SURVIVAL OF POLIOMYELITIC VIRUS IN THE BRAIN OF THE RABBIT.

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The consensus of opinion among investigators in America and Europe is that the causative microorganism of poliomyelitis is a so-called filter passer. The filterable organism or virus of poliomyelitis possesses definite properties through which it may be identified, and among these the most decisive is its ability to incite experimental poliomyelitis in the monkey. In common with other filter passers, the microbic cause of poliomyelitis is very minute. It has not been detected with certainty by microscopic examination in infectious filtered fluids. There are reasons for believing that in artificial cultures the microorganism gives rise to colonies visible to the naked eye and composed of masses of minute organisms which have been called globoid bodies. Similar globoid bodies have been detected in microscopic preparations of the nervous organs and once in the circulating blood of an infected monkey.

Another point of view, emphasized more recently, is based on the studies of Mathers, Rosenow, Nuzum, and their associates. According to these investigators, epidemic poliomyelitis is caused by a polymorphous streptococcus, which induces paralysis and histological changes characteristic of the disease in rabbits as well as in monkeys.

3 Amoss, H. L., J. Exp. Med., 1914, xix, 212.
Since the filtered virus of poliomyelitis, while highly active against monkeys, is practically without pathogenic power for rabbits, a wide difference of experimental results needs to be explained in order to bring the two points of view into harmony.

Bull,⁵ in this laboratory, attempted to confirm the work referred to with the polymorphous streptococcus, but without success. He carried out a large series of inoculations of streptococci derived from poliomyelitic human and monkey tissues without, in a single instance, either in rabbits or monkeys, inducing clinical symptoms or pathological lesions identifiable with those of epidemic poliomyelitis. Moreover, his efforts to immunize animals with the streptococcus so as to obtain a neutralizing serum for or to protect them against infection with the filtered virus, as Rosenow claims to have done, were wholly unsuccessful. More recently, Bull has again tried, unsuccessfully, to render a monkey immune to the virus by large intravenous injections of streptococci cultivated from the brain of a poliomyelitic human case. The protocols of this experiment follow. The question, therefore, arises as to the source as well as the significance of the streptococci found not infrequently in poliomyelitis. Smillie⁶ found that when the cultures are made from monkeys moribund and slowly dying, or from animals which have been dead some hours, streptococci are frequently present, not only in the nervous organs, but even more abundantly in the abdominal viscera. In other words, the streptococci exhibit the characters of secondary, agonal invading microorganisms. The unreported experiment of Bull with streptococci follows. Macacus rhesus monkeys were used.

**Monkey A.**—Apr. 25, 1917. Injected intravenously the centrifuged sediment from a 24 hour growth in 60 cc. of ascitic dextrose broth of the third generation of streptococcus obtained from human poliomyelitic brain. The monkey remained active. Apr. 27. Injected intravenously the growth from 56 cc. of the same medium, third generation of the same streptococcus, and intracerebrally the growth from 14 cc. of the same culture. The monkey became irritable, but remained active, and was normal on May 1 when another intravenous injection from 60 cc. of the third generation culture was made. May 5. A fourth injection was given.

This monkey, the serum of which was shown on May 22 to agglutinate the strain of streptococcus in a dilution of 1:4,000, was tested (a) for neutralizing action on the filtered poliomyelitic virus, and (b) for protection against an intracerebral inoculation of the same virus.

**Monkey B. Neutralization Test.**—May 22. 2 cc. of serum from Monkey A were mixed with 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic monkey cord (active virus), incubated for 2 hours at 37°C., and placed

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Autopsy.—Typical gross and microscopic lesions of poliomyelitis were present in the central nervous organs.

Protection Test of Monkey A.—May 22. After blood was withdrawn for the serum an intracerebral injection of 0.5 cc. of active virus was made. May 27. Monkey excited and ataxic. May 28. Prostrate. May 29. Died.

Autopsy.—Typical poliomyelitic lesions were present in the central nervous system.

EXPERIMENTAL.

In order to study further the relation of the filterable virus of poliomyelitis to the rabbit, with the special view of bringing out resemblances to or distinctions from the streptococcus and of determining its power of survival in the brain in vivo, inoculations of the virus were made into the brain of that animal. There was no expectation of inducing infection or of setting up paralysis. Bull injected streptococci isolated from the human poliomyelitic brain intravenously into a rabbit without producing symptoms. The rabbit was etherized after 131 days. Streptococci corresponding antigenically with the strain originally injected were found to have survived in the brain. Bull also observed that local injections of streptococci from poliomyelitic tissue sometimes produce focal lesions in which the organisms survive for long periods. In other words, the polymorphous streptococcus is, under certain conditions, sufficiently adapted to the central nervous system of the rabbit to survive there, and sometimes sufficiently pathogenic to produce focal lesions in the meninges, cerebellum, medulla, cerebrum, and even in the spinal cord, and to thus induce clinical symptoms. The lesions do not, however, partake of the nature of the characteristic lesions of poliomyelitis.®

Hence, if a relation exists between the polymorphous streptococcus and the filterable virus, the latter might at least be expected to exhibit a fair degree of ability to survive in the brain of the rabbit. As the protocols which follow show, the period of survival is short.

Unpublished experiment.
Survival of Poliomyelitic Virus in the Brain

Rabbit A.—Nov. 21, 1916. Under ether anesthesia 0.5 cc. of a suspension of equal parts of active poliomyelitic monkey cord and isotonic salt solution was injected intracerebrally. The rabbit remained well. Dec. 14. The animal was etherized and the brain removed aseptically. There was no visible lesion at the site of inoculation. A 10 per cent suspension of the brain tissue from the region below the point of needle penetration through the skull was prepared for injection into a Macacus rhesus (Monkey C).

Monkey C.—Dec. 14, 1916. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit A, which had received the poliomyelitic virus 22 days previously. The monkey remained well.

Rabbit B.—Jan. 3, 1917. Injected heavy suspension of poliomyelitic virus according to the method already described. Jan. 16. Killed. There was no visible lesion at the site of inoculation. From the brain substance around the site of inoculation, a 10 per cent suspension was prepared for injection into a Macacus rhesus (Monkey D).

Monkey D.—Jan. 16, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of brain at site of inoculation of Rabbit B, which had received poliomyelitic virus 12½ days previously. The monkey remained well.

Rabbit C.—Mar. 5, 1917. Injected intracerebrally heavy suspension of poliomyelitic virus according to the method already described. The rabbit showed no symptoms. Mar. 12. Killed. There was no visible lesion at the site of inoculation. The brain was removed aseptically and a 10 per cent suspension of the brain substance around the site of inoculation was prepared for injection into a Macacus rhesus (Monkey E).

Monkey E.—Mar. 12, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit C, which had received poliomyelitic virus 7 days previously. The monkey remained well.

Rabbit D.—Apr. 3, 1917. Injected intracerebrally a heavy suspension of active poliomyelitic monkey cord. The rabbit remained well. Apr. 7. Etherized and brain removed aseptically. No visible lesion at site of inoculation. A 10 per cent suspension was prepared from the brain tissue at the site of inoculation and injected intracerebrally into a Macacus rhesus (Monkey F).


Autopsy.—The central nervous organs showed macroscopic and microscopic lesions of poliomyelitis.

Discussion and Summary.

Suspensions of the central nervous tissues of monkeys, containing the active filterable virus of poliomyelitis, may be injected into the
brain of rabbits without setting up symptoms, provided the volume of
injection does not cause dangerous increased intracranial pressure.

Aside from the pressure effects which develop quickly, no other
symptoms or pathological lesions are produced by the suspensions.

The active virus of poliomyelitis survives in the brain of rabbits
for 4 days, as determined by tests in the monkey, into which the
excised site of injection in the rabbit brain is reinoculated. It cannot
be detected by this test after the expiration of 7 days.

The virus of poliomyelitis is unadapted to the rabbit, and neither in-
duces lesions nor survives long in the central nervous organs of that
animal. In this respect it differs from certain streptococci culti-
vated from poliomyelitic tissues.

A monkey immunized to streptococcus cultivated from human
poliomyelitic nervous tissues yielded a serum which agglutinated the
streptococcus in high dilution, but was without neutralizing action on
the filtered virus; and the streptococcus-immune monkey was not
protected against the effects of an intracerebral inoculation of the
filtered virus.

The experiments recorded provide additional reasons for conclud-
ing that the streptococcus cultivated from cases of poliomyelitis
differs essentially from the filterable virus and is not the microbic
cause of epidemic poliomyelitis.