A CONTRIBUTION TO THE PATHOLOGY OF EPIDEMIC POLIOMYELITIS.*

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In the course of our studies on experimental poliomyelitis we have collected certain data on the pathology of the disease which seem of sufficient interest to warrant publication. They relate to the distribution of the virus in the nervous tissues outside the cerebrospinal axis and its bearing on the pathogenesis of poliomyelitis, the resistance of the virus to glycerin, phenol, and freezing, and to immune bodies in the cerebrospinal fluid after recovery from infection.

THE VIRUS IN THE SPINAL, GASSERIAN, AND ABDOMINAL SYMPATHETIC GANGLIA.

That the virus of poliomyelitis is not present exclusively in the affected regions of the spinal cord but occurs also constantly in the brain was observed early in the course of the experimental inoculations of monkeys.[1] Later the virus was found to be present in the intervertebral ganglia of infected monkeys.[2] But no systematic study has been made from this point of view of the nervous organs that lie outside of and at a distance from the cerebrospinal axis. This we have now done with the results to be described, which have a bearing on the prevailing views on the pathogenesis of poliomyelitis.

The inoculation tests prove that the virus exists in the intervertebral, Gasserian, and abdominal sympathetic ganglia. Illustrative protocols follow of several positive inoculation experiments.

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

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

Protocol 1. Intervertebral Ganglia from the Monkey.—Feb. 2, 1911. Several intervertebral ganglia were removed aseptically from a monkey that had been inoculated intracerebrally with M A virus and become paralyzed. The ganglia were emulsified in 2 c.c. of salt solution and injected intracerebrally into a *Macacus rhesus*. Feb. 7. Back muscles weak. Feb. 8. Extremities and back paralyzed. Feb. 9. Death. Microscopical examination of the spinal cord showed typical lesions of poliomyelitis.

Protocol 2. Intervertebral Ganglia from the Monkey.—Dec. 26, 1913. Several intervertebral ganglia were removed aseptically from a monkey that succumbed to inoculation of K virus. An emulsion in salt solution was injected into the sciatic nerve and peritoneal cavity. Jan. 1, A. M. Excitability; ataxia. P. M. Legs paralyzed. Jan. 2. Prostrate, died. Lesions of poliomyelitis.

Protocol 3. Intervertebral Ganglia from a Child.—Aug. 8, 1911. Several spinal ganglia which had been taken from a fatal case of acute poliomyelitis in a child and placed in glycerin a few days previously were emulsified in salt solution and injected intracerebrally into a *Macacus rhesus*. Aug. 19. Excitability; paralysis of right arm. Aug. 25. Paralysis of left leg. Sept. 1. Condition improving. Finally recovery occurred, with residual paralysis of one arm.

Protocol 4. Gasserian Ganglia from the Monkey.—Nov. 15, 1913. Four sets of Gasserian ganglia were removed from monkeys dead of K virus. They were emulsified in salt solution and injected into the right sciatic nerve and the peritoneal cavity of a *Macacus rhesus*. Dec. 2. Excitability; tremor; paralysis of right leg. Dec. 3. Both legs paralyzed; back weak; protects right arm. Dec. 4. Both arms weak. Dec. 5. Prostrate. Etherized. Lesions of poliomyelitis.

Protocol 5. Sympathetic Ganglia from the Monkey.—Nov. 15, 1913. Four sets of the abdominal sympathetic ganglia (solar plexus) removed from monkeys dead of K virus (same as preceding) were emulsified in salt solution and injected into the left sciatic nerve and the peritoneal cavity of a *Macacus rhesus*. Nov. 26. Excitability; weakness; paralysis of left leg. Nov. 27. Left arm weak; right arm weaker. Nov. 28. Left arm paralyzed; back weak. Nov. 29. Prostrate. Etherized. Lesions of poliomyelitis.

Protocol 6. Sympathetic Ganglia from the Monkey.—Dec. 3, 1913. Sympathetic ganglia were removed from a monkey succumbing to K virus and suspended in salt solution which was injected into the left sciatic nerve and peritoneal cavity. Dec. 9. Excitability. Left arm weak, and later in the day paralyzed. Dec. 11. Weakness of back. Dec. 12. Left leg paralyzed; right leg weak; right arm weak. Dec. 14. Prostrate. Etherized. Lesions of poliomyelitis.

The protocols show that the ganglia inoculated contain the active virus of poliomyelitis. In view of the frequency with which the demonstration can be made with the ganglia and the rarity with which it has been made with non-nervous tissues, with possibly the one exception of the mesenteric lymph nodes, we may conclude again that the virus is strongly neurotropic. Apparently the virus is not stored in the peripheral nerves[3] but seeks the parenchymatous nerv-

[3] Landsteiner, K., in Kolle-Wassermann, Handbuch der pathogenen Mikroorganismen, 2d edition, Jena, 1913, viii, 445.

ous organs in which to multiply. But the selection among the latter appears to be small, since the virus occurs regularly not only in the spinal cord but in the brain and the several ganglia mentioned, of which some are remote from the cerebrospinal axis.

Moreover, the localization of the virus in the ganglia takes place early. Monkeys in the preparalytic stage of infection, before any detectable weakness of muscles has set in but while in the state of excitement, already show lesions of the intervertebral ganglia. Thus far the other ganglia have not been studied in the early period of the infection.

The histological lesions in the ganglia correspond accurately with those in the spinal cord: they are partly interstitial and perivascular, partly parenchymatous. The former extend in part from the pial investment of the ganglia to the fibrillar supporting tissue and small blood vessels which may be the seat of diffuse or of nodular accumulations of mononuclear cells. The degree to which the nerve cells are destroyed is variable. At times they escape entirely even when the interstitial changes are marked; again they are widely degenerated, or even necrotic, and have become invaded with neurophages. So far as the changes in the spinal ganglia are concerned they appear to arise through an extension of the cellular invasion from the subarachnoid spaces of the spinal cord. The changes in the Gasserian are less severe than those of the spinal ganglia, and the mode of invasion is not so evident. The cellular accumulations within the abdominal ganglia (solar plexus) are least marked and so constantly perivascular that infection by way of the blood is indicated. The nerve cells exhibit the slightest lesions of all the ganglia studied.

In view of the constancy with which the sensory ganglia are the seat of early and even profound histological changes undoubtedly caused by the presence of the virus, it becomes questionable whether any considerable support still exists for the belief that poliomyelitis is essentially a disease of the anterior grey matter of the spinal cord.[4] It would appear rather to be a disease of the nervous system in its entirety, although considered clinically it receives its significance from the muscular paralysis that is caused. Nor can it be held that

[4] Landsteiner, K., *loc. cit.*, p. 439.

the virus of poliomyelitis possesses a special affinity for nerve cells as such, since the lesions which it causes are impressed almost indifferently upon nerve cells and non-nervous elements within the nervous organs. Indeed, accurate studies of the cerebrospinal fluid in preparalytic and abortive human cases of poliomyelitis emphasize the early involvement of the leptomeninges in the pathological process.[5]

The experiments in which intrasciatic injections were made indicate that the virus travels to the spinal cord by the lymphatic channels and brings about paralysis first on the side of injection and later on the opposite side.[6] It is interesting to find that the virus may ascend by means of the lymphatics of the cord to the cervical level and set up paralysis in an arm before the leg into which the virus was injected shows distinct evidences of paralysis (protocol 6).

RESISTANCE OF THE VIRUS TO GLYCERIN, PHENOL, AND FREEZING.

Glycerin.—It was early noted that the virus of poliomyelitis resists glycerin for a time.[7] Römer and Joseph[8] found specimens still active after five months' glycerination. We have tested a specimen of virus K and found that it retained its activity undiminished after twenty-five months in 50 per cent. glycerin, the preparation having been kept constantly in the refrigerator at about 4° C.

Protocol.—Sept. 24, 1913. A *Macacus rhesus* was inoculated intracerebrally with an emulsion of spinal cord containing virus K, which was derived from a paralyzed monkey on Aug. 21, 1911. On Oct. 1, or seven days after the inoculation, the animal was excitable, and the arms and back were weak. Oct. 2. Paralysis of extremities and back complete. Etherized. Lesions of experimental poliomyelitis.

The M A strain withstands glycerination probably as well as the K strain. A Macacus inoculated with an emulsion prepared from nervous tissue in glycerin for eight and eleven months respectively developed paralysis in characteristic manner.

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

[6] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1913.

[7] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 227.

[8] Landsteiner, K., and Levaditi, C., *Compt. rend. Acad. d. sc.*, 1910, cl, 131; *Ann. de l'Inst. Pasteur*, 1910, xxiv, 833. Römer, P. H., and Joseph, K., *München. med. Wchnschr.*, 1910, lvii, 1059.

Phenol.—That the poliomyelitic virus withstands phenolization was shown by Kraus,[9] who proposed first to treat paper filtrates of the infected spinal cord of monkeys with 0.5 per cent. and later with 1 to 1.5 per cent. solutions of phenol in order to diminish their potency and thus to make possible their employment for purposes of active immunization. Since the inoculations of the phenolized virus were made exclusively into subcutaneous tissues no index of the degree of its infectiousness was obtained. Macacus monkeys react irregularly to subcutaneous injections of the virus even when highly active. Flexner and Clark[10] employed phenol for the purpose of rendering bacteria-free the virus contained in tonsillar and adenoidal tissues, and to prepare them for inoculation into monkeys. They observed no injurious action of 0.5 per cent. phenol in the virus at the expiration of fourteen days' exposure. The long survival of the virus in undiminished activity in glycerin suggested the testing for activity of a specimen of human spinal cord and brain put aside in the refrigerator fifteen months before in 0.5 per cent. phenol.

Protocol.—Oct. 20, 1913. A *Macacus rhesus* was inoculated into the sciatic nerve and peritoneal cavity with an emulsion obtained from the central nervous tissues from an actively fatal case of poliomyelitis in a child, and preserved since July 25, 1912, in 0.5 per cent. phenol. Nov. 5. Paralysis of legs and weakness of back; arms strong. Nov. 6. Prostrate. Etherized. Lesions of poliomyelitis present.

Two experiments were made to determine whether 0.5 per cent. phenol acting upon the virus contained within a Berkefeld filtrate almost devoid of coagulable protein would affect the activity of the virus. The mixtures were allowed to remain at 22° C. for twenty hours and five days respectively, at the expiration of which period both were found to be highly infectious and capable of causing experimental poliomyelitis after average incubation time.

Freezing.—In contrast to the long survival of the virus in an active state in glycerin and phenol is its shorter survival when the tissues containing it are kept continually frozen at a temperature of —2° to —4° C. Under these conditions we have found the virus

[9] Kraus, R., *Wien. klin. Wchnschr.*, 1910, xxiii, 233; *Ztschr. f. Immunitätsforsch.*, 1911, ix, 117.

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

active at the end of six weeks; but infection did not occur when material frozen for one and a half to three years was employed for inoculation.

Neutralization Tests with Cerebrospinal Fluid.—After recovering from experimental poliomyelitis monkeys are not subject to reinoculation; an active immunity has developed.[11] This state of immunity is associated with the occurrence in the blood of principles that neutralize the virus *in vitro*.[12] Since the poliomyelitic virus produces profound alterations in the cerebrospinal fluid and this fluid gains ready access to the interstices of the central nervous tissues, tests were carried out to determine whether neutralizing antibodies existed within it. It is known that antibodies are not secreted in appreciable quantities into the cerebrospinal fluid; hence any considerable quantity that might be present there would probably have been produced locally.[13] The fluids selected for the tests were obtained from patients who had recently suffered from typical paralytic poliomyelitis from which they were convalescent, and were scrupulously free from blood. The acute symptoms had begun from one to three months prior to the collection of the cerebrospinal fluid.

Seven fluids were tested. The experiments were practically uniform. 0.1 of a cubic centimeter of highly active filtrate was mixed with from two to three cubic centimeters of the cerebrospinal fluid, the mixtures were incubated for two hours at 37° C., and placed in the refrigerator over night. The inoculations were intracerebral. Six of the monkeys developed typical paralysis after the average incubation period. One monkey remained perfectly well, from which it may be concluded that neutralization of the virus was accomplished. The child from whom the neutralizing specimen was obtained had been attacked about six weeks before. It would therefore appear possible, but highly unusual, for neutralizing immunity principles to be present in the cerebrospinal fluid during convalescence from epidemic poliomyelitis.

[11] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 45.
[12] Levaditi, C., and Landsteiner, K., *Compt. rend. Soc. de biol.*, 1910, lxviii, 311.
[13] Flexner, S., *Jour. Am. Med. Assn.*, 1913, lxi, 447.

SUMMARY.

The virus of poliomyelitis is neurotropic, and localizes, and probably is capable of multiplying in the extramedullary parenchymatous nervous organs. It has been demonstrated by inoculation tests in the intervertebral, Gasserian, and abdominal sympathetic ganglia.

All the ganglia show histological lesions, more or less severe, similar to those of the spinal cord and brain. The severest occur in the intervertebral ganglia, those next in severity in the Gasserian, while the mildest appear in the abdominal sympathetic ganglia. The interstitial lesions predominate over the parenchymatous, and in preparalytic stages the intervertebral ganglia show interstitial lesions, especially pronounced at the pial covering.

Epidemic poliomyelitis is a general disease of the nervous system, although the most prominent and important symptoms are those following injury to the motor neurones of the spinal cord and brain.

The virus of poliomyelitis is highly resistant to glycerin, in which it survives for more than two years; to 0.5 per cent. phenol, in which it survives for more than one year; while it succumbs after having been kept frozen constantly for several months.

It is unsafe to employ phenol to modify the virus of poliomyelitis for the purpose of active immunization.

The cerebrospinal fluid of convalescents tends to be devoid of the neutralizing immunity principles for the virus of poliomyelitis, although they may exceptionally be present within this fluid. Doubtless the immunity principles are not produced locally in the nervous tissues, but elsewhere in the body, and are carried to the nervous organs by the blood.