

PENETRATION OF THE VIRUS OF POLIOMYELITIS  
FROM THE BLOOD INTO THE CEREBRO-  
SPINAL FLUID.\*

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An active poliomyelitic virus readily causes infection in monkeys when introduced into the brain, subarachnoid spaces, or peripheral nerves. When, however, the virus is injected into the circulation, far greater quantities are required to produce infection and the onset of the usual symptoms and the paralysis are delayed.<sup>1</sup> Were the virus withdrawn from the blood directly by the tissues of the central nervous system neither the greater dose nor the longer incubation should be necessary. It appears that the extranevous organs retain but little of the virus; hence we may suppose that the route by which the virus contained within the blood reaches the central nervous system is an indirect one.

It is now generally conceded that the poliomyelitic virus enters the human body by way of the upper respiratory passages, and in particular through the nasopharyngeal mucous membrane. Once within this membrane the virus may pass through the lymphatic channels surrounding the filaments of the olfactory nerve to the leptomeninges where it reaches the cerebrospinal fluid, or it may first enter the blood and be conducted to the central nervous organs by the general circulation. Flexner and Clark<sup>2</sup> have shown experimentally that when the virus is introduced into the upper nasal mucosa in monkeys its propagation can be followed from the olfactory lobes of the brain to the medulla oblongata and spinal cord. Had the distribution of the virus taken place by way of the general circulation the several parts of the nervous organs should have been

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<sup>1</sup> Clark, P. F., Fraser, F. R., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 223.

<sup>2</sup> Flexner, S., and Clark, P. F., *Proc. Soc. Exper. Biol. and Med.*, 1912-13, x, 1.

rendered infectious almost simultaneously. The virus of poliomyelitis has hitherto been regarded as strongly neurotropic; but it does not follow from the fact of this neurotropic affinity that the nervous tissues can under all circumstances remove the virus from the blood.

Another possibility exists, namely, that the virus introduced into the blood finds its way not directly to the nervous organs, but indirectly by way of the cerebrospinal fluid. When, therefore, a quantity introduced into the blood is insufficient to cause infection, although a much smaller quantity produces infection when introduced into the brain, the reason may be that the intact choroid plexus prevents the virus from reaching the cerebrospinal liquid. It is well known that this anatomical barrier excludes from the cerebrospinal fluid many substances contained within the blood, that the barrier is not absolute but is capable of being broken down, and that the most frequent source of injury is the pathogenic action of infectious microorganisms.

Hence we may consider that when insufficient quantities of the poliomyelitic virus are introduced into the blood they do not set up poliomyelitis because they fail to injure the choroid plexus; and when poliomyelitis is set up by larger quantities the plexus has been penetrated.

The virus has thus far not been detected by inoculation experiments in the cerebrospinal fluid obtained from human cases of poliomyelitis; and, indeed, when the virus is injected into the subarachnoid spaces in monkeys it remains within the fluid for a limited time and can no longer be detected there at the period of the onset of paralysis.<sup>3</sup> The conditions within the fluid are obviously unfavorable for the propagation of the virus; but the fluid constitutes the most immediate route for the passage of the virus to the interior of the nervous organs in which it multiplies and becomes fixed. The cerebrospinal fluid therefore acts merely as a medium for transporting the virus to the nervous tissues. Hence, should the passage of the virus contained within the blood proceed to the nervous organs by way of the cerebrospinal fluid it should be possible to detect it in transit. From the many failures to discover the virus in

<sup>3</sup> Clark, Fraser, and Amoss, *loc. cit.*

the early paralytic stages of poliomyelitis in man and the monkey it might appear that the probability of detecting the virus at all might be small. But it would now appear that when a large dose of the virus has been injected into the blood and a sufficient period allowed to elapse in order that the virus may act upon and injure the choroid plexus, it is possible to detect the presence of the virus in the cerebrospinal fluid by inoculation.

#### EXPERIMENTAL.

A passage strain of highly active K virus was employed for the intravenous inoculation. A Berkefeld filtrate of a 5 per cent. suspension caused paralysis and death when inoculated intracerebrally in doses of 0.2 to 0.3 of a cubic centimeter. For the purpose of intravenous inoculation the 5 per cent. suspension was merely centrifugalized and the clear supernatant fluid pipetted off. It was not filtered.

*Experiment A. Macacus rhesus 1.*—Jan. 30. 250 c.c. of the supernatant fluid were injected into a superficial vein of the leg. No effect was produced by the injection. No symptoms appeared until Feb. 14, when slight excitability was noted. Feb. 16. Weakness of the leg. Feb. 17. Excitability increased and arms paralyzed. Feb. 18. Legs and back paralyzed. Feb. 19. Died.

Lumbar puncture was performed as follows: on Feb. 1 (48 hours after the injection) 0.6 c.c. of cerebrospinal fluid was removed; on Feb. 2 (72 hours after the injection) 0.5 c.c. of fluid removed; on Feb. 3 (96 hours after the injection) 0.9 c.c. of fluid removed; and on Feb. 18 (19 days after the injection) 2.9 c.c. of fluid removed. The several samples of cerebrospinal fluid were free from blood. On Feb. 18 a sample of blood was also taken and defibrinated.

The specimens of cerebrospinal fluid and blood were injected intracerebrally into monkeys as follows:

*Macacus rhesus 2.*—Feb. 1. 0.6 c.c. of cerebrospinal fluid withdrawn from monkey 1 48 hours after an intravenous injection of K virus was inoculated intracerebrally. No symptoms were produced; the monkey remained well.

*Macacus rhesus 3.*—Feb. 2. 0.5 c.c. of cerebrospinal fluid withdrawn from monkey 1 72 hours after an intravenous injection of K virus was inoculated intracerebrally. Feb. 14. Monkey somewhat excitable, movements slow. The condition remained stationary until Feb. 25, when the excitability was more marked. No weakness of muscles was detected. Lumbar puncture yielded a clear fluid devoid of globulin and containing forty white corpuscles per c.mm. Feb. 26. Condition not so good; ataxia; slight weakness of both arms. Mar. 10. Condition stationary.

*Macacus rhesus 4.*—Feb. 3. 0.9 c.c. of cerebrospinal fluid withdrawn from

monkey 1 96 hours after an intravenous injection of K virus was inoculated intracerebrally. Feb. 7. Excitability. The condition did not change materially until Feb. 17 when excitability was greater. The condition again became stationary until Feb. 26, when a general paralysis of the muscles was present. Animal etherized. Lesions of poliomyelitis present.

*Macacus rhesus 5.*—Feb. 18. 2.9 c.c. of cerebrospinal fluid withdrawn from monkey 1 19 days after an intravenous injection of K virus and the 1st day of paralysis were inoculated intracerebrally. Feb. 26, A. M. Excitable; tremor; ataxic. P. M. Left arm flaccid. Feb. 27. Animal prostrate; all members were paralyzed except the tail and a few neck muscles. Etherized. Lesions of poliomyelitis present.

*Macacus rhesus 6.*—Feb. 18. 5 c.c. of defibrinated blood withdrawn from monkey 1 19 days after an intravenous injection of K virus and the 1st day of paralysis were inoculated intracerebrally. No symptoms developed; the animal remained well.

The experiments bring out several facts. (1) Even a very large dose of an active poliomyelitic virus when injected into the blood produces a much delayed infection. If we consider the certain effective dose of the specimen of K virus employed when injected intracerebrally at 0.2 of a cubic centimeter, then 1,250 doses were introduced into the blood of monkey 1. The average incubation period after an intracerebral inoculation is about 6 days;<sup>4</sup> in monkey 1 the period was 17 days. (2) The cerebrospinal fluid removed 48 and 72 hours, respectively, after the intravenous injection failed, when inoculated intracerebrally, to communicate definite poliomyelitis to rhesus monkeys; while the fluid removed 96 hours (monkey 4) and 19 days (monkey 5) after the intravenous injection caused typical poliomyelitis in monkeys of this species. In monkey 4 the incubation period was indefinite and in monkey 5 it was 8 days. (3) By the 19th day following the intravenous injection of the large dose of the virus and at the onset of the paralysis the virus had disappeared from the blood while it was still detectable in the cerebrospinal fluid by inoculation.

#### DISCUSSION.

The experiments emphasize in the first place the relatively great difficulty of infecting monkeys with the virus of poliomyelitis by introducing it directly into the blood. At first sight it may appear that this statement is in conflict with the effects of subcutaneous or

<sup>4</sup> Clark, Fraser, and Amoss, *loc. cit.*

even of intraperitoneal inoculations. It is, however, not improbable that in all external modes of inoculation practiced, except the intravenous mode, the virus actually penetrates to the central nervous organs by way of the nerves. In any event the difficulties in the way of accomplishing infection through the general blood provide another argument against the notion that the virus of epidemic poliomyelitis is communicated to man by the bite of infected blood-sucking insects.

On the other hand, the experiments afford valuable support to the hypothesis that infection of the nervous organs in man occurs through the mediation of the cerebrospinal fluid. The virus readily traverses the nasal mucous membrane to reach this fluid, which is capable of carrying the virus to the interstices of the nervous tissues. Apparently the virus enters the intimate structures of the nervous tissues not directly from the blood but indirectly after being passed from the blood to the cerebrospinal fluid. To accomplish this transfer time is required since the barrier of the choroid plexus must first be overcome. At the expiration of 48 hours following the intravenous inoculation the barrier appears still to be intact; at the expiration of 72 hours the passage of the virus seems to have begun, since infection of mild type followed the inoculation of the cerebrospinal fluid removed at this period. At the expiration of 96 hours it appears that the barrier had broken down; and it also appears that under the pathological conditions created the virus, in quantity sufficing to cause infection, still persisted in this fluid as late as the 19th day, although no longer detectable in the blood by the inoculation test. In no other instance has the virus been found in the cerebrospinal fluid at the period of the onset of paralysis.

When we consider the minute size of the microorganism constituting the virus of poliomyelitis we may well wonder at the failure to penetrate the capillaries to gain access to the interstices of the central nervous organs. The case is not wholly unique. von Behring discovered that the hen, which is insusceptible to the action of tetanus toxin injected into the blood, is subject to its effects when introduced into the cerebrospinal fluid. Lesions of the leptomeninges of an interstitial character are implicated in the develop-

ment of the specific lesions of poliomyelitis. They also suffice to produce at times in man and the monkey a poliomyelitic affection of the meninges in which the central nervous organs proper do not share specifically. Hence the meninges and the cerebrospinal fluid play a highly important and even a determining part in the pathogenesis of epidemic poliomyelitis.

#### CONCLUSIONS.

The virus of poliomyelitis introduced into the blood may pass indirectly by way of the cerebrospinal fluid to the interstices of the central nervous organs.

To reach the cerebrospinal fluid the virus must first penetrate the barrier of the choroid plexus, which operation requires time. By the inoculation test, no virus was detected in the fluid at the expiration of 48 hours, only small amounts at the expiration of 72 hours, while at the expiration of 96 hours the virus had passed more freely. The virus was still detectable in the fluid at the onset of paralysis 19 days after the intravenous injection.

Pathological conditions of the leptomeninges and the cerebrospinal fluid play an important part in the pathogenesis of epidemic poliomyelitis.