INTRASPINOUS INFECTION IN EXPERIMENTAL POLIOMYELITIS.*

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The routes by which the virus of poliomyelitis may be conveyed to the central nervous organs of monkeys so as to induce infection and paralysis are various but not of equal certainty. Undoubtedly the direct intracerebral injection yields the most constant results, next to which the intrasciatic and intranasal have been placed. Infection by way of the peritoneal cavity, subcutaneous tissues, and blood is obtained with far less certainty. Considerable interest attaches to the intraspinous route of inoculation of the virus, because of the bearing which it may have on the theory of the pathogenesis of poliomyelitis.

That the virus of poliomyelitis exhibits a great affinity for the nervous organs is obvious from the location of the main lesions of the disease. There is, however, lack of agreement as to the manner in which the lesions are produced; namely, whether through direct action of the virus upon the nerve cells or through indirect effect of lesions in the blood vessels and ground substance. The hypothesis that poliomyelitis is a specific affection of the anterior grey matter of the spinal cord has been abandoned. The lesions occur regularly throughout the structures of the cord including the intervertebral ganglia, frequently in the medulla and brain, and quite often in the Gasserian and abdominal sympathetic ganglia.[1] Probably other ganglionic masses, not yet studied, will show effects. Besides, lesions are present in the lymphatic and other somatic organs.[2]

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[1] Flexner, S., Clark, P. F., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 205.

[2] Flexner, S., Peabody, F. W., and Draper, G., *Jour. Am. Med. Assn.*, 1912, lviii, 109.

The pia-arachnoid membranes of the central nervous system are constantly involved in the pathological process, although the degree of involvement varies. Examination of the cerebrospinal fluid in human cases regularly shows an increase in cells and globulin, thus indicating an inflammation of the meninges.[3] Histological examination of the spinal cord of fatal human and experimental cases shows cellular infiltration of the pial membrane, particularly adjacent to the surface of the spinal cord and most marked near the blood vessels of the membrane. The pia projecting into the anterior and in a less degree into the posterior median columns contains this cellular infiltration, which, within the grey substance of the spinal cord, may be strongly marked about the blood vessels and even in the ground substance. While some degree of infiltration is never absent, cases in man and in the monkey occur in which it is slight. A similar infiltration exists in the intervertebral ganglia appearing to extend from the pial investment into their substance. Along with the cellular infiltration are nerve cell degenerations of various grades. Sometimes the infiltrations predominate in degree over these degenerations, sometimes the degenerations predominate over the infiltrations.

Flexner has emphasized the nasopharyngeal mode of infection in poliomyelitis, and according to his view the virus ascends probably by way of the lymphatics from the nasal mucous membrane and multiplies in the pia-arachnoid membranes and the adjacent nervous structures of the brain before becoming established in the medulla and spinal cord.[4] Hence it has seemed important to ascertain whether infection can be readily produced by introducing the virus directly into the subarachnoid spaces. This may be done by a subdural injection into the region of the brain after trephining or into that of the spinal cord by means of lumbar puncture. The latter is the simpler and safer method, as there is little risk of introducing the fluid into the nervous tissues themselves.

Thus far Flexner and Lewis[5] alone seem to have recorded a suc-

[3] Peabody, F. W., and Draper, G., *Am. Jour. Dis. Child.*, 1912, iii, 153.

[4] Flexner, S., *Jour. Am. Med. Assn.*, 1910, lv, 1105; Huxley Lecture, *Lancet*, 1912, ii, 1271, and *Science*, 1912, xxxvi, 685.

[5] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 535.

cessful infection among several failures by means of intraspinous inoculation of the virus, although Neustaedter and Thro caused poliomyelitis in three monkeys by means of combined intraspinous and subcutaneous inoculation,[6] and Römer,[7] without giving any experimental details, remarks that intraspinous infection takes place readily.

No special difficulty surrounds the intraspinous mode of infection. The requirements are an active virus, such as our M A and K strains, and a dose somewhat larger than that necessary for successful intracerebral inoculation. However, the immediate effects of the two modes of injection are different. When the virus is injected into the brain it is usually deposited in a small cavity within the cerebral hemisphere although some may escape into the meninges. It may safely be assumed that multiplication of the virus soon begins under the favorable conditions of temperature and location, and that gradually it passes into the adjacent membranes and general nervous tissue in which further multiplication occurs. When the virus is injected into the subarachnoid spaces in the lumbar region it quickly diffuses in the membranes along the length of the spinal cord and base of the brain, but it begins also immediately to pass with the cerebrospinal fluid into the circulating blood through the usual venous channels. Since the blood route is perhaps the poorest for producing infection the virus which escapes from the subarachnoid spaces into the veins may be regarded as lost.

Hence, instead of Berkefeld filtrates we have found it better to employ paper filtrates of the usual 5 per cent. emulsion of the infected nervous tissue containing the virus. In a series of nine monkeys inoculated by lumbar puncture with paper filtrates of M A virus, all developed poliomyelitis. Of this series six monkeys became paralyzed, while three developed definite symptoms without showing paralysis. The microscopical examination of the spinal cord and intervertebral ganglia of the latter three brought out the presence of typical poliomyelitic lesions of mild degree.

[6] Neustaedter, M., and Thro, W. C., *New York Med. Jour.*, 1911, xciv, 813.
[7] Römer, P. H., Die epidemische Kinderlähmung, Berlin, 1911, 132.

ILLUSTRATIVE PROTOCOLS.

Experiment 1. Macacus rhesus.—Feb. 18, 1913. A small quantity of cerebrospinal fluid was withdrawn by lumbar puncture and 1 c.c. of a paper filtrate from a 5 per cent. suspension of the spinal cord from a recently paralyzed M A monkey was injected. Feb. 22. Tremor; left arm paralyzed; extremities weak. Feb. 23. Paralysis of arms, back, neck, and right leg. Feb. 24. Prostrate. Etherized. Characteristic lesions of experimental poliomyelitis present in the spinal cord and intervertebral ganglia.

Experiment 2. Macacus rhesus.—Feb. 26. 0.5 c.c. of cerebrospinal fluid was withdrawn by lumbar puncture, and 0.5 c.c. of a paper filtrate prepared from a recently paralyzed animal was injected. Mar. 3. Weakness of left arm. Mar. 7. Extremities paralyzed; prostrate. Mar. 8. Etherized. Lesions of poliomyelitis present.

Experiment 3. Macacus rhesus.—May 22. Cerebrospinal fluid was withdrawn by lumbar puncture, and 1.2 c.c. of a paper filtrate prepared from a recently paralyzed animal was injected. May 29. Excitable; tremor. May 30. Legs weak. May 31. Legs and arms weak. June 2. Excitability increased; neck weak; weakness of arms and legs increased. June 7. Condition stationary. Etherized. The spinal cord and intervertebral ganglia showed typical but mild lesions of poliomyelitis.

The protocols given serve as examples of the remaining experiments. From them it can be concluded that given a specimen of virus of adequate virulence experimental poliomyelitis can be regularly produced by intraspinous inoculation.

The virus having been introduced into the subarachnoid spaces clearly gains access to the nervous tissues of the spinal cord and medulla, with the interstices of which the cerebrospinal fluid is in intimate communication. A part, therefore, becomes fixed to the tissues within the pial membrane and about the blood vessels and doubtless to the nervous structures proper. Another part readily leaves the subarachnoid spaces with the cerebrospinal fluid. Thus far no one has detected the virus in the cerebrospinal fluid in human cases of poliomyelitis and it is usually absent from the fluid in monkeys at the time of the onset of paralysis, although it may be present at an earlier period after intracerebral inoculation.[8] We have, therefore, tested the cerebrospinal fluid at intervals of twenty-four and forty-eight hours after the inoculation, and at the expiration of six days when the first symptoms of paralysis made their appearance.

[8] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

Macacus rhesus A.—Nov. 22. Intraspinous injection of 3 c.c. of a paper filtrate of K virus was given after withdrawal of 2 c.c. of clear cerebrospinal fluid. Nov. 23. 0.8 c.c. of turbid fluid was withdrawn by lumbar puncture. Nov. 24. 1 c.c. of less turbid fluid. The fluids were inoculated intracerebrally into rhesus monkeys. Nov. 26. Excitable; legs paralyzed; back weak. Nov. 27. Prostrate. Died. Typical poliomyelitic lesions present in the spinal cord.

Macacus rhesus B.—Nov. 13. Intracerebral injection made of the 24 hour specimen of fluid withdrawn by lumbar puncture from monkey A.

Nov. 30. Excitable. Dec. 2. Paralysis of left leg. Dec. 4. Muscles of extremities and back paralyzed; prostrate. Dec. 5. Etherized. Lesions of poliomyelitis present.

Macacus rhesus C.—Nov. 24. Intracerebral injection of 1 c.c. of spinal fluid withdrawn by lumbar puncture from monkey A 48 hours after intraspinous inoculation.

Nov. 29. Excitable; right arm and leg paralyzed; back weak. Nov. 30. Prostrate. Dec. 2. Etherized. Lesions of poliomyelitis present.

Macacus rhesus D.—Nov. 22. 1 c.c. of a paper filtrate of the virus was given by intraspinous injection. Nov. 28. First symptom of infection. 2 c.c. of cerebrospinal fluid were withdrawn, and on Nov. 29 injected intracerebrally into another rhesus monkey, E. No symptoms developed in the latter.

These experiments show again, first, that infection by way of the subarachnoid spaces readily occurs, and next, that the virus even when introduced directly into them tends to escape from these spaces partly into the central nervous tissues, where it is held and in which it multiplies, and into the general circulation by which it is probably carried to all the nervous organs which doubtless again in some degree remove it from the blood. The reasons for failure to detect the virus in the cerebrospinal fluid in human cases are now apparent.

SUMMARY.

By intraspinous injections of specimens of poliomyelitic virus of suitable virulence infection can be caused regularly in *Macacus rhesus* monkeys.

The virus passes from the subarachnoid spaces into the nervous tissues in which it multiplies, and into the blood.

The constant involvement of the pia-arachnoid membranes in poliomyelitis, even when no paralysis occurs, and the fact that infection can readily be produced by intraspinous inoculation suggests anew that in the pathogenesis of poliomyelitis the interstitial tissue changes within the meninges, blood vessels, and ground substance play a determining part.

While the virus injected into the subarachnoid spaces can be demonstrated there by inoculation tests forty-eight hours after the injection it can no longer be detected on the sixth day, at a time when the first symptoms of infection make their appearance. The failure of the cerebrospinal fluid from human and experimental cases of poliomyelitis to produce the disease when inoculated into monkeys is due to the fact that the virus is either fixed by the nervous tissues or passes into the blood.