AN IMMUNIZING STRAIN OF THE VIRUS OF POLIOMYELITIS.

By SIMON FLEXNER, M.D., and HAROLD L. AMOSS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, December 21, 1923.)

No means are known by which a strain of the virus of poliomyelitis, active for monkeys, can be attenuated artificially. All human strains hitherto described, once they have been adapted by implantation to the monkey, display high virulence. A certain number of strains, because of inherent low potency, fails to become adapted at all. These latter strains produce on injection no appreciable effect and leave the monkeys as subject to inoculation with a virulent strain as previously untreated animals. Thus up to the present time, two varieties only of the virus of poliomyelitis have become known: one too weak to excite any reaction, and the other so strong as to induce severe paralytic effects in the monkey.

Recently we have encountered a third variety. Ever since our experimental studies on poliomyelitis were begun in 1909, [1–4] we have been on the lookout for a strain of the virus which would be mildly effective in the monkey. Apparently such a strain has now come into our hands. It is of some interest to learn that the human case from which it was derived was unusually severe clinically, and exhibits histologically lesions of exceptional intensity and acuteness.[5]

Source of the Virus.

The patient, a child 5 years of age, from whom the specimen was obtained, was taken ill on Oct. 29, 1921, and died on Nov. 3. The symptoms were

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

[2] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 1780.

[3] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

[4] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685.

[5] We are indebted for the specimens of the nervous system of the case to the interest of Dr. George Draper of New York, and the cooperation of Dr. N. C. Russell and Dr. Perkins Arnold of Buffalo.

at first indefinite; there had been vomiting and anorexia. On the 2nd day of the illness, slight rigidity of the neck muscles and difficulty of swallowing were noted. Paralysis of the soft palate attended by nasal regurgitation developed, and was followed by increasing signs of bulbar involvement. Death occurred from respiratory paralysis.

EXPERIMENTAL.

The brain tissue as received in 50 per cent glycerol was in large sections. As no special precautions could be exercised in removing the brain at the necropsy, bacterial contamination was inevitable. The tissues were somewhat discolored and had a slightly putrefactive odor on their receipt. Test suspensions in agar Petri plates showed innumerable colonies of bacteria.

The large sections were cut up and placed in 50 per cent sterile glycerol. This device did not lead in a few days to any material reduction in the bacteria. Resort was therefore had to phenolation,[2, 3] as this means had previously been found effective against somewhat less degrees of bacterial contamination. The method consisted in immersing thin sections of the brain and spinal cord in abundant 0.5 per cent phenol for 24 hours, removing them for culturing, and again placing in 50 per cent glycerol. As the reduction in bacterial content was not regarded as sufficient, a second immersion in 0.25 per cent phenol was resorted to, and daily cultures were made. On the 4th day, few bacterial colonies developed in the Petri plates, hence animal inoculation was undertaken. Mention should be made of the fact that complete bacterial sterility was not found necessary, as the monkeys readily disposed of the saprophytic bacteria still remaining in the specimens injected. All the monkeys used in the experiments belonged to the species *Macacus rhesus*.[6]

Experiment 1.—Monkey A on Sept. 19, 1922, received intracerebrally 1 cc. of a 10 per cent suspension of the phenolated and glycerolated human material, which on plating showed about 100 colonies of bacteria per cc. No effect was detected until Oct. 1, when excitement, head tremor, and right facial paralysis were observed. These symptoms progressed, and in addition the right deltoid was noted to be weak; ataxia was also present. As on Oct. 6 the condition was stationary, the animal was etherized in order to obtain nervous tissues for further

[6] All inoculations were made in animals anesthetized with ether.

inoculation and for histological examination. The lesions disclosed by the microscope were typical of experimental poliomyelitis. They were of moderate grade of severity and involved the medulla and upper spinal cord chiefly.

Experiment 2.—Monkey B. An immediate transfer from Monkey A to a second *Macacus rhesus* was made. On Oct. 6, 1.5 cc. of a suspension prepared from the site of inoculation and medulla were injected intracerebrally into Monkey B. The suspension was sterile in aerobic culture. The first symptoms were noted on the 12th day; and on the 16th day, head tremor, right facial palsy, with ptosis and weakness of the deltoids were present. The animal was etherized on the 17th day. The lesions in the nervous tissues were those of experimental poliomyelitis.

Experiment 3.—Monkey C received intracerebrally on Oct. 27, 1 cc. of a 10 per cent suspension of the original phenolated and glycerolated human material and intraperitoneally 10 cc. The plated suspension showed about 300 colonies per cc. This animal remained well until Nov. 6, when head tremors and left facial palsy appeared. The next day, 15 cc. of the suspension were given intraperitoneally. On Nov. 8 the animal was ataxic and the right deltoid was weak. On Nov. 10 the back muscles were weak. The animal was etherized. The nervous tissues showed characteristic lesions of experimental poliomyelitis.

This experiment was repeated on three monkeys which were, however, permitted to recover and were subsequently tested with a potent M.A. strain of the virus of poliomyelitis for resistance. While the control animal developed paralysis and succumbed in the usual manner, the animals which developed symptoms from the inoculation of the Buffalo strain of human virus entirely resisted the inoculation. The fact should be mentioned that one monkey developed aphonia which became complete, then gradually receded, as other symptoms abated, until the voice was quite regained.

Experiment 4.—Monkey D. Nov. 22, 1922. Received intracerebrally 1 cc. and intraperitoneally 15 cc. of a 10 per cent suspension of the brain and spinal cord of Monkey C. No symptoms arose until Nov. 15, when the animal was observed to be unduly excitable. The next day ataxia and general tremors, including slight convulsive seizures, were present. As no progression of the symptoms took place, 15 cc. of the suspension were injected intraperitoneally. No effect was produced by the reinjection. The symptoms gradually abated and in a few days the animal was again apparently normal.

This animal was allowed to recover, after which it was (as in the previous examples) submitted to a test for immunity. On Dec. 18 a control *Macacus rhesus* and Monkey D were each given intracerebrally 0.5 cc. of a Berkefeld filtrate of a 5 per cent emulsion of M.A. virus. The control was prostrate on the 12th day and succumbed to experimental poliomyelitis. Monkey D on the other hand never showed any symptoms whatever.

Experiment 5.—Monkey E received intracerebrally on Jan. 3, 1923, 1 cc. of a 10 per cent suspension of the glycerolated brain and spinal cord of Monkey A. Ataxia, head tremors, and weakness of deltoid and back muscles were present

on Jan. 16. The legs became paralyzed on Jan. 18, on which date the animal was etherized. The lesions revealed by the microscope were typical of experimental poliomyelitis.

A mate to this animal, Monkey F, inoculated on the same day as Monkey E, but having received also 15 cc. of the suspension intraperitoneally, showed slighter symptoms and recovered.

These instances represent the second passage, and the next instances the third passage of the virus. As will be noted in the third passage, one monkey developed severe symptoms and two other monkeys much slighter symptoms of experimental poliomyelitis.

Experiment 7.—Monkey G. Sept. 29, 1923. Received intracerebrally 1 cc. of a 10 per cent suspension derived from Monkey E. On Oct. 15 the animal was excitable and tired easily. Reinjected with 1 cc. intracerebrally and 15 cc. intraperitoneally. No appreciable effect was produced by this inoculation. It was repeated on Oct. 23, without immediate result. But on Oct. 28, the animal was tremulous, showed ptosis, right facial palsy, and weakness of the back muscles. On Nov. 1 the monkey was etherized. The microscopic examination showed pronounced perivascular infiltrative lesions in the medulla and spinal cord.

Monkeys H and I of this series developed tremors, ataxia, and deltoid and leg paralyses. They were permitted to recover. The animal which showed a degree of leg involvement preventing standing, subsequently recovered with a trace, only detectable in its movements, of the original extensive paralysis.

DISCUSSION.

The preceding experiments present certain new facts regarding the virus of poliomyelitis. They show, apparently for the first time, that strains of the virus exist in nature which under some circumstances induce paralytic and lesser effects in monkeys, the inclination of which is toward amelioration terminating in recovery, instead of, as had been observed in the past, intensification leading to death. This latter consideration may in time come to have practical significance.

The qualities which distinguish the attenuated strain of the virus raise the question whether the mild grade of activity is an inherent property, or whether it is a product of the mild putrefactive changes taking place in the human tissues. No answer can be returned at present to this question, which, however, may not prove entirely beyond experimental determination.

It has been ascertained by experimental tests that the modified form of poliomyelitis through which the inoculated monkeys pass and from which they recover, leaves them protected against the action of a highly virulent strain of the virus of poliomyelitis. The Rockefeller Institute possesses several such potent strains, which are so active that fractions of a cubic centimeter of a Berkefeld filtrate of a 5 per cent suspension, injected intracerebrally, induce with certainty in 6 or 7 days paralysis leading to death. The several monkeys which had shown symptoms of mild poliomyelitis following injections of the attenuated virus and which were permitted to recover, were subsequently inoculated intracerebrally with a highly virulent (revived M. A. strain)[7,8] of the virus at the same time with controls. The results were consistent: the former never showed any appreciable symptoms, while the latter developed paralysis, usually by the 7th day, and succumbed by the 14th day.

The mild or attenuated strain of the virus seems to be of a fixed degree of potency, having neither increased nor decreased perceptibly in the 2 years during which it has been under study. Unless the virus was modified by the putrefactive process occurring in the original human brain and cord, it is obvious that wide disparity exists between the effects induced by the strain in the human being from whom it was derived and the monkeys into which it was inoculated. The former shows very severe, acute lesions in the spinal cord and medulla to which the death is attributable; the latter shows relatively mild lesions from which recovery, either complete or nearly complete, tends to take place.

The lesions present in the monkeys which were sacrificed explain not only the recovery in those allowed to go on to completion of the pathological process, but they afford an indication of the manner and degree of the resolution process. In the first place, the medulla is less often and less extensively affected than is the rule with the more potent virus. The severer lesions are found in the spinal cord and intervertebral ganglia. The spinal cord may show a degree of neuronophagocytosis, thus accounting for such slight residual paralysis

[7] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.
[8] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1924, xxxix, 191.

as sometimes occurs. But the chief interest centers about the extensive perivascular infiltrations and the milder interstitial infiltrations, which in time seem capable of undergoing complete resolution with which is connected the restitution of function taking place in paralyzed members.

SUMMARY.

In this communication we have described a strain of the virus of poliomyelitis derived from a rapidly fatal human case, which exhibits mild degrees of infective power and marked degrees of protective effect for the monkey. While *Macacus rhesus* displays perceptible differences in suceptibility to the attenuated virus, nearly all individuals respond to the inoculation and none succumbs to the infection induced. As compared with previously described examples of experimental poliomyelitis, the modified disease described in this paper is distinguished by its relatively benign nature and its tendency to end in recovery rather than, as with the earlier observed instances, in death.