THE PASSAGE OF MENINGOCOCCIC AGGLUTININS FROM THE BLOOD TO THE SPINAL FLUID OF THE MONKEY.

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The administration of antimeningococcic serum for the treatment of epidemic meningitis by other than the intraspinal route has hardly been considered until recently, since the publication of the papers by Flexner and his coworkers on the specific serum therapy of the disease on which the prevailing mode of treatment is based. The experience with a large number of cases of epidemic meningitis during the great war and under the unusual conditions of camp life has led to the revival of the employment of the serum by intravenous, usually associated with intraspinal, injection. That the intravenous administration was both indicated and called for is evidenced by the occurrence of cases of meningococcemia in some instances without, and in others before the onset of, the meningitis. Although other clinicians have from time to time employed antimeningococcic serum by intravenous injection the systematic use of it in that manner has been developed especially by Herrick.

The purpose of the intravenous injection may be regarded as threefold: (1) to combat the meningococcemia; (2) to diminish possibly the rapidity of the passage of the serum from the subarachnoid space into the blood; and (3) to bring remote portions of the meninges, not so readily accessible from the spinal fluid itself, under the influence.

2 Flexner, S., and Jobling, J. W., J. Exp. Med., 1908, x, 141, 690.
ence of the serum. Under normal conditions antibodies do not pass from the blood into the cerebrospinal fluid, but under circumstances of inflammation of the meninges the permeability is increased, and passage may in some degree take place. Of the three possibilities, the first, namely the control of the blood invasion by meningococci, may be regarded as the most important, since it may, if only in rare instances, prevent or abort a meningeval infection, and it may be even more effective in preventing the infections of joints, heart, eye, etc.

The experiments to be described relate to the question of the possibility and the degree of passage of antibodies, in this instance agglutinins, for the meningococcus from the blood into the cerebrospinal fluid.

EXPERIMENTAL.

*Macacus rhesus* monkeys were employed for the experiments because of the ease with which chemical meningitis may be induced in them and especially because they readily yield cerebrospinal fluid on lumbar puncture.

The first experiment was arranged to test two points: (a) whether the antimeningococcus agglutinins passed from the blood into the cerebrospinal fluid in normal animals, and (b) whether they passed in animals in which a chemical meningitis had been incited when only 10 cc. of the antiserum had been injected into a superficial vein. The protocol which follows shows not only that no such passage takes place in the normal animal but either none that is demonstrable, or at least very little, even in the presence of an aseptic meningitis.

*Experiment 1.*—Mar. 13, 1918. Three *Macacus rhesus* monkeys, weighing about 3 kilos each, received intravenously 10 cc. of polyvalent antimeningococcal serum. Monkeys A and B had received 18 hours previously 2 cc. of normal horse serum intraspinally. No intraspinal injection was given Monkey C. 7 and 24 hours after the intravenous injection spinal fluid was removed from each and tested for agglutination against the meningococcus. None was observed in either specimen from the monkey which received no intraspinal injection. The same was true of the specimens from one of the monkeys in which chemical meningitis had been induced. The spinal fluid removed 7 hours but not 24 hours after intravenous injection from the second monkey, which had received intraspinal injection of horse serum, agglutinated regular and parameningococcus in a dilution of 1:2 and 1:4.

The second experiment was made with 20 cc. of polyvalent anti-
meningococcic serum of high titer which was injected in each instance
into a superficial vein. It was devised to cover the following condi-
tions: (a) effect on normal cerebrospinal fluid, (b) effect on cerebro-
spinal fluid modified by a mild aseptic meningitis induced by an in-
traspinal injection of sterile normal salt solution, (c) effect on the
cerebrospinal fluid modified by a more severe chemical inflammation
induced by normal horse serum, and (d) a still more severe inflamma-
tion brought about by the injection of a salt solution suspension of
regular meningococcus culture. In the last instance the agglutinin
tested for was that of the para organism.

The result is clear: no agglutinins were present in the cerebro-
spinal fluid derived from the normal animal which received the intra-
venous injection; fluctuating amounts were present in the cerebro-
spinal fluid after salt solution, more after the horse serum injection,
and most after the injection of meningococcus. In other words,
the normal meninges were not permeable, while the inflamed mem-
branes were permeable in proportion to the intensity of the men-
ingitis experimentally induced.

Experiment 2.—Macacus rhesus D, weight 3.2 kilos; control. Mar. 21, 1918.
Injected intravenously 20 cc. of polyvalent antimeningococcic serum. 5.25 p.m.
Lumbar puncture, 0.5 cc. of clear fluid which did not agglutinate regular or
parameningococcus. Mar. 22, 10.10 a.m. Lumbar puncture, 1 cc. of clear
fluid which did not agglutinate the meningococci. 5.50 p.m. Injected intraspinal-
ly one-quarter of an 8 hour culture of regular meningococcus in 0.5 cc. of iso-
tonic salt solution. Mar. 23, 12 m. Lumbar puncture, 0.5 cc. of turbid fluid
which, after centrifugation, agglutinated parameningococcus ++ in a dilution
of 1:4. Culture from spinal fluid negative. Mar. 24, 10.35 a.m. Lumbar
puncture, 0.5 cc. of turbid fluid. Cultures negative. Centrifuged fluid agglu-
tinated parameningococcus + in a dilution of 1:2.

Macacus rhesus D, weight 3.5 kilos. Mar. 20, 1918, 4 p.m. Injected intra-
spinally 2 cc. of isotonic salt solution. Mar. 21, 11.10 a.m. Injected intra-
venously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.30 a.m.
Lumbar puncture, 1 cc. of slightly turbid fluid. The centrifuged fluid did not
agglutinate regular or parameningococcus in a dilution of 1:2. 5.45 p.m. In-
jected intraspinaly one-quarter of an 8 hour culture of regular meningococcus in
0.5 cc. of isotonic salt solution. Mar. 23, 11 a.m. Lumbar puncture, 0.5 cc. of
turbid fluid which yielded negative culture. The centrifuged fluid agglutinated
parameningococcus + in a dilution of 1:4. Mar. 24, 10.25 a.m. Lumbar punc-
Passage of Meningococcic Agglutinins

Meningococci, 0.5 cc. of slightly turbid fluid which yielded negative culture. The centrifuged fluid agglutinated parameningococcus +++ in a dilution of 1:4.

Macacus rhesus F, weight 3.3 kilos. Mar. 20, 1918, 4 p.m. Injected intraspinally 2 cc. of normal horse serum. Mar. 21, 10.38 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.20 a.m. Lumbar puncture, 0.5 cc. of turbid spinal fluid. The centrifuged fluid agglutinated regular meningococcus ++ 1:4, - 1:2, and parameningococcus + 1:4 and - 1:2. 6.15 p.m. Lumbar puncture, 0.5 cc. of turbid fluid which after centrifuging agglutinated parameningococcus + 1:4. Injected intraspinally one-fourth of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 11.30 a.m. Lumbar puncture, 0.5 cc. of turbid fluid; culture negative; the centrifuged fluid agglutinated regular meningococcus + in a dilution of 1:2, ++ 1:4, and parameningococcus + 1:2, - 1:4.

The next experiment brings out conclusively the effect of degree of chemical inflammation in promoting the passage of agglutinating bodies from the blood into the spinal fluid. The order of the experiment was first to inject intraspinally into Macacus rhesus monkeys either isotonic salt solution or normal horse serum, and about 20 hours later to give 20 cc. of polyvalent antimeningococcic serum intravenously. The spinal fluid is then withdrawn at stated intervals and tested. When salt solution or horse serum is injected intraspinally and the turbid fluid withdrawn later and tested for agglutinins of the meningococcus no agglutination takes place unless antimeningococcic serum has been given also. The results of this experiment are given in Table I. The far greater effect of the horse serum over isotonic salt solution is at once apparent.

Attention has been drawn to the practice now becoming more common of combining the intravenous with the intraspinal injection of the antimeningococcic serum. Time and experience alone will decide in how far this practice is superior to the intraspinal injection alone. The serum introduced into the subarachnoid space soon begins to escape into the blood; hence the necessity for its reintroduction if the meninges are to be kept bathed in the antiserum. The passage of the antiserum from the subarachnoid space into the blood leads to a general distribution throughout the organs. Possibly the higher dilution thus produced compared with the greater concentration secured from a direct injection of the larger volume of serum into the blood gives to the latter a special advantage in overcoming the so-called meningococcemia.
It seemed desirable to determine, by direct agglutination tests, the agglutination titer of the blood and spinal fluid under the three sets of conditions; namely, after an intraspinal injection alone, after an intravenous injection alone, and after combined intraspinal and intravenous injection. This determination was made in monkeys; it could of course be carried out better with cases of epidemic meningitis under serum treatment. Unfortunately we were restricted by the scarcity of monkeys to one test of each condition. The result given in Text-fig. 1, a, b, and c must be regarded, therefore, as tentative only. Certain factors affecting the results are entirely obscure; such, for example, as the delay after intravenous injection before the full expression of the agglutination titer declares itself. The three curves are, however, not only distinct, but suggest first that passage of the serum from the cerebrospinal fluid into the blood begins almost at once (Text-fig. 1, a), and second that the persistence of the titer in the blood and spinal fluid (Text-fig. 1, c) is sensibly affected by combined intraspinal and intravenous injection. However, we desire to repeat that not too much stress should be laid upon the results of these single tests.

Experiment 3.—Macacus rhesus G. May 14, 1918, 9.15 a.m. Injected intraspinally 3 cc. of polyvalent antimingococcic serum. Blood was taken for

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<td>Isotonic salt solution.</td>
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<td>Normal horse serum.</td>
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agglutination tests at 10.30 a.m., 1.15, 5.15, and 9.30 p.m. Lumbar puncture at 10.50 a.m., 1.25, 5.45, and 9.45 p.m. May 15. Bleedings at 10.45 a.m. and 4.18 p.m. Lumbar puncture at 10.50 a.m. and 4.23 p.m. The samples were tested May 16 for agglutinins with a parameningococcus, and the results are recorded in Text-fig. 1, a.

Text-fig. 1, a, b, and c. (a) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intraspinal injection of 3 cc. of antimeningococcic serum. (b) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intravenous injection of antimeningococcic serum and the intraspinal injection of normal horse serum. (c) Meningococcic agglutinins in the blood and spinal fluid of the monkey after combined intravenous and intraspinal injection of antimeningococcic serum.
Macacus rhesus II. May 13, 1918, 5 p.m. Injected intraspinaly 2 cc. of normal horse serum. May 14, 8.10 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Specimens of spinal fluid and blood were taken at 9.10 a.m., 12.10 and 8.10 p.m., and at 9.10 a.m. on May 15. The results of the agglutination tests with parameningococcus on May 16 are recorded in Text-fig. 1, b.

Macacus rhesus I. May 14, 1918, 9.30 a.m. Injected intraspinaly 3 cc. and intravenously 20 cc. of polyvalent antimeningococcic serum. Specimens of blood and spinal fluid were removed at 11 a.m., 1.30, 6, and 9.40 p.m. May 15. Specimens taken at 10.50 a.m. and 4.25 p.m. The results of agglutination tests with parameningococcus on May 16 are shown in Text-fig. 1, c.

CONCLUSIONS.

Agglutinins for the meningococcus were not found in the spinal fluid of normal monkeys which had received antimeningococcic serum intravenously.

The intraspinal injection of isotonic salt solution, normal horse serum, or a culture of living meningococci allows agglutinins for the meningococcus to pass from the blood to the spinal fluid of the passively immunized monkey; and the rate of the passage is affected by the severity of the inflammation induced in the meninges.

The rates of elimination from the blood and spinal canal of meningococcic antibodies, as shown by the agglutination reaction, were compared in monkeys treated with immune serum (a) intraspinally, (b) intravenously, and (c) intraspinally and intravenously in combination.

(a) When immune serum is given intraspinally the agglutinins are very much diminished after 8 hours and practically disappear at 12 hours. They appear in the blood at the 4th hour after injection and quickly diminish.

(b) After intravenous injection of immune serum, when the meninges are inflamed, agglutinins appear in the spinal fluid in small amounts in about 12 hours and increase to the 25th hour. More than one-half of the agglutinins disappear from the blood within 8 hours and remain in low concentration at 25 hours.

(c) After combined intraspinal and intravenous injection the agglutinins remain in higher concentration in the spinal fluid and for a longer time than by method (a) or (b). The curve descends after 12 hours, and agglutinins are present at 25 hours. They remain in maximum concentration in the blood for 25 hours.