THE PASSAGE OF NEUTRALIZING SUBSTANCES FROM THE BLOOD INTO THE CEREBROSPINAL FLUID IN POLIOMYELITIS.

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Recovery in poliomyelitis as in other infectious diseases is accomplished through a process of active immunization. The immunity substances on which recovery depends have been detected by neutralization\textsuperscript{1} and protection\textsuperscript{2} tests, but not by other biological methods. Similarly, monkeys in which experimental poliomyelitis is induced by inoculation yield corresponding immunity bodies. Evidence exists to show that the neutralizing principles arise irrespective of the intensity of the clinical symptoms,\textsuperscript{3} and apparently as early as the 6th day of the disease (page 501). Similar conditions exist in respect to the experimental disease in monkeys. Both protective and neutralizing principles have been discovered in animals which have passed through an attack induced by inoculation of filterable virus from human or from monkey sources.

The manner in which the immunity principles act in arresting the pathologic processes has become of especial interest on account of the treatment of cases of epidemic poliomyelitis by intraspinal injections of immune human and other sera. This method is based on the therapeutic experiments of Flexner and Lewis\textsuperscript{4} with monkeys, the results of which were later extended to human beings by Netter and his coworkers\textsuperscript{5} and since by many others.\textsuperscript{6,7,8}

\textsuperscript{1} Levaditi and Landsteiner, Compt. rend. Soc. biol., 1910, lxvii, 311.
\textsuperscript{7} Weekly Bull. Dept. Health, City of New York, 1916, iv, s. v, 345.
\textsuperscript{8} Fischer, L., Med. Rec., 1917, xci, 52.
Recently, however, both normal human serum and normal horse serum have been employed for intraspinal injection. It is still too early to state whether these latter sera exerted any definite influence on the pathologic processes.

All sera introduced into the subarachnoid spaces act as foreign bodies, and if sterile give rise to aseptic inflammation. The severity of the inflammation is less with homologous, than with heterologous serum. But experiments have shown that in monkeys very slight changes in the permeability of the meninges allow infection to take place after intravenous injection of the virus which otherwise is incapable of causing infection.

Hence we have attempted to detect in the cerebrospinal fluid immunity principles such as exist in the blood. In the course of other experiments carried out by us at a much earlier period, neutralization tests were made with the cerebrospinal fluid. Seven fluids were tested, of which only one proved neutralizing. This specimen came from a convalescent child paralyzed 6 weeks earlier. No examination was made at the time to discover whether the fluid indicated persistence of the inflammatory processes in the meninges. The conclusion reached was to the effect that, while possible, it was unusual for neutralizing principles to be contained in the cerebrospinal fluid during convalescence from epidemic poliomyelitis. Incidentally, it was determined that neutralizing bodies were not produced locally.

The present inquiry has arisen from the idea that under certain circumstances immunity substances enter the cerebrospinal fluid from the blood and assist materially in the healing process. If this supposition is founded on fact, we might view the inflammatory conditions occurring in the meninges, which increase their permeability to circulating proteins otherwise excluded, as beneficial; and from this it may follow that any advantage actually shown to be derived from the intraspinal administration of normal human or horse serum.

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*Flexner, S., Clark, P. F., and Amoss, H. L., J. Exp. Med.*, 1914, xix, 205.

may be the result not of the effects of the serum as such, but of a further increase in this permeability.

Neutralization Tests with Cerebrospinal Fluid.

Three preliminary tests were made with human cerebrospinal fluid and one sample of human blood. Two of the former were drawn on the 2nd and 18th days, respectively, of the disease. One of them contained 920 cells per c. mm., and gave a positive test for globulin; the other contained 215 cells, and also gave a positive test for globulin. 2 cc. of each fluid were added to 0.1 cc. of filtrate of active monkey spinal cord, the mixture was incubated for 2 hours at 37°C., kept at 4°C. for 16 hours, and then inoculated intracerebrally, under ether anesthesia, into two Macacus rhesus monkeys. One monkey became paralyzed on the 6th, and the other on the 10th day. Both died. The third test with cerebrospinal fluid was made with an equal mixture of two specimens, one taken on the 6th, and the other on the 8th day of illness. Both contained an excess of cells and globulin. The inoculated monkey became paralyzed on the 7th day and afterwards developed contractures. It was etherized for histological study 6 weeks after inoculation. Hence none of the specimens tested contained demonstrable neutralizing substances.

The blood test was made with human serum obtained on the 6th day of illness. 1 cc. was mixed with 0.1 cc. of filtrate of active monkey virus, incubated, and kept as described above. The mixture was injected intracerebrally into a Macacus rhesus. It remained well. Hence it appears that adequate neutralizing substances were present in the specimen.

The next experiment was made in order to determine whether the neutralizing substances may be made to pass from the blood into the cerebrospinal fluid. For this purpose normal monkeys were chosen. Each animal was given an intraspinal injection of normal horse serum and 16 hours later an intravenous injection of immune monkey serum from recovered poliomyelitic monkeys. The cerebrospinal fluid was withdrawn at different periods and tested for neutralizing properties. Every care was exercised to avoid admixture with blood. When

Ether anesthesia was employed for the intracerebral inoculations.
blood appeared in the puncture needle, the fluid was discarded and
the monkey yielding it was not used again for that experiment.

Experiment 1.—Dec. 5, 1916. 5 p.m. 2 cc. of normal horse serum were in-
jected intraspinally into a Macacus rhesus. Dec. 6, 9.10 a.m. Injected 10 cc. of
immune monkey serum intravenously. The cerebrospinal fluid was withdrawn
6, 9, and 24 hours later. The 6 and 9 hour specimens were combined in order
to produce a total of 1 cc. Two neutralization tests were performed.

Monkey A.—Dec. 8. A Macacus rhesus received intracerebrally a mixture
consisting of 0.1 cc. of active filtrate of poliomyelitic virus and 1 cc. of the com-
bined 6 and 9 hour cerebrospinal fluids which had been incubated for 10 hours
at 37°C. and kept for 3 hours at 4°C. No symptoms developed.

Monkey B.—Dec. 8. This test was repeated with the 24 hour specimen of
cerebrospinal fluid. Dec. 18. Animal excitable and slow; protects right arm;

Autopsy showed marked lesions of poliomyelitis in the spinal cord.

In these experiments the fluids withdrawn at the 6th and the 9th
hours exerted a neutralizing effect, while the 24 hour specimen did
not. The test was repeated as follows:

Experiment 2.—Jan. 23, 1917. 4 p.m. 2 cc. of normal horse serum were in-
injected intraspinally into a Macacus rhesus. Jan. 24, 8.45 a.m. Injected in-
travenously 10 cc. of immune monkey serum. The cerebrospinal fluid was
withdrawn 6 and 9 hours later and combined. One neutralization test was made
with active filtered virus.

Monkey C.—Jan. 26. A Macacus rhesus received intracerebrally a mixture
consisting of 0.1 cc. of filtrate virus and 1 cc. of combined spinal fluid incubated at
37°C. for 2 hours and kept at 4°C. for 15 hours. No symptoms developed.

This experiment confirms the previous one. On the other hand, in
one test with fluid withdrawn at the 6th hour, neutralization was not
effected. The protocol follows.

Experiment 3.—The procedure was identical with the two preceding exper-
iments, except that the puncture was made at the end of 6 hours only. The cere-
brospinal fluid was incubated with 0.1 cc. of filtrate virus and injected intracere-
brally into a Macacus rhesus on Jan. 4, 1917. Jan. 13. Ataxia; widespread

At autopsy typical lesions of poliomyelitis were present.
This series of experiments was controlled with the cerebrospinal fluid withdrawn from normal monkeys which had received an intraspinal injection of horse serum 18 hours before, but which had not received an intravenous injection of immune serum. An illustrative protocol follows.

Experiment 4.—Dec. 23, 1916. A Macacus rhesus was injected intracerebrally with an incubated mixture of 0.1 cc. of filtrate virus and 2 cc. of cerebrospinal fluid obtained from two monkeys which 18 hours before had received intraspinal injections of normal horse serum. Dec. 29. Tremor of head; ataxia; facial asymmetry; widespread muscular weakness. Dec. 30. Prostrate. Jan. 5, 1917. Etherized.

At autopsy typical lesions of poliomyelitis were present.

DISCUSSION.

The detection of the neutralizing immunity substances in the cerebrospinal fluid of human beings, at relatively early and later periods in the course of poliomyelitis, has been accomplished so rarely as to constitute a marked exception. At first sight, this observation would seem to indicate that the neutralizing principles do not pass into the fluid.

On the other hand, the experiments with passively immunized monkeys show unmistakably that the neutralizing substances are capable of passing into the cerebrospinal fluid under conditions in which the meninges have been inflamed. Later, however, when the inflammation is subsiding, the neutralizing substances either do not pass into the cerebrospinal fluid in detectable amounts or are fixed by the nervous tissues. However, cognizance must also be taken of the possibility in lightly passively immunized animals that the falling concentration of the immune bodies in the blood due to elimination may explain their failure to pass into the cerebrospinal fluid after the 9th hour.

While this point has not been settled, it is probable that in actively immune animals the passage of the neutralizing substances from the blood into the cerebrospinal fluid would continue as long as the inflammation present in the meninges rendered the structures easily permeable to the protein constituents of the blood. Probably the human fluids investigated by us were taken either at too early a period (2nd
day of the disease) or at too late a period (after the meningeal inflammation had subsided) to yield neutralizing effects. Until sufficient time has elapsed to permit the active immunization of the body to occur, no neutralizing substances are, of course, available. We have recorded an instance in which on the 6th day of illness the blood contained these substances in detectable amounts. Moreover, once the meningeal lesions are healed, these substances would no longer pass through. If, therefore, the healing process is inaugurated or facilitated by the passage of immunity substances from the blood into the cerebrospinal fluid, the height of that process would probably come in the first week of the illness.

What the experiments show conclusively is that once the neutralizing substances are contained within the blood, they can be made to pass into the cerebrospinal fluid by the production of an aseptic meningitis. We know that the injection of immune serum intrathecally tends to arrest the pathologic poliomyelitic process in monkeys and apparently are learning that it performs this purpose also in man. It is desirable, therefore, to consider what reason may exist for the therapeutic employment of non-immune sera, such as normal human and normal horse serum, in the treatment of cases of human poliomyelitis.

As far as present results can be interpreted, the therapeutic value of immune serum is largely confined to the early period of the disease corresponding with its onset. The value is much less definitely shown, although not excluded, for the later stages when paralysis has already appeared and is extending. It is during the early period that the immunity processes are still in abeyance and that the blood is probably devoid of neutralizing substances. Hence no diversion into the cerebrospinal fluid could be accomplished at that time by increasing the permeability of the meninges through injections of normal serum. It is not easy to see what benefit may be expected at this most favorable time by the injection of normal serum, while it is obviously rational to employ an immune serum. Whether at later periods, when the immunity response has already taken place, the normal serum may hold out more promise cannot be stated; but as even immune serum is far less effective then, the promise seems to be small.
CONCLUSIONS.

The cerebrospinal fluid taken very early and quite late in the course of acute poliomyelitis exhibits no neutralizing action on filtered poliomyelitic virus.

The blood serum on the 6th day of the disease already contains the neutralizing principles.

The injection of sterile horse serum into the cerebrospinal meninges in monkeys increases their permeability, so that they permit the immunity neutralizing principles passively injected into the blood to pass into the cerebrospinal fluid.

The passage in passively immunized monkeys takes place during a relatively brief space of time and apparently only while the inflammatory reaction produced by the horse serum is at its height.

It is established for monkeys and rendered probable for man that the intraspinal injection of immune serum in poliomyelitis is curative. In monkeys normal serum exerts no such action, and at present nothing can be stated definitely regarding the therapeutic effect of normal serum in man except that probably any benefits which may arise from its employment would be attributable not to the action of the serum as such, but to the escape of circulating immunity principles in the blood made possible by the aseptic inflammation set up by it in the meninges.

As the immunity principles appear in the blood only after several days, and the reported favorable effects of the immune serum treatment relate to the first days of illness, the employment of normal serum is thus not indicated, while that of an immune serum is.