EXPERIMENTS ON INSECT TRANSMISSION OF THE VIRUS OF POLIOMYELITIS.*

By C. W. HOWARD and PAUL F. CLARK.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

In considering the epidemiology of epidemic poliomyelitis we are confronted with a complex problem. Not only do we find cases which can be traced either through direct or indirect contact to other persons ill with the disease, but also we meet sporadic cases occurring over widely scattered areas and apparently well isolated from any acute source of contagion.

Both in human and in experimental poliomyelitis the virus has been repeatedly demonstrated in the tonsils and the secretions of the nasopharyngeal mucosa.[1] As the experimental disease can also be readily produced by swabbing the nasal mucosa with the virus, it has been held by many that the nasal mucosa is one, at least, of the sources of the virus in the outside world and also the means of its entrance to the body. The marked viability of the virus under adverse conditions, such as drying, low temperature, etc., and the fact demonstrated by Neustaedter and Thro[2] that the virus remains alive in dust, must also be considered as making for a fairly adequate explanation of the occurrence of cases in neighboring localities. More recently Kling, Wernstedt, and Pettersson[3] have brought forward experimental evidence that the nasal secretions may also be an important factor in the sporadic cases, as they claim to have found the virus in nasal washings from persons in contact with acute cases, as well as from the cases themselves.

* Received for publication, October 10, 1912.

[1] Flexner, Simon, and Lewis, Paul A., *Jour. Am. Med. Assn.*, 1910, liv, 535; Osgood, R. B., and Lucas, W. P., *idem*, 1911, lvi, 495; Landsteiner, K., Levaditi, C., and Pastia, C., *Compt. rend. Acad. d. sc.*, 1911, clii, 1701.

[2] Neustaedter, M., and Thro, W. C., *N. Y. Med. Jour.*, 1911, xciv, 813.

[3] Kling, C., Wernstedt, W., and Pettersson, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1911–12, xii, 316, 657; 1912, xiv, 303.

The fact that the seasonal incidence of the disease corresponds in general with the annual wave of insect life still suggests, however, the possibility that some insect may play a part in the transmission of this disease.

We have carried out experiments with several insects that are in close relation to human beings with reference to their ability to carry the poliomyelitic virus. Two types of insects come at once into consideration. One inhabits man's surroundings and acts as a mere contaminator of objects with which he is in intimate relation. These insects do not bite and are not to be viewed as possible active inoculators of the virus. An example of this group is the common house fly, *Musca domestica.* The other type comprises some of the biting insects. Among these are species that possess limited means of locomotion, such as the bedbug (*Cimex lectularius*) and lice (*Pediculus capitis* and *Pediculus vestimenti*), and others, such as the different mosquitos which have comparatively a much greater power of locomotion. These may conceivably act as active inoculators.

The domestic fly is by reason of its habits readily subject to gross contamination provided it can find access to infectious material. The existence of the poliomyelitic virus in the secretions of the nose and throat and also in the discharges from the intestine (Kling, Wernstedt, and Pettersson,[4] and Flexner, Clark, and Dochez[5]) makes such access possible. Whether the contamination ever occurs in nature is not known.

Our experiments were, in general, performed in two ways. In the first, insects were permitted to feed directly on infected cord or upon materials containing a suspension of infected cord. After the removal of the infected materials, the insects were placed in a fresh receptacle and after varying periods of time were killed and used whole or in part for the preparation of bacteria-free Berkefeld filtrates that were inoculated into monkeys. In this way we sought to determine whether the poliomyelitic virus survived either within the bodies of the insects or upon their external parts. In the

[4] Kling, C., Wernstedt, W., and Pettersson, A., *loc. cit.*

[5] Flexner, Simon, Clark, Paul F., and Dochez, A. R., *Jour. Am. Med. Assn.*, 1912, lix, 273.

second group, biting insects were made to feed upon monkeys inoculated with the virus of poliomyelitis. It has been shown by Flexner and Lewis[6] that the virus is present in the blood stream of infected monkeys, but only in rather minute quantities. The dilution is so great that twenty cubic centimeters or more of the blood from a recently paralyzed monkey are usually necessary to produce the disease in a normal animal. On account of this high dilution, it is especially valuable to determine whether the virus may be taken up by blood-sucking insects in sufficient quantity to produce infection when a filtrate of their bodies is employed for inoculating healthy monkeys. This experiment was carried out with bedbugs and lice.

EXPERIMENTS WITH FLIES.[7]

The flies used were the ordinary species, *Musca domestica,* bred in the laboratory. The technique of the experiment has been described above. After being allowed to feed on infected cord, the flies were placed in fresh receptacles from which certain numbers were removed at intervals, killed with ether, ground up with sand and physiological salt solution, and passed through a Berkefeld filter. The filtrate was injected as usual into the brain of *Macacus rhesus* monkeys. Protocols A and B show that the flies retained the virus either in or on their bodies for at least twenty-four and forty-eight hours respectively.

In order to determine the presence or absence of the virus actually within the bodies of the flies, the viscera of certain flies that had fed on infected cord were removed with aseptic precautions. Protocol C gives the history of such an experiment proving that the virus may remain alive in the body of the fly for at least six hours after ingestion.

Protocol A.—May 2, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 3 c.c. of filtrate from the bodies of 7 flies which had had an opportunity to feed on infected cord for 5 hours and had then lived in clean surroundings for 24 hours before they were killed. The monkey recovered from the ether anesthesia and was apparently normal.

May 8. The monkey shows slight weakness.

[6] Flexner, Simon, and Lewis, Paul A., *Jour. Exper. Med.,* 1910, xii, 227.

[7] Flexner, Simon, and Clark, Paul F., *Jour. Am. Med. Assn.,* 1911, lvi, 1717.

May 9. Animal is nervous and irritable. Right arm is weak, left arm definitely paralyzed. During the day the paralysis affected the right arm.

May 10. The monkey is completely prostrate. The animal was killed with ether. At autopsy the cord showed the characteristic macroscopic lesions of experimental poliomyelitis. The microscopic sections were also typical.

Protocol B.—May 3, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 4 c.c. of filtrate from the bodies of 10 flies which had fed on infected cord 48 hours previously. The monkey was apparently normal after recovery from ether anesthesia and remained well until May 13.

May 14. Animal is completely prostrate with paralysis of back muscles and all four extremities.

May 15. Animal was killed with ether. The autopsy showed characteristic lesions of experimental poliomyelitis in the spinal cord, and the sections, typical histological lesions.

Protocol C.—May 17, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 5 c.c. of filtrate representing the viscera of 9 flies which had had an opportunity to feed on infected cord for about 18 hours. They were then removed to a clean receptacle for 6 hours, when they were etherized, and their legs, wings, and heads removed. The viscera were then taken out with sterile instruments, ground and filtered as usual, and injected as above. The monkey carries its head towards the left side after recovery from ether and seems somewhat dazed.

May 18. The monkey is active and apparently normal.

May 29. The animal shows a definite tremor and excitability and has the characteristic worried expression. The coat is fluffed up. Cerebrospinal fluid shows marked lymphocytosis and gives a positive test for globulin. 5.00 P. M. Arms very weak. Tremor is more marked. Animal gets up on perch with great difficulty.

May 30. Animal is prostrate. Legs and back muscles are partly but definitely paralyzed. Arms are completely flaccid.

May 31. Animal is moribund. Killed with ether. At autopsy the gross lesions in the cord were characteristic and the microscopic sections were also typical.

EXPERIMENTS WITH MOSQUITOS.

The mosquito experiments were performed in much the same way as were those with flies, except that the sticky, viscid nature of the incised cord caught the mosquitos as a piece of sticky fly-paper would, so a variation in the method of bringing them in contact with the virus was adopted. A mixture of defibrinated blood and a heavy suspension of cord from a recently paralyzed monkey was injected into washed figs, and these figs were suspended in the jars with the mosquitos. In this way the mosquitos apparently took the blood-virus mixture very well. After being fed on this mixture for two days the mosquitos were placed in clean jars with non-injected figs and water and kept alive until wanted.

In two experiments the mosquitos used were the common *Culex pipiens*. In all the others the mosquitos were the salt marsh varieties, about 90 per cent. in each lot being *Culex sollicitans* and 10 per cent. *Culex cantator*.

Numbers of them were removed at intervals varying from three hours to twenty days after they had fed on the virus mixture. They were then killed, ground up, passed through a Berkefeld filter, and injected into the brain of a *Macacus rhesus* monkey. The purpose in allowing longer periods to elapse after the mosquitos had fed on the virus was to determine if possible whether there might be some biological transformation of the virus in the body of the mosquito as a secondary host. Fifteen monkeys were injected with mosquitos removed at different intervals and not one of these monkeys showed any symptoms of poliomyelitis. A control monkey inoculated with a minute portion of a fig injected with the blood-virus mixture came down typically with poliomyelitis.

Two illustrative protocols follow, showing the negative nature of the results, and also the protocol of the control animal (protocols D, E, and F).

Protocol D.—Aug. 12, 1911. Injected intracerebrally into a *Macacus rhesus* monkey 3.5 c.c. of filtrate representing the bodies of 20 mosquitos which had fed on the blood-virus mixture 24 hours previously. The animal remained normal and active.

Protocol E.—Sept. 16, 1911. Injected into the brain of a *Macacus rhesus* monkey 4 c.c. of filtrate representing the bodies of twelve mosquitos that had had an opportunity to feed on active poliomyelitis virus in figs for 2 days and had lived in clean surroundings for 20 days thereafter. The wings and legs were removed, the mosquitos ground up, filtered and injected as above. The monkey showed a slight weakness on the day following the injection but no other symptoms or paralysis.

Protocol F.—(Control of blood-virus mixture in figs.)

June 1, 1912. Injected into the brain of a *Macacus rhesus* monkey 3 c.c. of the following filtrate: On May 29 about 5 c.c. of a blood-virus mixture (2.5 c.c. of defibrinated horse blood and 2.5 c.c. of a 5 per cent. suspension of the cord from a recently paralyzed monkey) were injected into a fig weighing about 20 grams and allowed to remain at room temperature until June 1. A 5 per cent. suspension was then made of this fig and the material shaken, centrifugalized, and filtered. The filtrate was injected as above, thus introducing into the monkey filtrate which originally contained approximately 0.013 gm. of the cord.

June 6. Monkey somewhat inactive.

June 7. Slightly excitable. Arms and legs are weak, especially arms.

June 8. The arms, legs, and back muscles are paralyzed.

June 9. Found dead in the morning. At autopsy there were slight characteristic lesions in the cord and the microscopic sections were typical for experimental poliomyelitis.

EXPERIMENTS WITH LICE.

The experiments with *Pediculus vestimenti* and *Pediculus capitis* belong in the second general group; that is, the insects were allowed to suck the blood of several monkeys infected with epidemic poliomyelitis and then to remain alive for a number of days before they were killed, ground up in salt solution, filtered, and injected into monkeys. These insects proved to be difficult to manipulate, the mortality among them being very high. After their last feeding upon an infected monkey, it was found impossible to keep them alive for more than two to four days even though they were fed daily on a normal monkey. In only one experiment were we able to keep sufficient numbers of *Pediculus capitis* alive for as long as two days after their last feeding. The *Pediculi vestimenti* were used in five experiments in which they received from one to six feedings on monkeys with poliomyelitis, some in the preparalytic stage and others definitely paralyzed. The feedings were usually given either every day or on alternate days, so that in no case did any of the insects remain alive for more than thirteen days after the first feeding.

The results were entirely negative. The following are two illustrative protocols (protocols G and H).

Protocol G.—Dec. 19, 1911. Injected a *Macacus rhesus* monkey intracerebrally with 3 c.c. and intraperitoneally with 5 c.c. of filtrate representing in all 24 *Pediculi capitis* that had fed on a poliomyelitic monkey from Dec. 7 to Dec. 18. They were then made into a filtrate and injected as above on Dec. 19. The monkey showed no untoward symptoms or paralysis.

Protocol H.—Jan. 17, 1912. Injected a *Macacus rhesus* monkey intracerebrally with 2.25 c.c. of filtrate and intraperitoneally with 0.5 c.c. of filtrate representing 13 body lice which had fed on paralyzed monkeys from Jan. 9 to Jan. 13. On Jan. 15, the lice were fed on a normal monkey and on Jan. 16 they were ground up and passed through a Berkefeld filter. The monkey remained active and well.

EXPERIMENTS WITH BEDBUGS.

The experiments with bedbugs (*Cimex lectularius*) also belong to the second group and were carried out as were those with the lice, except that the greater ease of manipulation made it possible to perform a more complete series.

The bedbugs were in all cases fed at least six times on infected monkeys in various stages of the experimental disease. More of the feedings were made on monkeys in the preparalytic stage than on those in a paralyzed condition because of the probably greater concentration of the virus in the blood stream during that period. It was found impossible to induce the bedbugs to feed until the previous meal had been digested, and, in the case of the immature forms, until the skin had been molted. Hence four or five days to a week passed between the successive feedings. After the last feeding on infected monkeys, periods ranging from a few hours to twenty-five days were allowed to elapse before the insects were ground up in salt solution and filtered preparatory to injection into a normal monkey. As in the case of the other insects this period between possible ingestion of the virus and the killing of the bedbugs was designed to answer the question of a possible life cycle in the body of the insect. Since many of the bedbugs were fed more than six times, some of them remained alive from seven to nine weeks after their first feeding.

In all, sixteen monkeys were injected with filtrates from the bodies of these bedbugs, and of this number only one showed evidence of infection with the virus of epidemic poliomyelitis. A repetition of the successful experiment, however, with the same time limits, etc., proved unsuccessful.

Two illustrative protocols are given.

Protocol I.—Dec. 18, 1911. Injected into the brain of a *Macacus rhesus* monkey 3 c.c. of filtrate, and intraperitoneally 7 c.c. of filtrate representing in all 20 bedbugs that had fed on poliomyelitic monkeys on the following dates: Nov. 8, 13, 15, 20, 29, and Dec. 5.

The filtrate was made on Dec. 15; thus an interval of 10 days occurred between the last feeding and the killing of the insects. The monkey showed no symptoms or paralysis.

Protocol J.—Nov. 24, 1911. Injected intracerebrally a *Macacus rhesus* with 2.5 c.c. of filtrate representing 10 bedbugs fed on infected monkeys on the following dates: Oct. 23, 24, 27, Nov. 3, 8, 13, and 17. On Nov. 24, 7 days after the last feeding, the bedbugs were killed, filtered, and injected as above.

Nov. 30. Monkey somewhat slow. Arms are weak.

Dec. 1. Arms are partly paralyzed. The back and legs seem weak. Animal lies down most of the time.

Dec. 2. Animal moribund. Killed with ether. At autopsy the animal was found to have a severe tuberculosis of the spleen and left lung. The liver, mesenteric nodes, and right lung were also involved, but to a less degree. The right lung had only three or four tubercles. There were no definite macroscopic lesions in the cord. The microscopic sections, however, showed the typical lesions of experimental poliomyelitis.

A suspension of the glycerinated cord from this monkey was inoculated into another animal and this second animal came down typically, developing a definite paralysis on the sixth day. This animal was killed with ether. At the autopsy marked characteristic lesions were evident in the cord and the microscopic sections confirmed the result. The histological alterations in both spinal cords were characteristic of experimental poliomyelitis in the monkey and showed both infiltrative lesions about the blood-vessels in the interstitial substance and also degeneration and necrosis of ganglion cells and neurophagocytosis.

During the series of feeding experiments both with the lice and the bedbugs, the insects were sometimes allowed to feed on normal monkeys when no infected monkeys happened to be available at the time a feeding was necessary. Also during the varying intervals occurring after the last feeding on infected animals, normal animals were used in order to keep the insects alive. While this was not a definite attempt to study direct transmission by means of these insects, it is worthy of note that none of these normal animals so used ever showed the least symptom of poliomyelitis.

DISCUSSION.

The deductions from these experiments are clear. The domestic fly may become contaminated with the poliomyelitic virus which it may obtain in nature from infected discharges from the nose and throat or intestine. Because of the resistance of the virus to ordinary physical changes, especially when combined with mucous secretions, it can be transported in a living state on the surface of their bodies for two or more days, and because of the resistance it displays to digestive secretions, within the esophagus and stomach for at least several hours. The survival in the gullet permits of the contamination of objects during the feeding process through the act of regurgitation which the fly performs in moistening and dissolving its food.

The flight of the house fly is considerable. Copeman, Howlett, and Merriman[8] found marked flies from one half to three quarters of a mile from the place of their liberation. On this account these potential passive contaminators are theoretically capable of carrying and depositing the virus at a considerable distance from the point at which the original contamination occurred. Through the ordinary active habits of the fly the virus may be transferred to persons or to things with which persons come into close relation, and by the death of the flies they may through disintegration liberate surviving virus that may possibly be converted into dust. The finding of the virus in the secretions of the nose, throat, and gastro-intestinal tract of human beings suggests that through the agency of such a passive contaminator as the fly, the virus may be taken into the bodies of human beings in which, conditions favoring, it may develop and cause infection.

Our experiments tend to exclude at least the species of mosquitos with which we worked as carriers of the virus. The pediculi also seem incapable of taking the virus out of the blood or of maintaining it in a living state within their bodies.

The experiments with the bedbug have a special interest. They were not designed either to ask or answer directly the question whether the bedbug acts as the source of infection in man. The question asked was whether they (or other biting insects) could take up the virus from the blood and maintain it for a period in a living state. When it is recalled that the virus exists in a highly dilute condition in the blood, this question assumes considerable importance. Although only a single successful experiment was secured, it is none the less clear in its meaning, which is that it is possible for a blood-sucking insect both to obtain living virus from the blood and to maintain it in a living state for at least seven days,

[8] Copeman, S. M., Howlett, F. M., and Merriman, G., *Reports of the Local Government Board on Public Health and Medical Subjects,* 1911, No. 53, 1.

at which time it can be inoculated successfully into the monkey and experimental poliomyelitis produced. Since, however, the experiment succeeds so exceptionally, the bedbug would seem not to be a carrier of the poliomyelitic virus in nature, although this possibility is not entirely excluded.

CONCLUSIONS.

The domestic fly (*Musca domestica*) can carry the virus of poliomyelitis in an active state for several days upon the surface of the body and for several hours within the gastro-intestinal tract.

Mosquitos (*Culex pipiens, Culex sollicitans,* and *Culex cantator*), in our experiments, have not taken up and maintained in a living state the virus from the spinal cord of monkeys.

Lice (*Pediculus capitis* and *Pediculus vestimenti*) have not taken the virus out of the blood of monkeys or maintained it in a living state.

The bedbug (*Cimex lectularius*) has taken the virus with the blood from infected monkeys and maintained it in a living state within the body for a period of seven days.