THE EFFECT OF CATAPHORESIS ON POLIOMYELITIS VIRUS

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Recent investigations in the field of filterable viruses have been marked by a tendency to apply to the disease-producing agents of this class certain biological principles which govern the reactions of bacteria. Of particular interest have been the observations on the behavior of viruses in an electrical field (1, 2, 3). By means of cataphoresis of virus suspensions, it has been found that the effective agent could be revealed in tissues which, by the usual tests of animal inoculation, failed to show activity. This results from the migration of the virus, or possibly, of the material in a suspension carrying the virus, to one or the other pole, depending on the charge. Thus vaccine virus under ordinary conditions of hydrogen ion concentration migrates to the anode (2, 3). By this method, furthermore, vaccine virus has been isolated from rabbits wholly recovered from infection and several weeks or months after vaccination (3, 4). Finally, cataphoresis has been of use in separating vaccine virus from neutral virus-immune serum mixtures (5). Since these observations have a bearing on immunity in virus diseases, it was determined to ascertain the behavior of the poliomyelitic virus in an electrical field.

Method

The apparatus used was a modification of that described by Field and Teague (6) and by Todd (1). The details will be given by Olitsky and Long (4) in another communication. It is sufficient to say here that three large U tubes were employed, the combined capacity of which was about 96 cc. of fluid. The ends of the connecting tubes which dipped into the fluid under observation contained 1 per cent agar and 1 per cent NaCl. This furnished a convenient method for collecting the particles containing the virus, or the virus itself, which migrated with the current. From 114 to 117.5 volts and 0.8 to 4.2 milliamperes were passed for 3 hours through the fluid under examination between non-polarizable electrodes.

At the end of the period the agar plugs were removed under sterile conditions, ground in 1 cc. of Ringer's solution, and inoculated intracerebrally in monkeys.[1] The mode of preparation of the fluid will be given with each of the following experiments.

The first step was to determine whether the virus of poliomyelitis possessed the property of migrating in an electric field.

Experiment 1.—A piece of spinal cord of a monkey dead of typical experimental poliomyelitis was used. The nervous tissue was emulsified in 100 cc. of distilled water under aseptic conditions. The cataphoresis test was done as follows: milliamperes current, 0.8; potential drop, 117 volts; time, 3 hours; pH of the suspension, 6.9. After 30 minutes the fluid at the cathodes was clear and that at the anodes cloudy. After 2 hours the cathodic material was quite clear for a distance of 2 cm. from the surface and the remainder was partly clear. The agar from the positive poles contained a diffuse whitish cloud and that from the negative pole was translucent. *Macacus rhesus* 1 was inoculated intracerebrally with 1 cc. of Ringer's solution containing the ground agar from the positive poles. On the ninth day after inoculation the animal was excited and tremulous and both legs were paralyzed. On the tenth day the prostrate monkey was etherized. The gross and microscopical lesions of the spinal cord and brain were typical of poliomyelitis. *Macacus rhesus* 2 received a similar intracerebral inoculation of Ringer's solution containing the agar from the negative poles of the same experiment. On the fifteenth day after inoculation all four limbs were paralyzed and the animal was prostrate. The monkey was etherized and the gross and microscopic lesions of the nervous system were typical of poliomyelitis.

From the fact that the animal inoculated with anodic material developed poliomyelitis first, we were led to suppose that the virus probably carried a negative charge. Since the monkey inoculated with the cathodic material also contracted the disease, although after a somewhat longer incubation period, it was thought that probably the virus suspension was too strong, and part of the virus adhered to the cathodic agar. To obviate this difficulty we next employed a Berkefeld "V" filtrate of a 5 per cent suspension of the active nervous tissue.

Experiment 2.—The spinal cord from a monkey with poliomyelitis was made into a 5 per cent suspension in distilled water. The emulsion was passed through a Berkefeld "V" candle and placed in the cataphoresis apparatus. The conditions

[1] All experimental procedures on animals were made with the aid of deep ether anesthesia.

were: milliamperes, 0.8; potential drop, 116 volts; time, 3 hours. The pH of the filtrate was 6.5. Contrary to the previous experiment, in which suspension was used, the clearing in this case appeared at the positive pole and the cathodic material remained cloudy. *Macacus rhesus* 3 was inoculated intracerebrally with 1 cc. of Ringer's solution containing the ground agar from the positive pole. The fifth day after inoculation the animal was excited, weak, and ataxic. The next day a marked tremor and partial paralysis of all limbs were present. On the seventh day the animal was prostrate, and on the eleventh it was etherized. The lesions of the nervous system were typical of poliomyelitis. *Macacus rhesus* 4 (control) was inoculated the same day with the material from the negative pole. This monkey remained well throughout the period of observation.

It will be noted that the virus collected at the positive pole. It is of interest that the clouding due to migration of proteins in the fluid appeared at the pole opposite to the one at which the virus was found.

The next experiment was designed to recover by cataphoresis the virus from a non-infectious, neutral mixture of poliomyelitis virus and convalescent serum.

Experiment 3.—Poliomyelitis material of the pooled mixed virus strain which had been glycerolated for some time was made into a 5 per cent emulsion in salt solution and passed through a Berkefeld "V" filter. 50 cc. of the filtrate was mixed with an equal volume of human convalescent serum. 4 cc. of this mixture was inoculated intraspinally and intracerebrally in *Macacus rhesus* 5 (control). No symptoms developed. The material was therefore considered to have been neutralized. The remainder of the mixture was subjected to cataphoresis under the following conditions: milliamperes, 4.2; potential drop, 114 volts; time, 3 hours; pH = 6. *Macacus rhesus* 6 was inoculated intracerebrally with 1 cc. of Ringer's solution containing the agar from the positive pole. On the eleventh day after inoculation the animal was excited, slow in movement, and tremulous. Ptosis and a right facial paralysis were present. On the twelfth day the monkey was ataxic and partially paralyzed in all four limbs. The thirteenth day the prostrate animal was etherized. The gross and microscopic lesions in the spinal cord and brain confirmed the diagnosis of poliomyelitis. *Macacus rhesus* 7 (control) was inoculated intracerebrally on the same day with the emulsified agar from the negative pole of the same experiment. No symptoms developed.

The experiment shows unmistakably that active poliomyelitis virus can be separated from a non-infective, neutral mixture of the virus and its specific immune serum. We can thus conclude that the virus is not killed by the antiserum but only held in some sort of an ineffective combination. The result also confirms the previous obser-

vation that under the conditions of the experiment poliomyelitis virus bears a negative charge.

Since it was found possible to regain active poliomyelitis virus from the neutral mixture, it was thought advisable to attempt to isolate virus from an animal which had recovered from the disease.

Experiment 4.—A monkey, *Macacus rhesus* 8, was selected which had developed typical poliomyelitis 6 days after an intracerebral inoculation of virus. Almost complete paralysis of both arms and partial paralysis of the legs had existed for 4 days, followed by rapid recovery, so that by the eleventh day there was little disability. During the next 12 days the monkey ran and jumped about with almost normal vigor. At this time, or 23 days after infection, the animal was etherized and the spinal cord made into a 5 per cent suspension in salt solution. Cataphoresis was carried out under the following conditions: milliamperes, 0.8; potential drop, 117.5 volts; time, 3 hours; pH = 6.8. *Macacus rhesus* 9 was inoculated intracerebrally with the agar suspension from the positive pole. On the eighteenth day tremor, ataxia, and partial paralysis of both arms appeared. On the nineteenth the monkey was prostrate and was etherized. The gross and microscopic examination of the central nervous system confirmed the diagnosis of poliomyelitis. *Macacus rhesus* 10 (control) was inoculated intracerebrally with the material from the negative pole and 11 (control) with the suspension of central nervous system before cataphoresis had been performed. Neither animal developed poliomyelitis.

This experiment brings out the interesting fact that active virus persists in the nervous tissues for a period after recovery from poliomyelitis. Since ordinary inoculation of central nervous tissues before cataphoresis was without effect, the positive result indicates either that the antibody concentration in the tissues is sufficient to have neutralized the virus associated with it on injection in our experiment, or that in the recovered monkeys the virus is present in too minute quantity to be detected by the usual mode of inoculation. The tests described demonstrate that the virus of poliomyelitis may be separated in effective quantity from neutral mixtures of virus and immune serum or further that it can be concentrated at the positive pole from dilute virus filtrates.

CONCLUSIONS

1. Under ordinary conditions of hydrogen ion concentration the virus of poliomyelitis, as such, or associated with particles in fine sus-

pension, migrates in an electrical field to the anode. It follows that the virus bears an electronegative charge.

2. By means of cataphoresis, the virus can be recovered from a non-infective mixture of virus and specific immune serum.

3. By the same means it is possible to reveal the presence of virus in the central nervous system of a monkey which has recovered from the active stage of experimental poliomyelitis.

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