LOCALIZATION OF THE VIRUS AND PATHOGENESIS OF EPIDEMIC POLIOMYELITIS.*

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

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

Since epidemic poliomyelitis is an affection chiefly of the central nervous tissues, it may be supposed that the nervous organs possess a special affinity for its microbic cause.[1] Once the virus of the disease has gained access to, and multiplied within, the nervous tissues, it survives there, apparently, longer than it does in other organs in which, under ordinary conditions, it occurs far less regularly. It has been shown by experiment that the poliomyelitic virus readily reaches the central nervous system when it is brought into relation with the peripheral nerves. It is in this manner that infection is induced when the virus is brought into contact with the nasal mucosa, sciatic nerves, and probably also when it is injected into the subcutaneous tissues and peritoneal cavity.[2] Thus deposited, the virus ascends by way of the nerves to the olfactory lobes of the brain or to the spinal cord and intervertebral ganglia. It is to be assumed that in the case of the spinal nerves the ascent is by way of the afferent or sensory fibers; in the case of the nasal membrane, along the olfactory fibers. Hence the virus is carried both by nerves of common and of special sensation. In these instances the virus is brought into relation, not with special end organs, but with nerve fibrils, along which it travels. It appears, however, that the virus may enter the nerves by way of specialized end organs, which are themselves not appreciably injured by it.

INTRAOCULAR INOCULATION.

The demonstration that the virus of poliomyelitis may penetrate the uninjured sensory end organs has been made in connection with the optic nerve. An emulsion of the spinal cord carrying the active

* Received for publication, July 1, 1914.

1 Flexner, S., *Jour. Am. Med. Assn.*, 1910, lv, 1105.

2 Flexner, S., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 411.

virus and free from bacterial contamination can be injected into the vitreous chamber of the eye without causing appreciable inflammation. A small quantity of the vitreous humor is aspirated, under ether anesthesia, by means of a tuberculin syringe carrying a very fine needle, and replaced by an emulsion of the virus. The cloudiness thus caused tends to disappear, and the vitreous body to return to normal. After a variable incubation period, symptoms of experimental poliomyelitis appear, paralysis develops, and the pathological findings are characteristic of the disease.

Experiment 1.—Macacus rhesus. May 29. Withdrew under ether anesthesia as stated 0.1 c.c. vitreous humor by puncture of sclera and introduced 0.2 c.c. emulsion of active virus. June 7. Excitable. June 9. Weakness of arms. June 10. Ataxic. June 12. Paralysis of right arm; weakness of left leg; both eyes normal. June 15. Arms and legs weak. From this date the condition remained stationary until June 22, when death occurred from diarrhea.

The autopsy revealed old and recent dysenteric lesions of the large intestine. No visible changes were observed in the spinal cord or eyes. However, microscopical examination of the spinal cord, medulla, and intervertebral ganglia shows typical lesions of poliomyelitis. The retina of the inoculated eye appears normal.

Experiment 2.—Macacus rhesus. June 12. Withdrew, in the manner of experiment 1, 0.1 c.c. vitreous humor from the left eye and injected 0.2 c.c. paper filtrate of an emulsion of virus. The cloudiness of the vitreous humor did not entirely clear up. June 19. Excitable. June 22. Tremor of head; weakness of left leg; ataxia. June 23. Paralysis of left arm; weakness of right arm; double ptosis and slight left facial paralysis; weakness of left leg. June 24. Prostrate; etherized.

The spinal cord, medulla, and intervertebral ganglia present typical lesions of poliomyelitis. The ophthalmic ganglion on the inoculated side shows also a diffuse cellular infiltration.

The two experiments given confirm the supposed affinity which the poliomyelitic virus possesses for nervous tissues and they indicate also that the virus is capable of penetrating highly specialized end organs in order to reach peripheral nerves, along which it penetrates to the central nervous system. Incidentally it shows that the virus may pass from peripheral nerves into the adjacent ophthalmic ganglion, but whether directly from the eye, or indirectly after infection of the central nervous organs as occurs with the Gasserian ganglia, does not appear.[3]

[3] Landsteiner, K., and Levaditi, C. (*Compt. rend. Soc. de biol.*, 1909, lxvii, 787) succeeded in one instance in producing paralysis by inoculating the virus into the anterior chamber of the eye.

DISTRIBUTION OF THE VIRUS AFTER INTRAVENOUS INOCULATION.

It is established that experimental poliomyelitis may be caused with more or less regularity by insuring that the virus reaches the central nervous organs by way of the peripheral nerves. When the virus is brought directly into relation with the central nervous system by intracerebral and intraspinous injections the most constant results are secured. Probably the less constant effects which follow injection of the virus into the peripheral nerves result from the fact that the greater the distance the virus is compelled to travel along nerves, the more chances there are for miscarriage of infection, either by reason of too great dilution, or failure of the virus to reach the central organs at all.

In all these instances the affinity of the nervous organs for the virus may be exerted directly, since the virus is brought either immediately into relation with the nervous tissues, or reaches them directly through lymphatic communication. When the virus is brought to the nervous organs by means of the blood, it is at first separated from the tissues themselves by the vessels and other structures interposed between the blood itself and the nervous tissue. For this reason it has been observed that, while small or even infinitesimal doses of the virus suffice to induce quite constant infection by the intranervous mode of inoculation, large quantities of the virus produce only occasional and inconstant infection, when injected directly into the blood.

The cause of this discrepancy has already been traced to an apparent inability of the virus to enter directly the substance of the brain and spinal cord from the blood.[4] In order to reach these organs, the virus must, it appears, leave the blood and pass into the cerebrospinal fluid, with which it reaches the interstices of the tissues. Since the cerebrospinal liquid is a product of the activity of the choroid plexus, it has been assumed that the virus must first penetrate that structure. Experiments have been performed in order to study this phase of the subject more closely.

Infectivity of Organs.—When the virus is injected directly into the blood it is quickly distributed throughout the circulatory system

[4] Flexner and Amoss, *loc. cit.*

in the manner in which other microörganisms are distributed. In due time it may be assumed that the virus is deposited in certain organs, since experiment has shown that it does not remain long in the circulating blood.[5] Hence it is readily possible to ascertain the distribution of the virus by sacrificing at intervals the infected animals, and inoculating emulsions of the organs themselves. In this manner it can be determined whether the virus is distributed mechanically, or according to the affinity which the several organs display toward it.

The next experiments to be described have been devised to answer this question.

Experiment 3.—Macacus rhesus. May 4. 250 c.c. of a centrifugalized suspension of the spinal cord and medulla containing the active virus were injected into one of the superficial veins of the leg. Three days later, on May 7, the animal was etherized and the spinal cord, medulla, crura cerebri, and intervertebral ganglia were removed aseptically. These were made into 5 per cent. suspensions which were injected intracerebrally into three *Macacus rhesus* monkeys, A, B, and C.

Monkey A.—May 7. 2 c.c. of emulsion of intervertebral ganglia injected. May 10. Excitable. May 12. Right arm weak; left arm paralyzed. May 13. Prostrate; etherized. Typical poliomyelitis.

Monkey B.—May 7. Injected 2 c.c. of emulsion of spinal cord and medulla. This animal developed no symptoms and remained well indefinitely.

Monkey C.—May 7. Injected 2 c.c. of emulsion of crura cerebri. No symptoms of poliomyelitis developed and the animal remained well indefinitely.

Experiment 4.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus were injected intravenously. Four days later, Apr. 20, the animal was etherized. There were removed aseptically for inoculation: spleen, bone marrow, kidneys, spinal cord and medulla, which were made into 5 per cent. emulsions and injected intracerebrally into *Macacus rhesus* monkeys, D, E, F, and G.

Monkey D.—Apr. 21. Received 2 c.c. of the suspension made from portions of spinal cord and medulla. No symptoms of poliomyelitis developed, and the animal remained normal.

Monkey E.—Apr. 21. Received 2 c.c. of a suspension of portions of each kidney. No symptoms of poliomyelitis developed, and the animal remained normal.

Monkey F.—Apr. 21. Received 2 c.c. of a suspension of the spleen. Apr. 21. Excitable; ataxic. Apr. 25. Both legs and right arm paralyzed. Apr. 27. Died. Typical poliomyelitis.

Monkey G.—Apr. 21. Received 2 c.c. of suspension of bone marrow of both femurs. Apr. 24. Excitable. Apr. 29. Tremor; weakness of neck. Apr. 30. Prostrate; etherized. Typical poliomyelitis.

[5] Clark, P. F., Fraser, F. R., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 223.

Experiment 5.—Macacus rhesus. Mar. 31. Intravenous injection of 250 c.c. of centrifugalized virus. Five days later, Apr. 5, etherized and portion of spleen, bone marrow, spinal cord and medulla were removed aseptically and made into 5 per cent. emulsions and injected intracerebrally into *Macacus rhesus* monkeys, H, I, and J.

Monkey H.—Apr. 5. Injected 2 c.c. of suspension of spinal cord and medulla. No symptoms developed and the animal remained normal.

Monkey I.—Apr. 5. Injected 3 c.c. of the suspension of the spleen. Apr. 8 Excitable. Apr. 11. Ataxic. Apr. 12. Tremor; prostrate. Apr. 13. Died. Typical poliomyelitis.

Monkey J.—Apr. 5. Injected 3 c.c. of suspension of bone marrow from both femurs. Apr. 10. Excitable; ataxic; weakness of left leg. Apr. 17. Legs and left arm paralyzed; prostrate; etherized. Typical poliomyelitis.

The sections of the central nervous organs show not only marked lesions of poliomyelitis, but those of the fourth ventricle reveal an infiltration of the choroid plexus with mononuclear cells.

This series of experiments is consistent in exhibiting that, in spite of the essential affinity which the spinal cord and brain exhibit for the poliomyelitic virus, they are, nevertheless, unable to remove it directly from the blood, prior to some change taking place in the structures (vascular or secretory) that preside over the production of the cerebrospinal fluid; while the spleen and bone marrow, but not the kidney, readily remove it from this source. The fact that the intervertebral ganglia are capable of readily removing part of the virus from the blood shows, first that their relation to the blood vessels differs from that of the brain and spinal cord, and second explains the constant and early involvement of these structures in the poliomyelitic process. The ganglia, therefore, appear capable of obtaining the virus from two sources, namely, directly from the blood, and indirectly from the cerebrospinal fluid.

Moreover, the poliomyelitic virus may be retained alive in the body for a considerable period of time without gaining access to the interior of the central nervous organs.

Experiment 6.—Macacus rhesus. Apr. 18. Injected intravenously 250 c.c. of centrifugalized virus. No symptoms of poliomyelitis developed and seventeen days later, May 5, the animal was etherized. 5 per cent. suspensions of spleen and central nervous tissues were prepared and inoculated intracerebrally in monkeys K and L. Microscopic examination of sections of the spinal cord, medulla, and intervertebral ganglia prove them to be free from lesions of poliomyelitis.

Monkey K.—May 6. 2 c.c. of emulsion of medulla and spinal cord injected. No symptoms developed, and the animal remained normal.

Monkey L.—May 6. 2 c.c. of suspension of spleen injected. May 12. Excitable; ataxic. May 16. Arms weak; etherized. Poliomyelitis.

This experiment indicates, first that the virus of poliomyelitis is capable of surviving for a considerable period in the interior of the body, without inducing an infection of the central nervous system; and next that this long sojourn is not without effect on the quality of the virus, which would appear to have been weakened by the action of the spleen.

Effect of Aseptic Meningitis.—The permeability of the meninges for the contents of the blood is increased by inflammation of those structures. The introduction of sterile alien blood serum into the subarachnoid spaces causes an aseptic inflammation of mild degree that reaches its maximum in twenty-four hours, and then subsides. The inflammation is marked by emigration into the pia-arachnoid, cerebral ventricles, and choroid plexus of polymorphonuclear leucocytes chiefly, and by the escape of plasma. Neither the ependymal epithelium nor the perivascular lymphatics show appreciable change.

Experiments were conducted to ascertain the effect of this inflammation on the penetration of the virus of poliomyelitis into the central nervous organs.

Experiment 7.—Control. *Macacus rhesus.* Apr. 23. Injected intravenously 25 c.c. of centrifugalized suspension of spinal cord and medulla of paralyzed monkey. No symptoms developed and the animal remained normal.

Experiment 8.—Control. *Macacus rhesus.* Apr. 23. 50 c.c. of the centrifugalized suspension of virus used in the previous experiment were injected intravenously. The animal remained normal.

Experiment 9.—*Macacus rhesus.* Apr. 22. Injected intraspinously 3 c.c. of 40 per cent. inactivated horse serum. Apr. 23. Lumbar puncture; 0.5 c.c. of turbid fluid containing large numbers of white corpuscles was obtained. Injected intravenously 25 c.c. of centrifugalized suspension of virus as in experiments 7 and 8. Apr. 26. Excitable; legs weak. Apr. 30. Arms also weak; etherized.

Microscopic examination of the medulla, spinal cord, and intervertebral ganglia reveals pronounced lesions of poliomyelitis. While vascular lesions are everywhere pronounced, lesions of the nerve cells, interstitial substance, and meninges are also marked. The choroid plexus of the fourth and lateral ventricles is included in the sections. The plexus of the fourth ventricle shows definite infiltration with mononuclear (lymphoid) cells; the plexus of the lateral ventricle is less infiltrated. The blood vessels in the floor of the fourth ventricle are heavily infiltrated, while the deeper vessels are less affected, and those beneath the lateral ventricle are unaffected.

Experiment 10.—Macacus rhesus. May 5. Injected intraspinously 3 c.c. of 40 per cent. inactivated horse serum. May 6. Injected intravenously 25 c.c. of centrifugalized suspension of active virus. May 9. Excitable. May 13. Right arm paralyzed. May 14. Died.

The microscopic examination of sections of the medulla, spinal cord, and ganglia shows poliomyelitic lesions of moderate degree. The most pronounced lesions occur in the floor of the fourth ventricle. Vascular lesions are nowhere severe.

The foregoing observations raise anew the question as to the path traversed by the virus from the blood to the cerebrospinal fluid, and thence to the nervous tissue. Since the cerebrospinal liquid is the product of the secretory activity of the choroid plexus, it has been assumed that the impermeability of the plexus for most foreign products results in the exclusion also of microörganisms, as long as the secreting structures remain intact. Apparently such moderate quantities of the poliomyelitic virus as are contained in twenty-five to fifty cubic centimeters of a clear suspension of the spinal cord and medulla, taken from a paralyzed monkey, may be insufficient to inflict the necessary damage upon the choroid plexus, while still larger quantities may suffice to accomplish this. When, however, the superficial structures of the nervous organs, such as the meninges and choroid plexus, are put into a state of mild chemical inflammation, their permeability is increased, so that what was before an inadequate quantity of virus is now rendered sufficient to cause poliomyelitic infection.

Another fact has emerged from these experiments: while the blood vessels of the spinal cord and brain may show no unusual degree of pathological alterations, definite lesions of an infiltrative nature may appear in the choroid plexus itself. Before, however, a decision is reached as to the relation of the histological lesions to the escape of the virus from the blood, it is desirable to determine the nature of the lesions in animals that have developed paralysis from large unaided injections of the virus, administered intravenously.

Lesions Caused by Intravenous Injections.—While rhesus monkeys show almost no difference in susceptibility to the action of the poliomyelitic virus when introduced directly into the brain, they exhibit distinct differences when injections are made into the peripheral

parts of the nervous system, or into the blood. Moreover, the quality of the virus itself is brought out by the site of inoculation, since a specimen that is of less than maximal activity may be infectious even in minute doses, when introduced into the brain, and either not active at all, or slightly infectious when injected into the blood, or even into the sciatic nerves. As the experiments that follow show, the quantity of the centrifugalized suspension carrying the virus required to cause infection by the unaided blood route usually exceeds fifty cubic centimeters, while even 200 or more sometimes fail, although a few tenths of a cubic centimeter of the same virus succeed when introduced into the brain.

Before describing the lesions present in the spinal cord, brain, and intervertebral ganglia, caused by intravenous injections, it is desirable to present briefly the nature of the lesions of experimental poliomyelitis such as result from other modes of inoculation. The several lesions may be considered as they affect first the meninges, second the spinal cord, third the medulla and pons, fourth the cerebrum, and fifth the ganglia.[6]

The meninges of the cord and medulla show, as a rule, mononuclear cellular infiltration most pronounced adjacent to, or surrounding, the blood vessels which enter the fissures of the cord and are present in the floor of the fourth ventricle. The general infiltration of the pia-arachnoid is interstitial and as a rule not heavy, while the invasion about the vessels within the perivascular lymphatics is usually heavy, and sometimes is nodular. The spinal cord presents lesions most pronounced in the anterior gray matter, less marked in the posterior gray matter, and least present in the white matter. They are perivascular, interstitial, and parenchymatous. The vascular lesions, which are often pronounced, extend inward from the meninges; the interstitial ones are associated with the presence of mononuclear, to a less extent of polynuclear cells, rarely of red corpuscles, and commonly of serum. Actual necrosis of the ground substance arises, but is uncommon on a large scale. The anterior gray matter is rarely wholly destroyed at certain levels. The interstitial lesions can, in some instances, be traced outwards, directly from affected vessels. The lesions of the parenchyma consist of degeneration and necrosis of ganglion cells, occurring chiefly but not exclusively in the anterior gray matter. The necrotic cells are commonly invaded by phagocytes, the so called neurophages. It is not usual for definite relation to be obvious between the altered blood vessels and the affected interstitial substance or parenchyma. The lesions of the medulla resemble those of the spinal cord, except as they are modified by differences in structure. The vessels most infiltrated are those present im-

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

mediately beneath the fourth ventricle; the deeper lying vessels tend to be less affected, and the very small branches throughout the part are involved inconstantly. The focal interstitial lesions tend to be smaller than those of the cord. No definite relation can, as a rule, be made out between the vascular and interstitial changes. Because of the smaller size and less uniform distribution of nerve cells, the parenchymatous lesions are less conspicuous; they are, however, essentially identical with those of the cord. Lesions similar to those in the medulla occur in the pons and crura cerebri, but less frequently. The cerebrum is affected far less constantly than other parts of the nervous system. When present, the lesions are perivascular and focal interstitial. The cerebral meninges, as a rule, escape affection. The choroid plexus of the lateral and fourth ventricles has not been studied in all instances. When the virus has been introduced into the brain, cerebrospinal fluid, or nerves, the rule appears to be that the plexus escapes. However, exceptions to this rule occur, in which case lesions similar to those to be described as occurring after intravenous injection of the virus may arise (compare experiment 5, monkey J). The intervertebral ganglia are invariably affected. The lesions are of two main kinds, interstitial and parenchymatous, and are always focal. The cellular invasion proceeds from two sources; the pial investment and the blood vessels. In the former, direct extension may take place from the spinal meninges, or extension may occur by way of the connective tissue of the nerve roots. In the latter, extension seems to proceed from the blood vessels. It remains, however, to state that the involvement of the blood vessels may not arise through the general blood, but through inclusion of the vessels in the infiltrative process within the septa of the nerve roots. In rare instances the blood vessels present, as compared with other parts of the ganglia, an unusual degree of surrounding infiltration. The ganglionic nerve cells are destroyed in two ways: first, they are obliterated by focal accretions of mononuclear cells, and, second, by necrosis and neurophagocytosis, in the same manner as in the corresponding condition in the spinal cord.

With this description before us, we may now proceed to describe the lesions which arise as a result of infection by the intravenous mode of inoculation.

Experiment 11.—Macacus rhesus. Nov. 14. 100 c.c. of a Berkefeld filtrate of the virus were injected into the right saphenous vein. Nov. 24. Excitable; paralysis of the left leg and left side of the face. Nov. 25. Paralysis of arms and legs; weakness of back. Nov. 26. Prostrate. Dec. 1. Died.

The spinal cord, medulla, and ganglia show pronounced lesions. Those of the spinal cord affect the blood vessels, interstitial tissue, and nerve cells. They are not distinctive, and do not differ from the usual lesions. This fact is also true of the lesions in the medulla, which are most pronounced in the blood vessels in the floor of the fourth ventricle, and of the ganglia, where they are wide-spread.

Experiment 12.—Macacus rhesus. Nov. 7. Injected intravenously 54 c.c. of a Berkefeld filtrate of the virus. At the same time, under ether anesthesia, 5 c.c. of salt solution were introduced into the left cerebral hemisphere. Nov. 16. Excitable; ataxic. Nov. 17. Arms paralyzed; back weak. Nov. 18. Prostrate. Nov. 19. Etherized.

The lesions of the spinal cord, medulla, ganglia, and cerebrum are pronounced. Not only are the usual lesions present, but in addition severe affection of the blood vessels in the cord, medulla, and cerebrum occurs, from which infiltrations extend into the substance of the nervous tissues. The cerebral meninges at the site of the injection of salt solution also show infiltration.

Experiment 13.—Macacus rhesus. Jan. 30. 250 c.c. of centrifugalized suspension injected intravenously. Feb. 14. Excitable. Feb. 16. Weakness of legs. Feb. 17. Paralysis of arms. Feb. 18. Weakness of back. Feb. 19. Died.

The lesions are typical of poliomyelitis. The meninges of the spinal cord are diffusely infiltrated. The vascular lesions are moderate. There is widespread degeneration of the nerve cells, but none of the lesions are distinctive, or differ from those usually occurring.

Experiment 14.—Macacus rhesus. Oct. 30. Intravenous injection of 180 c.c. of centrifugalized virus. Nov. 9. The animal shows weakness and disturbance of vision. During the night it died.

The medulla, spinal cord, and ganglia are the seat of marked lesions of poliomyelitis. The vascular infiltration is heavy but the usual larger vessels only are affected. The cerebrum is devoid of lesions, while the crura cerebri are the seat of interstitial, but not of striking vascular lesions.

Experiment 15.—Macacus rhesus. Feb. 28. Intravenous injection of 250 c.c. of centrifugalized virus. Mar. 4. Excitable. Mar. 5. Arms and legs weak; ataxic. Mar. 6. Died.

The lesions of the spinal cord are perivascular, interstitial, and meningeal; of the ganglia, perivascular, with extension into the nerve roots. The medulla shows a high degree of affection of the blood vessels. The cerebrum has escaped, but the choroid plexus of the lateral ventricle, but not of the fourth ventricle, shows edema and perivascular cellular infiltration. The ependymal cells appear normal.

Experiment 16.—Macacus rhesus. Apr. 16. Intravenous injection of 250 c.c. of centrifugalized virus. During the injection a needle was kept in the lumbar spinal canal. Apr. 21. In the morning the arms and back were paralyzed. In the afternoon death occurred.

The lesions in this instance are very pronounced. The blood vessels within the spinal cord, medulla, and pons show wide involvement, while the interstitial tissue and nerve cells are affected only moderately. The cerebrum is devoid of lesions, while the choroid plexus of the lateral ventricles contains a slight accumulation of mononuclear cells about the blood vessels. The plexus of the fourth ventricle appears normal.

Experiment 17.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus injected intravenously. Apr. 20. Lumbar puncture yielded a fluid containing an excess of white corpuscles. Apr. 21. Excitable; no paralysis; etherized.

The spinal cord, medulla, and pons show early vascular, but no other lesions. The ganglia, however, contain focal cellular infiltrations of small size, and a small number of single necrotic nerve cells. No changes were detected in the choroid plexus.

Experiment 18.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus injected intravenously. Apr. 20. No symptoms appeared; etherized.

No lesions were detected in the spinal cord, medulla, or choroid plexus, while the ganglia show early infiltrative lesions about the blood vessels, and a few instances of necrosis of single nerve cells.

We may consider this series of experiments according as the lesions affect the nervous tissues proper, or as they affect the choroid plexus.

Within the nervous tissues proper, the lesions are, at times, precisely similar to, and indistinguishable from, those produced by intraneural modes of inoculation. However, in certain instances, the lesions present not only resemble those caused by the intraneural modes of inoculation, but differ from them in the extent and degree to which the blood vessels, and those especially in the medulla and pons, are affected. While the degree of perivascular infiltration does not afford a basis of discrimination, a sharp distinction may be drawn between the usual degree of vascular involvement, and the unusual extent in which it occurred in several cases of intravenous injection. What is especially impressive in the latter instances is the diffuse participation of small vessels, down to those of capillary size, in the process and the extension of the infiltrative process from them to the surrounding nervous tissues. Vessels so greatly altered as those under consideration may be considered as contributing to the permeation of the virus from the blood into the tissues. The early lesions of the intervertebral ganglia should be emphasized in this place since they antedate those of the spinal cord and medulla, and extend apparently from vascular lesions.

What appear, however, to be especially important are the changes detected in the choroid plexus, in which infiltrative lesions have hitherto not been observed. That definite lesions of the plexus may occur is clearly indicated by the experiments. As yet no evidence has been obtained of morphological alterations in the ependymal cells, but merely in the blood vessels beneath them. That the secretory functions of the plexus are altered in the direction of greater permeability may be safely assumed, from which it follows that the experiments indicate that when the poliomyelitic infection is induced by the intravenous injection of the virus, there arise, not only the common lesions of poliomyelitis, but also certain additional lesions of the blood vessels and choroid plexus which are of peculiar and distinctive nature.

Since the precise mode of infection in human cases of poliomyelitis may be regarded still as an open question, this criterion of a blood invasion may prove of assistance in the solution of the problem. So far as can be judged from the study of the tissues from several human cases, a corresponding wide-spread vascular involvement to that arising in the experiments would seem not to have occurred.

INTRASPINOUS SERUM PROTECTION.

The data presented confirm and extend the observations already made concerning the passage of the poliomyelitic virus in transit to the central nervous organs from the blood to the cerebrospinal fluid. Since the virus is known to pass successively over several days into the cerebrospinal fluid,[7] in which it seems not to accumulate, but from which it is transferred to the nervous tissues, it was thought that the introduction of a potent immune serum into the meninges at intervals over a number of days would suffice to neutralize the translated virus and thus prevent infection. For this purpose immune serum was available from several monkeys which had recovered from poliomyelitis and had been subsequently reinforced by large subcutaneous injections of the virus.

Earlier experiments had shown that the intraspinous injection of an immune serum is effective under circumstances in which the intravenous injection is not, in delaying or preventing poliomyelitic infection in the monkey. For the next series of experiments it was necessary at the outset to insure that the intravenous injections of the virus would alone induce paralysis, which was accomplished by employing the device of setting up an aseptic meningitis with horse serum, in the animals about to be inoculated.

SERUM AFTER INTRAVENOUS INOCULATION.

Experiment 19.—Macacus rhesus. May 26. Intraspinous injection of 3 c.c. of inactivated 40 per cent. horse serum. May 27. Intravenous injection of 50 c.c. of centrifugalized virus, followed immediately afterwards by an intraspinous injection of 3 c.c. of normal monkey serum. The normal serum was injected intraspinously on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. June 3. Excitable. June 9. Paralysis of arms and back. June 10. Prostrate; etherized. Typical poliomyelitis.

[7] Flexner and Amoss, *loc. cit.*

Experiment 20.—Macacus rhesus. May 29. Intraspinous injection of 3 c.c. of inactivated 40 per cent. horse serum. May 30. Intravenous injection of 50 c.c. of centrifugalized virus, followed immediately afterward by an intraspinous injection of 3 c.c. of immune monkey serum. The immune serum injections were repeated May 31, June 1 and 2, and, after a two days' interval, on June 5, 6, and 7. No symptoms developed, and the animal remained normal.

Since these experiments show that the virus may be neutralized by an immune serum in process of passage by way of the cerebrospinal fluid to the nervous tissues, it seemed desirable to ascertain whether a similar neutralization could be affected in a case in which the virus was introduced directly into the meninges by means of lumbar puncture.

SERUM AFTER INTRASPINOUS INOCULATION.

Experiment 21.—Macacus rhesus. May 27. Intraspinous injection of 1 c.c. of emulsion of virus. Two hours later, intraspinous injection of 3 c.c. of normal monkey serum. The injection of normal serum was repeated on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. June 3. Excitable. June 7. Ataxia; arms and neck weak. June 8. A.M. Arms and back paralyzed; legs weak. P.M. Died. Typical poliomyelitis.

Experiment 22.—Macacus rhesus. May 27. Intraspinous injection of 1 c.c. of emulsion of virus. Two hours later, injected 3 c.c. of immune monkey serum intraspinously. The immune serum injections were repeated on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. No symptoms developed and the animal remained well.

The preceding experiments show unmistakably that by introducing an immune serum into the subarachnoid spaces, the poliomyelitic virus is capable of being neutralized within the cerebrospinal fluid into which it is directly introduced, or to which it passes in transit from the blood to the nervous tissues. Probably the neutralization in the latter instance is effected at successive stages in process of transfer of the virus to the central nervous organs. Normal serum lacks this power of neutralization.

It may be considered as highly probable that the neutralization is accomplished before any quantity of the virus becomes attached to the nervous tissues themselves. Earlier experiments had shown that when such minute amounts of the virus as one fiftieth to one tenth of a cubic centimeter are inoculated intracerebrally, neutralization is either wholly impossible to accomplish, or is accomplished with very great difficulty even by intraspinous injections of immune

serum.[8] Hence the experiments described carry a step further the demonstration that the virus introduced into the blood passes by way of the cerebrospinal fluid to the substance of the nervous tissues in those instances in which paralysis results.

RELATION OF CARMIN TO THE CHOROID PLEXUS AND PERIVASCULAR LYMPHATICS.

That the virus of poliomyelitis is capable, in some instances, of passing from the blood to the cerebrospinal fluid may be considered as demonstrated. This passage takes place probably by way of the choroid plexus and possibly also, to some extent, through the blood vessels in the meninges as well as in the substance of the nervous tissues. Although certain lesions have been detected in the choroid plexus, no morphological alterations have been discovered in the ependymal cells themselves. Hence the question arose whether by the use of pigments the ependymal cells in certain pathological states, including poliomyelitis, might be shown to react in a manner supplying ocular evidences of a disturbance of function.

Carmin is a non-toxic pigment which can be sterilized and suspended in a fine state of subdivision. In this form its introduction into the cerebral ventricles and subarachnoid spaces causes no discomfort in monkeys. Its presence in the meninges and ventricles is followed by an inflammation and rich cellular exudation. The emigrated cells are polynuclear chiefly, but mononuclear cells which take up pigment granules emigrate also. The effects of the carmin injections were studied in normal monkeys, and in monkeys in which an aseptic inflammation had been set up twenty-four hours earlier by means of horse serum, or in which poliomyelitis had been induced by intracerebral inoculation of the virus.

A suspension of the pigment was made in 20 per cent. glycerin, and ammonia was added until solution was complete. This solution was autoclaved and immediately before use was slowly neutralized by the repeated addition of small amounts of sterile 2 N hydrochloric acid, until litmus paper indicated change of reaction. The injections were made under ether anesthesia into the lateral ven-

[8] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1910, liv, 1780; 1910, lv, 662.

tricle, the volume of fluid injected being determined by a needle in the lumbar meninges, from which the colored solution was allowed to flow before the injection was stopped.

Within twenty-four hours the pigment is distributed over the surfaces of the spinal cord and brain, and within the cerebral ventricles. The base of the brain is deeply and uniformly pigmented. The intervertebral ganglia are either unaffected or mottled with pigment. The nerve roots are visibly pigmented. The choroid plexus appears a vivid red color.

Normal monkeys etherized respectively twenty hours and five days after the injection of the pigment show the inflammatory reaction of the meninges and ventricles mentioned. Interest centers especially in the relation of the pigment to the ependymal cells, choroid plexus, perivascular lymphatics, and intervertebral ganglia. The differences in this respect between the twenty-hour and the five-day specimens are inconspicuous and unimportant.

The pigment appears in two states of division, namely, as excessively minute particles, smaller than many bacteria, and as coarser grains. The latter are contained largely in the mononuclear cells of the inflammatory exudate. The very fine particles have been taken up by the ependymal cells covering the walls of the ventricles and the surfaces of the choroid plexus. Not all, but many of the ependymal cells contain the pigment particles in varying number. The minute and larger grains occur also within cells in the subependymal layer, in close proximity to the ventricles. The latter pigment-containing cells do not seem to have emigrated from the interior of the ventricles, so it is considered probable that the pigment has passed from the ependymal to the subependymal cells. A small quantity of pigment occurs also in the superficial perivascular spaces in the cortex, but not in the spinal cord. The pigment penetrates to the interior of the ganglia with difficulty, along two courses: first the pia capsular investment, second the septa of the nerve roots. About the pigment there is a marked cellular reaction, and pigment-containing leucocytes come to lie against or near nerve cells, but no wide diffusion occurs within the ganglia. The meninges of the brain and spinal cord show a rich cellular exudation containing pigment.

When an aseptic inflammation has been set up previously, and the animal etherized twenty-four hours after the pigment has been injected, the distribution is identical with that described. The single difference noted is a greater amount of pigment within the ependymal and subependymal cells of the ventricles. In the case of an animal in which the pigment was injected during the early paralytic stage of poliomyelitis and which was etherized twenty-four hours later, the cellular accumulations were greater because of the addition of the polynuclear cells to the usual mononuclear infiltration. The choroid plexus of the lateral ventricle showed marked lymphoid, nodular aggregations beneath the ependymal cells, and a rich leucocytic emigration outside. The quantity of pigment taken up by the ependymal and subependymal cells is somewhat greater than that observed in the other instances.

The experiments with the carmin may be interpreted as indicating that the ependymal cells in a living state can be entered by particulate substances. Whether the strictly normal ependymal cells take up and pass on, in the manner described, pigment particles cannot be deduced from the experiments, as the carmin itself causes an inflammatory reaction and consequently may act injuriously upon the cells. Aside from the presence of the pigment, the ependymal cells exhibit no morphological alteration. Apparently a previous inflammation, such as that caused by horse serum and the virus of poliomyelitis, has the effect of rendering the ependymal cells more permeable for the pigment.

PATHOGENESIS OF POLIOMYELITIS.

A consideration of the experiments described in this paper should deal with the question of the pathogenesis of poliomyelitis which they are believed to elucidate.

We are confronted with the problem as to the site of entrance of the virus of poliomyelitis into the human body, as well as the manner in which the specific lesions of the disease are produced. The latter question has already been cleared up in large measure.[9]

[9] Flexner, *Jour. Am. Med. Assn.*, 1910, lv, 1105. Flexner and Lewis, *Jour. Exper. Med.*, 1910, xii, 227.

It is now sufficiently obvious that the virus possesses affinity for nervous tissues in general, but for no element of these tissues in particular. The constancy with which meninges, blood vessels, interstitial parts, and nerve cells are affected indicates that they all react to the presence of the virus. On the basis of actual observations it cannot be stated that virus is attracted by the nerve cells, either alone or necessarily in advance of the other structures mentioned; while the experiments here recorded show that it is only when the virus is brought to the nervous organs otherwise than by the general blood that the tissues composing them are able readily to remove and attach it to themselves.

This latter fact is a cardinal point, and one from which we may derive valuable information on the pathogenesis and mode of infection of the disease.

It may be regarded as established that all intraneural means of infection are successful, and that the virus travels with more or less ease and certainty along the nerves to the interstices of the central nervous organs, probably utilizing the lymphatic channels of communication. The experiments given in this paper show that the central nervous organs, excepting the intervertebral ganglia, are incapable of removing the virus from the general blood prior to changes induced in the blood vessels and in the choroid plexus. They indicate, also, that in the monkey these preliminary lesions are of a nature that permits of differentiation from the lesions caused by the intraneural modes of infection. The lesions in human cases of poliomyelitis would seem to correspond with those caused by intraneural and not by intravenous inoculation.

In general it should be stated that the intraneural modes of inoculation are effective in proportion to the degree with which they bring the virus into intimate relation with the central nervous tissues. On that account intracerebral inoculation is the most effective, because it not only insures contact between the virus and the mechanically injured tissues, but also because it isolates the virus in the brain tissue, under conditions favorable to multiplication and gradual diffusion into the ventricles and cerebrospinal fluid. Intraspinous injection is somewhat less effective for the reason that a part, and sometimes perhaps all, of the virus may be carried into the gen-

eral blood before it can reach and become attached to the nervous tissues. Intranasal infection is, in keeping with the general statement made above, more certain in its results than subcutaneous or intrasciatic inoculation, because of the proximity of the short olfactory nerve fiber to the brain tissue. It may fail, because the virus is washed away before it reaches the olfactory fibers and can be carried to the brain. Possibly intraocular inoculation may prove among the most successful, because the virus cannot escape and has only a short distance to travel to the brain; while the vitreous humor may even prove a favorable medium for its multiplication.

In the main, under natural conditions, it is the upper respiratory mucous membrane that would most often become contaminated with the virus, and most readily favor its conveyance to the brain. This series of events is determined by the manner in which the virus is thrown off by the infected body,[10] by the fact of its presence upon the nasal mucosa, even in healthy persons in contact with cases of poliomyelitis,[11] and by the demonstration that it passes, on the whole easily, along the olfactory nerve fibers to the brain, medulla, and spinal cord.[12] Although the virus is conveyed to the nervous organs from without by the lymph, the distribution throughout the nervous system is, in large part at least, effected through the medium of the cerebrospinal fluid. Even when the virus passes from the blood into the nervous organs, it takes the indirect course through the cerebrospinal fluid. This important fact has been established, not only by the finding of the virus by inoculation tests in the cerebrospinal fluid[13] after a blood injection, but also through the prevention of infection by the injection of immune serum into the subarachnoid spaces after lumbar puncture following the intravenous infusion of the virus under conditions insuring infection.

[10] Flexner, *Jour. Am. Med. Assn.*, 1910, lv, 1105. Flexner and Lewis, *Jour. Exper. Med., loc. cit.*

[11] Flexner, S., Clark, P. F., and Fraser, F. R., *Jour. Am. Med. Assn.*, 1913, lx, 201.

[12] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1910, liv, 1140. Flexner and Clark, *Proc. Soc. Exper. Biol. and Med.*, 1912–13, x, 1. Flexner, *Lancet*, 1912, ii, 1271; *Science*, 1912, xxxvi, 685. Landsteiner and Levaditi, *Ann. de l'Inst. Pasteur*, 1910, xxiv, 833.

[13] Flexner and Amoss, *loc. cit.*

Thus the experimental evidence, which is upheld by observations in human cases of poliomyelitis, supports the view that epidemic poliomyelitis is caused by the entrance into the body of its specific microbic cause or virus, through the upper respiratory mucous membrane to the olfactory lobes of the brain, from which by means of the cerebrospinal fluid it is distributed throughout the substance of the nervous organs; but, since the virus may reach the brain by way of any nervous channel, and even, although with great difficulty, from the blood, it is, of course, possible that in exceptional instances other modes of infection may arise.

SUMMARY.

The virus of poliomyelitis is capable of penetrating the retina without producing apparent injury, to reach the central nervous organs.

The virus injected into the blood is deposited promptly in the spleen and bone marrow, but not in the kidneys, spinal cord, or brain.

Notwithstanding the affinity which the nervous tissues possess for the virus, it is not removed from the blood by the spinal cord and brain until the choroid plexus and blood vessels have suffered injury.

The intervertebral ganglia remove the virus from the blood earlier than do the spinal cord and brain.

An aseptic inflammation produced by an intraspinous injection of horse serum facilitates and insures the passage of the virus to the central nervous organs, and the production of paralysis. The unaided virus, even when present in large amounts, passes inconstantly from the blood to the substance of the spinal cord and brain.

When the virus within the blood fails to gain access to the central nervous organs, and to set up paralysis, it is destroyed by the body, in course of which destruction it undergoes, as a result of the action of the spleen and, perhaps, other organs, diminution of virulence.

The histological lesions that follow the intravenous injections of the virus in some but not in all cases differ from those which result from intraneural modes of infection.

In escaping from the blood into the spinal cord and brain, the

virus causes a lymphatic invasion of the choroid plexus and widespread perivascular infiltration, and from the latter cellular invasions enter the nervous tissues. A similar lymphoid infiltration of the choroid plexus may arise also from an intracerebral injection of the virus.

The histological lesions present in the central nervous organs in human cases of poliomyelitis correspond to those that arise from the intraneural method of infection in the monkey.

The virus in transit from the blood through the cerebrospinal fluid to the substance of the spinal cord and brain is capable of being neutralized by intraspinous injection of immune serum, whereby the production of paralysis is averted.

Carmin in a sterile and finely divided state introduced into the meninges and ventricles sets up an aseptic inflammation, but is quickly taken up by cells, including ependymal cells. When an aseptic inflammation has been previously established by means of horse serum, or when the nervous tissues are already injured by the poliomyelitic virus, the pigment appears to enter the ependymal cells more freely.

The experiments described support the view that infection in epidemic poliomyelitis in man is local and neural, and by way of the lymphatics, and not general and by way of the blood. Hence they uphold the belief that the *infection atrium* is the upper respiratory mucous membrane.