

## SURVIVAL OF THE VIRUS OF POLIOMYELITIS FOR EIGHT YEARS IN GLYCEROL.

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The occurrence of epidemics of poliomyelitis raises the question of the length of time which the etiological agent or virus of the disease will survive outside of the animal body. The power of this virus to resist adverse conditions is of interest not only because of its relation to the natural history of the disease, but also in view of the possible application of such information to experimental methods. It was therefore considered worth while to record an instance of the survival of poliomyelitis virus for 8 years in glycerol, particularly since the infectivity of the material remained almost unchanged during this period.

The mechanism responsible for the epidemic nature of the disease has aroused considerable speculation. Flexner and Amoss (1) have pointed out the great variation in the infecting power of a strain of virus much used in experimental work. They (2) suggested that the ability of the virus to survive a long time in mild antiseptics might indicate that a recrudescence of poliomyelitis in a given locality was due to renewed activity of virus which had been dormant since a previous epidemic.

Many attempts have been made to immunize monkeys against poliomyelitis by the use of attenuated virus. The methods used to decrease the infectivity of the material, while preserving its antigenic power, have usually been based on the use of chemical or physical agents, such as phenol, glycerol, heat, and drying. The results have almost always been disappointing, due to the fact that a certain number of animals contracted the disease during the treatment designed to immunize them against it. Such failures were probably caused by the extraordinary resistance shown by some samples of

virus. The persistence of infectivity after long exposure to glycerol is an indication of the hazard of attempts to immunize human beings by the use of virus attenuated by the ordinary methods.

The possibility of exposing poliomyelitis virus to mild antiseptics over long periods is important also from its application to the views of those investigators who consider that the disease is due to a streptococcus. Although Long (3) has cultivated streptococci from pieces of brain tissue which had been kept in 50 per cent glycerol for 303 days, it seems improbable that the organisms could survive in the chemical for a period of 8 years. Needless to say, the material used by us was cultured and streptococci proved absent before it was inoculated into animals.

The first report of the survival of poliomyelitis virus in glycerol is that of Flexner and Lewis (4), published in 1910. These investigators kept the material in 50 per cent glycerol for 7 days and then found it infectious for a monkey. In the same year, Römer and Joseph (5) stated that they had produced poliomyelitis in monkeys with material which had been kept in 100 per cent glycerol for 5 months. 4 years later, in 1914, Flexner, Clark, and Amoss (6) described an experiment in which spinal cord was kept 25 months in 50 per cent glycerol, after which it produced the disease when inoculated in a monkey. Flexner and Amoss (7), in 1917, recorded the successful transfer of virus which had been in glycerol for 6 years. In this case, repeated inoculations of large doses were required to infect the animal, and the authors felt that there had been a definite decline in virulence of the virus.

In the course of investigations on poliomyelitis at The Rockefeller Institute, specimens of the central nervous systems of monkeys which succumb to the disease are removed under rigid aseptic precautions, cut in pieces about 1 cm. in diameter, and placed in a considerable volume of 50 per cent glycerol. Tightly stoppered glass bottles are used and the stopper covered with tin-foil to prevent evaporation. The specimens are kept in the refrigerator at a temperature of approximately 4°C. The glycerol used is free from oxalates and sulfates.

The material employed in the following experiment was a mixture of two strains (M. A. and K.) which had been used for a long time to produce experimental poliomyelitis in monkeys (8). The animals from which the virus was obtained were prostrate on or before the 7th day after inoculation. Spinal cords from seven different monkeys were pooled before grinding and inoculating.

*Experiment 1.*—October 31. Monkey (*Macacus rhesus*) inoculated intracerebrally<sup>1</sup> with 1.5 cc. of a 20 per cent suspension of pooled 1920 mixed virus. November 8, excitement, tremor, and well marked ataxia were noted, with a tendency to fall when jumping about the cage. There was weakness of arms and legs, with inability to raise the arms above the head. November 9, the animal was prostrate, unable to move either arms or legs, and the respiration was barely perceptible. The monkey was killed by ether and the central nervous system removed under aseptic precautions.

Macroscopic examination: The cord was rather soft and edematous. Tiny, punctate, hemorrhagic areas were scattered through the cord and medulla.

Microscopic examination: The meninges showed a slight infiltration with lymphocytes and endothelial cells. Perivascular infiltration with lymphocytes was noted in the cord and medulla. Various degrees of degeneration of nerve cells of the cord were seen, particularly marked in the anterior horns. Here many examples of active neuronophagia were found, and in some places a polymorphonuclear reaction was present around necrotic nerve cells. Some increase in glia elements was noted. The intervertebral ganglia showed diffuse lymphocytic infiltration and some nerve cell degeneration.

*Experiment 2.*—November 9. Monkey (*Macacus rhesus*) was inoculated intracerebrally with 1 cc. of a fresh 5 per cent suspension of spinal cord and medulla of the previous monkey. November 12, the animal was slow in its movements about the cage, was somewhat excited, and showed definite ataxia. November 13, weakness of both arms and legs was observed, and the ataxia was more marked. November 14, the condition was slightly more marked. November 15, there was little change. November 16, the animal was much more excited and weaker, and both arms were paralyzed. November 17, the monkey was found prostrate and moribund. It was promptly killed with ether and an autopsy performed.

Macroscopic examination: The cord was somewhat injected and bulged from edema when cut. Scattered, small, soft hemorrhagic areas were found unevenly distributed throughout its length and the medulla.

Microscopic examination: Fairly well marked lymphocytic reaction was present in the meninges. The blood vessels were congested. Perivascular infiltration with lymphocytes was present throughout the cord and medulla. The nerve cells of the cord showed marked degenerative changes, ranging from slight to complete dissolution. A sharp reaction of lymphocytes and mononuclear cells about the degenerated nerve cells was present. The intervertebral ganglia showed diffuse lymphocytic infiltration.

The virus was passed through three more animals in series, with gradually decreasing doses. All the animals were prostrate within 8 days, even when injected with a dose as small as 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension. The gross and microscopic changes, as well as the symptoms produced, were in all instances characteristic.

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<sup>1</sup> All monkey inoculations were carried out under ether anesthesia.

## DISCUSSION.

In a paper by Flexner and Amoss, in which the successful transmission of poliomyelitis material kept for 6 years in glycerol was described, appreciable difficulty was experienced in obtaining infection in the first monkeys inoculated. A considerable length of time intervened between the introduction of the nervous tissues and the development of symptoms. In several instances, repeated injection of virus was required to produce the disease. These facts were recorded in order to emphasize the long survival as well as reduction in activity of the virus following the long glycerolation. In view of the results described in this paper, in which the virus has been shown to maintain maximal potency after glycerolation for 8 years, the attenuating action of the chemical may well be questioned. That the specimens of virus should have retained the original high infectivity so that on the first inoculation of the glycerolated tissues the monkeys should have developed typical symptoms and lesions of experimental poliomyelitis is not only an unusual occurrence but perhaps one of epidemiological significance.

## CONCLUSIONS.

1. An instance of successful inoculation of poliomyelitis virus after preservation for 8 years in 50 per cent glycerol is reported.
2. The virulence of the material injected remained essentially unchanged during this period.
3. The fact that poliomyelitis virus will survive in glycerol for so great a period may be taken as further indication of the improbability of streptococci as the inciting organisms.
4. Poliomyelitis virus would seem to vary in its resistance to glycerolation.
5. The remarkable persistence of active virus outside of the body may have a bearing on the epidemiology of poliomyelitis.

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