundance of poliomyelitic virus were found to be incapable of causing the infection when allowed in large numbers to bite normal *Macacus* monkeys.

2. *Culex pipiens* which were fed on infected poloimyelitic monkeys during different stages of the disease were found to be incapable of transmitting the infection when allowed in large numbers to bite normal *Macacus* monkeys. A previous disturbance of the meninges by an injection of horse serum into the intrathecal space did not alter the result, which was negative.

3. The offspring of the mosquitoes which were either reared in the infected tanks or fed on infected monkeys were found to be entirely harmless when allowed to feed in large numbers on a normal monkey. There was no hereditary transmission of the virus from one generation to another.

4. No trace of the virus of poliomyelitis was demonstrable in the filtrate of an emulsion of adult flies and pupæ of the common housefly and bluebottle fly which were reared in the laboratory on slices, emulsion, or filtrate of monkey brain containing the poliomyelitic virus. The intracerebral injection of the filtrate produced no poliomyelitic infection in the normal monkey.