THE RAT AND POLIOMYELITIS: AN EXPERIMENTAL STUDY.

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(Received for publication, July 1, 1918.)

Under the title of "The rat and infantile paralysis: a theory," Richardson has brought together such hypothetical data in favor of the rat and its parasite, the flea, as the active agents in the transmission of poliomyelitis as he could assemble. He has also brought these evidences to bear on the epidemiology of the disease and has concluded that:

"In the transfer of the infection from the rat to man, the agency of the flea is assumed, although the possible contamination of food by rodent excretions might well be considered. The insect transfer might well be simply mechanical or it might require a preliminary cycle of development of the virus in the flea. The foregoing theoretical considerations have been put forward as explaining better than any other hypothesis as yet submitted, the epidemiological facts as observed in infantile paralysis."

Richardson further states that extended experimental investigation will be necessary to determine the validity of his theory.

Various kinds of animals have from time to time been incriminated in the spread of epidemic poliomyelitis. Perhaps the one most often cited is poultry because of the paralytic disease, limber-neck, to which they are subject. Doty in his epidemiological report of the New York epidemic of 1916 gives consideration to and excludes from account the rat as an agent of transmission of the disease, and he quotes Professor L. O. Howard to the effect that no marked epizootics among rats have been noted in connection with the larger epidemics of infantile paralysis.

Along with the failure to discover a reservoir of the causative microorganism outside man, by inoculating the nervous tissues from animals suspected to have suffered from poliomyelitis into monkeys,
unsuccessful attempts have been made to induce paralysis in various kinds of animals, including the white rat, by direct injection of infected nervous tissues into the brain. It is, however, not evident that any systematic effort has been made to detect the virus of poliomyelitis in the rat or to discover whether, when experimentally injected, it is capable of surviving in that animal. Richardson refers in a parenthesis to a statement by Rosenau that while he has apparently been able to produce paralytic disease in rats by inoculation of the virus, yet the experimental data are not sufficiently advanced to justify conclusions. As no confirmation of this important point has been offered in the 2 intervening years, we may assume that convincing evidence has not been obtained.

EXPERIMENTAL.

Our experiments have taken two directions. In the first place, we undertook to transmit poliomyelitis to monkeys by employing for inoculation the central nervous organs of wild rats caught in the localities in Brooklyn in which numerous cases of the epidemic disease prevailed in the summer of 1916. Through the cooperation of the Deputy Commissioner of Health, Dr. John S. Billings, we secured a considerable number of rats. The animals, trapped in cages, were chloroformed and the central nervous and other organs carefully removed and preserved in 50 per cent glycerol in the refrigerator. In the next place, we injected active poliomyelitic virus (suspensions of brain and spinal cord of paralyzed monkeys) into the brain of white rats. As pressure effects which may cause death in a few hours are readily produced, several rats are injected at once. The purpose was to determine whether the virus is capable of surviving in the brain of the rat for any length of time even though poliomyelitis is not induced. Should the virus be quickly destroyed, the conclusion would be justified that the rat organism is not favorable to the virus, from which the further deduction, that this animal improbably serves as a reservoir of the virus in nature, might be made.

Rosenau, M. J., cited from Richardson, p. 400.
Series I. Inoculation of Organs of Rats into Monkeys.

Experiment 1.—April 11, 1917. 10 per cent suspension was made of the brain and spinal cord of Rats 1, 2, and 3 collected in Brooklyn. 2 cc. of the suspension were injected intracerebrally and 15 cc. intraperitoneally into a Macacus rhesus. April 25. No symptoms having appeared, the inoculations were repeated in the same manner and with a 10 per cent suspension prepared from the nervous organs of the same rats. No symptoms whatever developed.

Experiment 2.—April 11, 1917. 10 per cent suspension was made of the brain and spinal cord of Rats 4, 5, and 6 obtained through Dr. Billings. 2 cc. were injected intracerebrally and 15 cc. intraperitoneally into a Macacus rhesus. April 25. No symptoms having developed, the injections were repeated with a 10 per cent suspension prepared in the same manner from the nervous organs of the same rats. The animal never showed symptoms.

Experiment 3.—April 11, 1917. The spleen, liver, and kidney of Rats 1, 2, and 3 were made into a 10 per cent suspension. Of this, 2 cc. were injected intracerebrally and 12 cc. intraperitoneally into a Macacus rhesus. April 25. No symptoms having appeared, the inoculations were repeated in the same manner and with a 10 per cent suspension prepared from the same viscera. No symptoms developed subsequently.

The significance of this experiment is obvious. Materials from six rats collected in Brooklyn in the vicinity in which the epidemic of poliomyelitis was severe, were injected into three Macacus rhesus monkeys under conditions sufficing to incite infection in these animals provided the poliomyelitic virus had been present in the internal organs in any considerable amount and of any real virulence. Moreover, not only did the monkeys fail to respond to a single inoculation of large quantities of the organs of the rats, but they failed equally to react to a second injection of this material made 2 weeks later. It may therefore be concluded that none of the six rats tested carried demonstrable amounts of the poliomyelitic virus.

The next tests are a repetition of those carried out by Amoss on the power of survival of an active virus of poliomyelitis when injected into the brain of rabbits. The tests had been made in the course of a critical examination of the contentions of Rosenow and

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his coworkers and Nuzum and his associates that poliomyelitis may be induced in rabbits by means of streptococci. Amoss found that the virus of poliomyelitis when injected into the brain of rabbits did not survive there as long as 7 days. The rabbit was therefore regarded as unfavorable to the mere presence within its body of the true virus of poliomyelitis although permitting, on the contrary, streptococci both to survive and multiply in its organs.

Series II. Survival of the Virus in Rats.

Experiment 4.—Under ether anesthesia 0.15 cc. of a suspension of equal parts of active glycerolated monkey virus (spinal cord) and isotonic salt solution was injected intracerebrally into each of four white rats. Two died from pressure within 24 hours. The remaining two were observed for 7 and 14 days respectively and then etherized. Neither developed any symptoms, and on autopsy showed any lesions at the site of inoculation. In each instance the brain tissue surrounding the site of inoculation was made into a suspension with equal parts of isotonic salt solution and injected intracerebrally into a Macacus rhesus monkey. No symptoms of poliomyelitis developed.

Experiment 5.—Five white rats were given intracerebral injections of 0.1 cc. of a 50 per cent suspension of active virus in the manner described in the previous experiment. Four succumbed to pressure, and only one survived. The rat which survived and which had shown no symptoms was etherized on the 4th day and the excised site of inoculation in the brain suspended in 2 cc. of isotonic saline solution and injected intracerebrally into a Macacus rhesus. The monkey remained well. Because of the brief period of time elapsing between the inoculation of the rat and of the monkey, this experiment was repeated, with the same result.

The tests thus far conducted indicated that the virus of poliomyelitis was incapable of surviving as long as 4 days in the brain of the rat. The question arose whether this result might not be accounted for not by the rapid destruction of the injected virus, but by the small amount which was introduced; in other words, whether the minimal quantities of virus, capable of being injected into the brain without causing pressure effects, were not below the infecting dose when transferred with the rat's brain tissue to the monkey. As a matter of fact 0.1 to 0.15 cc. of the 50 per cent suspension of the spinal cord contains from 10 to 50 minimal lethal doses of the virus for monkeys.

according to its initial potency. However, the next experiment was
designed to answer this question and it showed that the failure to
infect is not due to subminimal doses of virus introduced into the rat.

Experiment 6.—A white rat was given an intracerebral injection of 0.1 cc. of
a 50 per cent suspension of the virus. 1½ hours later, and after the recovery from
ether, the animal was killed with ether and the brain removed. The site of
inoculation was excised, suspended in isotonic salt solution, and injected intra-
cerebrally into a *Macacus rhesus*, of which the following is the history:

May 20, 1918. Received intracerebrally 1 cc. of the suspension described.
May 26. Somewhat excited. May 27. Tremor, ataxia, weakness of left and
complete paralysis of right arm. May 28. Prostrate, etherized.

The autopsy showed typical lesions of poliomyelitis confirmed by microscopic
examination of the medulla, spinal cord, and intervertebral ganglia.

DISCUSSION.

The above experiments bring out a remarkable similarity between
the tests on the rat and those made by Amoss on the rabbit. Indeed,
it now appears that the rat is an even more unfavorable host for the
virus than is the rabbit, since in the former animal an effective dose
of the virus for the monkey was no longer detectable at the expiration of 4 days. This fact is hardly consistent with the theory that
the rat constitutes a natural reservoir in nature of the virus of polio-
myelitis. Rather the experiments would indicate that the rat’s
organism is wholly unadapted to its multiplication and survival.

CONCLUSIONS.

The central nervous organs and other viscera of six rats, collected
in a district in Greater New York in which many cases of epidemic
poliomyelitis occurred, have been proved incapable of inciting, on
inoculation, experimental poliomyelitis in *Macacus rhesus* monkeys.

The virus of poliomyelitis injected into the brain of white rats
does not survive there as long as 4 days in a form or in amounts
sufficient to cause infection when inoculated intracerebrally into
monkeys.

The failure of the virus injected into the brain of rats to incite in-
fec tion in monkeys is not due to the quantity introduced, since at the
expiration of 1½ hours after the injection, the excised inoculation site when injected into the monkey caused typical experimental poliomyelitis.

It does not appear probable, therefore, that the rat acts in nature as the reservoir of the virus of poliomyelitis.