SURVIVAL OF THE POLIOMYELITIC VIRUS FOR SIX YEARS IN GLYCEROL.

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Plates 40 to 43.

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The reappearance of poliomyelitis in severe form in the United States during the summer and autumn of 1916 has revived the question of the conditions under which the microbic cause, or virus, of the disease survives outside the human body. The problem of the source of the epidemic is still unsolved: whether, for example, the virus was newly imported or merely a survival from previous epidemics of the disease. We are not now in a position to answer this question. Our present knowledge indicates that the virus does not multiply in nature outside the human body, but it is possible that it may survive upon human carriers or elsewhere without actually multiplying.

The question of the power of the virus to resist extraneous influences has been sharpened by recent publications connecting the streptococcus with the etiology of poliomyelitis. Experience has shown that streptococci in animal tissues, for example, do not retain viability over long periods of time. We ascertained early in the experimental investigation of poliomyelitis that the virus in the central nervous tissues resists glycerolation,[1] and later we reported instances of survival after 25 months' immersion in glycerol.[2] In the course of investigations on poliomyelitis conducted at The Rockefeller Institute, specimens of nervous tissues from human beings and monkeys, the subjects of poliomyelitis, have been regularly put aside in 50 per cent glycerol in the refrigerator, the temperature of which has been

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

[2] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 205.

kept at approximately 4°C. Some of the tissues were set aside in 1910, and others later. We have recently tested certain specimens for activity and ascertained that monkeys may be infected with samples 6 and 4 years old.

The spinal cord and medulla were cut into small cubes which were immersed in a large excess of 50 per cent glycerol previously sterilized in the autoclave. When portions were removed for purposes of inoculation, tests were made for bacteria by imbedding fragments of the tissue in deep tubes of glucose-ascitic-fluid-agar and inoculating the tissue on the surface of tubes of that medium. The tubes were incubated for several days. On microscopic examination no growth was discovered.

Experiment 1.—Human spinal cord and medulla kept in glycerol since August, 1911, and September, 1912. Fragments from the two specimens were combined for inoculation. Hence the more recent sample of tissue had been put aside more than 4 years before. Dec. 7, 1916. A *Macacus rhesus* was given an intracerebral injection, under ether anesthesia, of a heavy suspension of the tissues. Dec. 11. 3 cc. injected into the peritoneal cavity. Jan. 4, 1917. No symptoms. A fresh suspension was prepared of the same tissues, of which 3 cc. were given intracerebrally and 5 cc. intraperitoneally. Jan. 17. Excitement; ataxia; tremor of head; facial asymmetry; weakness of right deltoid. Jan. 22. The symptoms have advanced slightly. Jan. 27. Injected 2 cc. of suspension intracerebrally and 5 cc. intraperitoneally. Jan. 31. Symptoms slowly increasing. Etherized for histological examination and for purposes of reinoculation.

Histology.—Lesions occur in the medulla, spinal cord, and intervertebral ganglia. Those present in the medulla consist (1) of lymphocytic infiltration of the vessels in the meninges and (2) of slight perivascular infiltration of the superficial internal vessels. There is no general lymphocytic infiltration and no necrosis of nerve cells. The lesions in the spinal cord are focal. The cervical cord shows diffuse lymphocytic infiltration, necrosis of anterior horn nerve cells, and a small degree of neurophagocytosis. The blood vessels of the gray matter are moderately infiltrated with mononuclear cells. The lumbar cord is the seat of marked perivascular infiltration affecting chiefly the gray matter, but the meningeal vessels are also heavily surrounded (Fig. 1). The anterior gray matter is diffusely, but not severely, infiltrated with lymphocytic cells and no neurophagocytosis is present in the sections studied. The intervertebral ganglia show slight infiltration with lymphocytes and no nerve cell degeneration.

Experiment 2.—Monkey spinal cord and medulla. Several specimens of tissue from monkeys inoculated with M. A. virus in the spring and autumn of 1910 were combined for testing. They had been kept in 50 per cent glycerol for 6 years or longer at 4°C. Culture tests, similar to those described in Experi-

ment 1, were negative. Dec. 9, 1916. A *Macacus rhesus* received, under ether anesthesia, an intracerebral inoculation of 3 cc. of a heavy suspension. Dec. 12. 13 cc. of the same suspension were injected into the peritoneal cavity. Jan. 4, 1917. No symptoms had appeared. Injected 2 cc. of a fresh suspension made from the same tissues intracerebrally and 7 cc. intraperitoneally. Jan. 13. Ataxia; shoulder and leg muscles weak. Jan. 14. Weakness advancing; animal unable to climb. Jan. 17. Prostrate. Etherized.

Histology.—Sections prepared from the medulla, spinal cord, and intervertebral ganglia were studied. The medulla shows marked perivascular lymphocytic infiltrative lesions of the meninges and less of the nervous tissue itself. But within the latter are small focal lymphocytic accumulations sometimes about degenerating nerve cells. The spinal cord shows more pronounced lesions, especially of the anterior gray matter. Diffuse lymphocytic and edematous infiltration as well as marked perivascular cellular infiltrations are prominent (Fig. 2). The blood vessels of the white matter are widely affected. Hyaline degeneration of motor nerve cells is frequent, but little neurophagocytosis is going on (Fig. 3). The intervertebral ganglia show typical focal infiltrative lymphocytic lesions, but no necrosis of ganglion cells (Fig. 4).

The lesions in the nervous organs of the second monkey exceed in extent and severity those of the first. This is in conformity with the greater pathogenicity displayed for monkeys of the virus which has become adapted to that species.

The experiments described show that the virus of epidemic poliomyelitis as contained in the central nervous organs of human beings, the subjects of epidemic poliomyelitis, and of monkeys in which the experimental disease has been induced, survives for many years in the weak disinfectant glycerol. The manner in which thereinoculations which led to paralysis and to characteristic histological lesions were made indicates that during the periods mentioned, the virus suffered reduction in activity either because of mere diminution in the number of microorganisms still surviving or through qualitative modifications of the viable organisms themselves.

On the other hand, the experiments again bring out the value of reinoculation in establishing the viability and activity of the microorganism causing poliomyelitis.[3] The virus of this epidemic disease is peculiar in that the injection into the monkey does not afford an immunity unless obvious symptoms of an infection appear. An unsuccessful inoculation increases susceptibility to a subsequent

[3] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

injection of the same relatively ineffective material. It seems as if an accumulation of the virus takes place until the quantity or number of viable microorganisms becomes sufficient to induce an infection. Repeated inoculations, therefore, rather lead to infection than to specific protection. This phenomenon was noted first with the active monkey virus used in subminimal doses for purposes of immunization[4] and later with the globoid bodies[5] of which a single inoculation failed, but repeated injections sufficed to cause experimental poliomyelitis.[3]

The demonstration of the survival of the virus in the presence of a weak antiseptic compound for the long periods given may have a bearing on the epidemiology of poliomyelitis. The reappearance in a given place of epidemic disease is usually ascribed to a new importation of virus, when possibly the explanation is sometimes to be sought in the persistence and survival of specimens of the virus which flourished previously in that or a neighboring community.

CONCLUSIONS.

The virus of poliomyelitis contained within the spinal cord and medulla of human beings and monkeys withstands glycerolation for many years. The specimens tested had been kept for 4 and 6 years respectively in 50 per cent glycerol at refrigerator temperature.

The symptoms and lesions caused by this virus are identical with those produced by that contained in the more recently collected spinal cord and medulla.

The specimens had lost a part of their activity under the conditions described, necessitating larger and repeated doses to induce infection. Whether this difference is due merely to quantitative reduction in number of viable microorganisms or to qualitative alterations under the influence of the mildly antiseptic glycerol has not been determined.

An ineffective inoculation of tissues containing the virus does not increase resistance, but rather diminishes it, so that a subsequent injection, inadequate in itself, may cause experimental poliomyelitis.

This power of survival under adverse conditions may not be without

[4] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780.

[5] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

significance in respect to the recrudescence of poliomyelitis in a given locality and after a lapse of years. Hitherto this phenomenon has been accounted for by assuming a fresh importation of a virus of pronounced pathogenic power. It is possible that the explanation in some instances resides in the renewed activity of specimens of the virus surviving from a previous epidemic, while in other instances a fresh introduction actually takes place from a remote focus of infection.

The infectious nervous tissues employed in these experiments did not yield in culture streptococci or other ordinary bacteria.

EXPLANATION OF PLATES.

PLATE 40.

FIG. 1. Spinal cord of the monkey in Experiment 1, showing perivascular infiltration of meningeal and intramedullary vessels and focal accumulations of lymphoid cells. × 62.

PLATE 41.

FIG. 2. Spinal cord of the monkey in Experiment 2, showing perivascular and diffuse lymphocytic infiltration of the white and gray matter. × 62.

PLATE 42.

FIG. 3. The same as Fig. 2. Perivascular infiltrative necrosis of nerve cells and neurophagocytosis. × 400.

PLATE 43.

FIG. 4. Intervertebral ganglion of the monkey in Experiment 2, showing focal accumulations of lymphoid cells. × 62.

FIG. 1

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

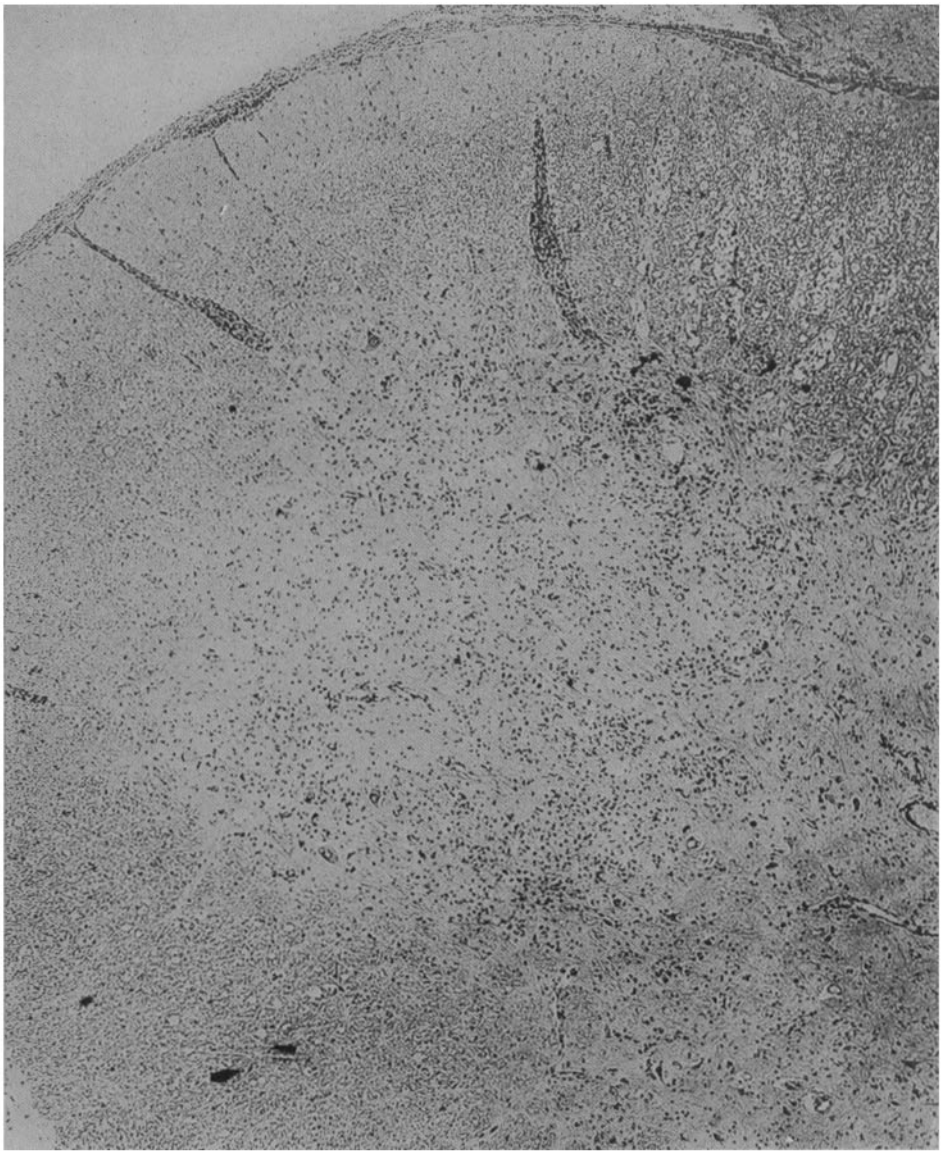

FIG. 2.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

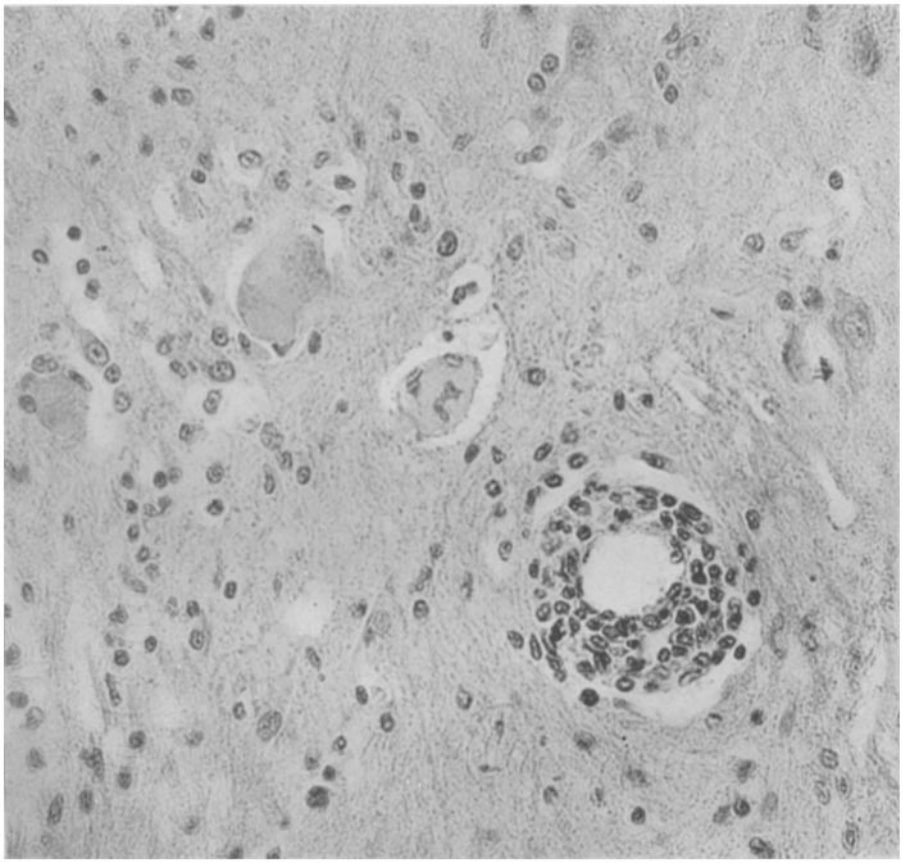

FIG. 3.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

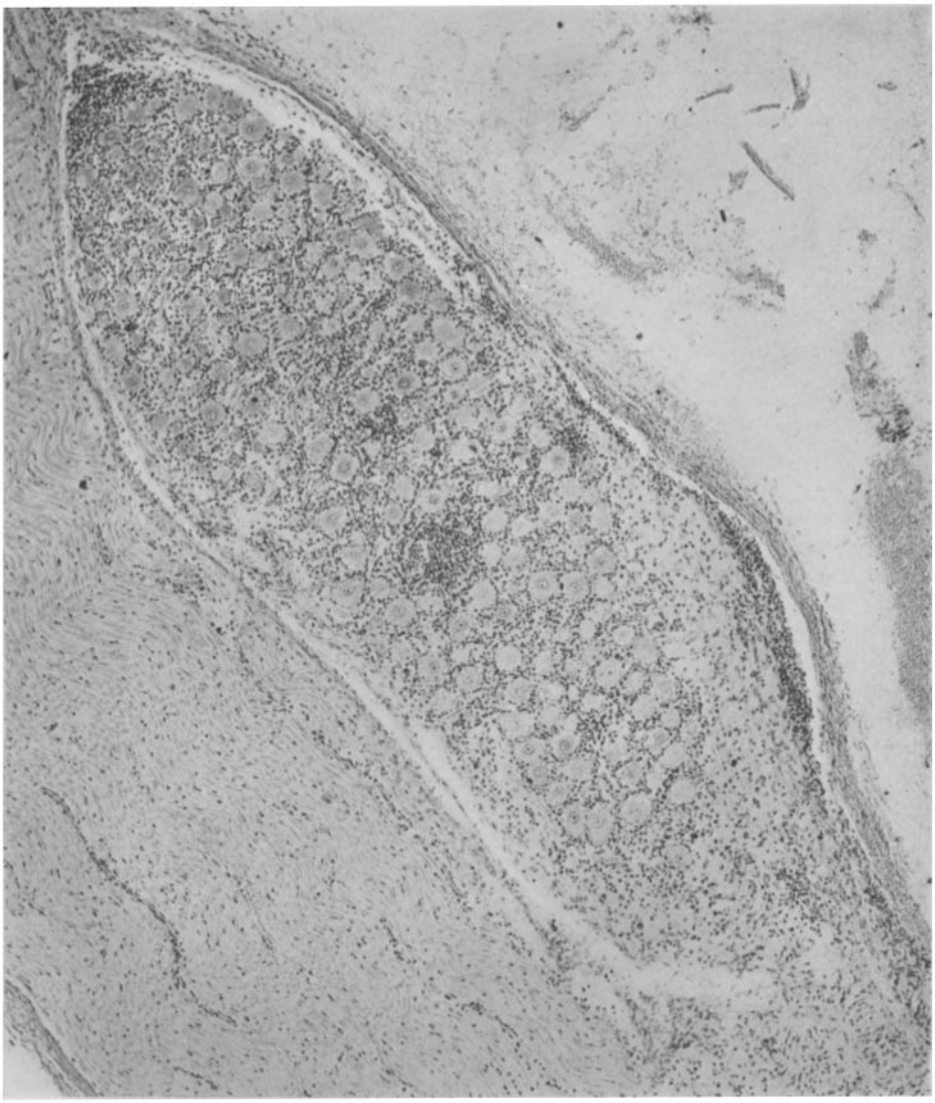

FIG. 4.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)