Epidemiological and Experimental Observations on the Possible Significance of Rodents in a Suburban Epidemic of Poliomyelitis*

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IT is the purpose of this communication to record briefly preliminary observations bearing (a) on the recovery of a rodent-paralyzing virus from house mice collected in an endemic area of human poliomyelitis, and (b) on the apparent transfer of poliomyelitis virus to rodents from one of the human cases involved in this epidemic. Since the two sets of observations were made in the course of the same epidemic and the results serve to supplement each other, the pertinent data are here reported together.

I. RECOVERY OF A RODENT-PARALYZING VIRUS FROM HOUSE MICE IN ASSOCIATION WITH A LOCALIZED OUTBREAK OF HUMAN POLIOMYELITIS

Within a sharply circumscribed half square mile area in White Plains, Westchester County, N. Y., bounded by the Bronx River and a tributary brook, 5 cases of poliomyelitis occurred in the fall of 1942. The cases followed each other in rapid sequence between the end of September and the beginning of October. They were the only cases of this disease reported in the entire city for the current year, though 2 cases of poliomyelitis had been reported in this locality during the previous year. The epidemic involved 2 adults and 3 children, all of whom were treated at the Grasslands Hospital, Valhalla, N. Y. Two patients died of bulbar paralysis and 3 recovered with extensive peripheral paralyses. Postmortem examination of both fatal cases revealed severe and typical lesions in the cord and medulla.

In searching for a possible extrahuman local source of contagion among rodents, a dead gray house mouse was discovered in the basement of the home of one of the fatal cases (M.M.), shortly after the patient's admission to the hospital; the animal had obviously been dead for only a short time. A second gray house mouse was trapped in this basement on the same day. Additional house mice, dead or alive, were later collected in other parts of the epidemic area. The brains of all mice were removed and, after storage in

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glycerin for a few days, failed to cause any growth upon inoculation of ordinary bacteriological media. Saline suspensions (10 per cent) were prepared from these mouse brains and injected intracerebrally into albino mice. Definite symptoms were observed in some albino mice following injection with brain material from 3 different house mice, *i.e.*, the dead mouse and the trapped mouse found in the home in which one of the fatal cases had occurred (M.M.), and another house mouse trapped in the neighborhood.

The symptoms appeared after an incubation period of from 5 to 14 days and were characterized by ruffled fur, excitement, strong tremors, nervous movements of the forepaws, and occasional convulsions with respiratory failure. In some mice these initial symptoms progressed to paresis of both front and hind legs and terminated in death; in others, the symptoms were of a transitory character and the animals recovered. This syndrome was further transmissible in albino mice by intracerebral but not by intraperitoneal transfer of 10 per cent brain and cord suspensions from the affected mice.

While all lines of transmission originating from trapped healthy house mice came to an abrupt end after one or two laboratory passages, transmission of the infectious agent recovered from the dead house mouse has been maintained, so far, over twelve serial passages in albino mice. During the early passages the ratio between symptomless survival, convalescence, or death varied greatly from generation to generation. With subsequent passages the incubation period shortened and the described nervous symptoms became more pronounced, resulting in death of most of the injected animals and frequently in complete flaccid paralysis of one or more extremities. With later passages, mice could also be infected by the intraperitoneal route. Intracerebral injection of passage-mouse brains into rabbits, guinea pigs, or rhesus monkeys failed to produce any symptoms; however, transfer to one cotton rat and two hamsters succeeded with the third and ninth mouse brain passage, respectively, leading to prostrating paralysis in the injected rodents. Sterility tests of several mouse passage brains showed them to be free from microörganisms cultivable on ordinary bacteriological media. On the other hand, the infectious agent passed easily through a Seitz filter.

Neutralization tests were carried out with later mouse brain passages by injecting young albino mice intracerebrally with mixtures of 10 per cent virus suspensions and various sera. The results of repeated tests indicated that the serum from 2 of the 3 convalescent patients neutralized virus to an appreciable extent (90 per cent survival among the injected mice), whereas neutralization was less marked with the serum from one of these patients collected during the early stages of the disease (60 per cent survival). Definite neutralization was also obtained with normal adult mouse serum (90 per cent survival), but not with normal rabbit serum (45 per cent survival). Pathological examination of mice which presented the fully developed paralytic disease showed extensive destruction of the anterior horns in various levels of the spinal cord, recalling the picture of "rodent poliomyelitis." In other words, the lesions were generally similar to those observed in mice suffering from Theiler's disease,[1] or in mice paralyzed by infection with either the mouse-adapted Lansing,[2] or the SK [3] murine strains of human poliomyelitis virus.

The exact nature of this virus is not known at present. In view of its relatively low pathogenicity for albino mice, it has not been possible accurately to titrate potency, determine limits of

filterability, study various routes of invasion, investigate the range of susceptible hosts, or carry out more extensive neutralization tests. However, the filterability of the infectious agent, together with the symptomatology and pathology of the disease which it produces in albino mice, may be taken as suggestive evidence that we are dealing with a virus of the poliomyelitis group, perhaps identical with or related to the FA strain of Theiler's virus of "spontaneous mouse encephalomyelitis."[4] This conclusion would be strengthened by the fact that a small group of mice which had survived this virus infection resisted intraperitoneal reinfection with a standard strain (GD VII) of Theiler's virus, whereas normal control mice developed paralysis. It should be noted, however, in this connection that heretofore Theiler's virus has not been encountered in healthy gray house or field mice (brain or intestines) trapped at a distance from the laboratory.[5] Final identification of the described infectious agent and the relationship, if any, which may exist between its local carriage by house mice and the synchronous outbreak of human poliomyelitis are problems which are at present under investigation.

II. ISOLATION OF A RODENT-PARALYZING VIRUS BY PASSAGE OF HUMAN POLIOMYELITIS VIRUS TO SYRIAN HAMSTERS, EASTERN COTTON RATS AND ALBINO MICE

Attempts to corroborate the clinical and pathological diagnosis of poliomyelitis in the 2 fatal cases referred to above were made by tests in rhesus monkeys. Intracerebral injection of a monkey with brain and cord material from one of the fatal cases (T.D.) failed to produce any symptoms. However, prostrating paralysis, with typical cord lesions, occurred in another monkey following intracerebral injection with a suspension from cord and medulla of the second fatal case (M.M.). Intracerebral transfer of this monkey cord to another monkey failed to transmit paralysis but caused death in a hamster on the 20th day after injection. At the same time the original human material was also injected intracerebrally into other laboratory animals, i.e., 1 rabbit, 3 guinea pigs, 19 albino mice, 2 cotton rats, and 1 hamster. None of the injected animals showed any definite signs of disease, except for the hamster, which appeared sick on the 18th day after injection and was found dead the next morning. Intracerebral transfer of this hamster's brain to another hamster caused in the latter animal complete flaccid paralysis of both hind legs within 5 days. Further intracerebral passage of the paralyzed hamster's brain to 2 new hamsters, 2 cotton rats, 5 albino mice, 1 rabbit, and 1 rhesus monkey was followed by prostrating paralysis, within 2 to 3 days, in all injected small rodents. The monkey developed a transient fever but remained free from recognizable paralysis; no symptoms were observed in the rabbit. From this point it was possible to transmit the disease serially through passages of hamsters and mice, using both intracerebral and intraperitoneal routes of injection.

The infectious agent proved filterable through Seitz filters; it passed readily through graded collodion membranes of 50 mu APD and with considerable difficulty through membranes of 26 mu APD, but was completely retained by membranes of 10 mu APD. The probable size of the virus particle may therefore be assumed to lie in the neighborhood of 10 mu. Of special interest is the enormous potency and invasiveness of this virus. In both intracerebral and intraperitoneal tests dilutions between 10^{-3} and 10^{-5} were capable of inducing paralysis in hamsters, while dilutions as high as 10^{-11} sufficed to paralyze albino mice, by either intracerebral or intra-

peritoneal injection, within an incubation period of from 24 hours to 5 days.

Pathological examination of paralyzed hamsters and albino mice revealed the presence of typical and severe poliomyelitic lesions in the entire central nervous system. Neutralization tests were carried out by injecting albino mice intraperitoneally with mixtures of mouse passage virus and various sera. The results of repeated tests may be summarized as follows: A high degree of virus neutralization was obtained with a rabbit antiserum against Theiler's virus (1 million paralytic doses) and with a rabbit antiserum against SK murine virus (10,000 paralytic doses)[6]; a moderate or slight degree of virus neutralization occurred with an antipoliomyelitis (monkey virus) hyperimmune horse serum (100 paralytic doses) and with three convalescent sera collected from surviving patients in this epidemic (10 paralytic doses). Control sera which failed to neutralize the virus at the indicated levels were normal horse, rabbit, and hamster serum.

From what has been said before, it is clear that the cord and medulla of the fatal case (M.M.), upon direct inoculation, was capable of causing paralysis in one rhesus monkey only. It also appears that the human material was primarily non-pathogenic for cotton rats and albino mice, but that intermediary hamster passage had evidently brought about some change in the virus which made it possible subsequently to maintain multiple paralytic passages in several species of rodents, including hamsters, cotton rats, and albino mice.

The above facts, while suggesting that a direct transfer of poliomyelitis virus was obtained from man to hamster—with further transfer from hamster to cotton rats and white mice—are admittedly insufficient actually to prove such transmission. The possibility that accidental contamination may have occurred with a latent virus, or with a virus carried in the laboratory, cannot be ignored. However, the circumstances under which these observations were made and the trend of the available data would tend to minimize the chances for such a contamination. It is hoped that further work, which is in progress, will help to clarify not only this problem but make it possible to pass final judgment on the identity or non-identity of the two paralyzing viruses isolated from man and mouse in the course of this epidemic.

CONCLUSIONS

A rodent-pathogenic virus which paralyzes albino mice, cotton rats, and hamsters was isolated from the brain of a house mouse found dead in the home of a fatal case of human poliomyelitis. Another rodent-pathogenic virus capable of inducing paralysis in albino mice, cotton rats, and hamsters was isolated from the brain stem of this fatal case. The two viruses, when passaged in albino mice, are similar in that both are completely inactivated by antisera against Theiler's virus of mouse encephalomyelitis and show some neutralization with convalescent sera from patients involved in this epidemic. They differ however markedly in virulence for albino mice in that the human virus is much more potent than the mouse virus.

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