THE PASSAGE OF NEUTRALIZING SUBSTANCE FROM THE BLOOD INTO THE CEREBROSPINAL FLUID IN ACTIVELY IMMUNIZED MONKEYS.

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Previous experiments\(^1\) have shown that in passively immunized monkeys neutralizing antibodies for the poliomyelitic virus can be made to pass from the blood into the cerebrospinal fluid by merely increasing the permeability of the meningeal-choroidal complex, through an aseptic inflammation induced by means of normal horse serum. The passive immunization is effected through the injection into normal monkeys of the blood serum of monkeys which have survived an attack of experimental poliomyelitis and which subsequently have had the immunity reinforced by subcutaneous injection of active virus (suspensions of spinal cord and brain of recently paralyzed monkeys preserved in glycerol). The order of the experiment was as follows: An aseptic meningitis was induced in normal monkeys by an intraspinal injection of 2 cc. of normal horse serum. The next morning, or about 16 hours later, about 10 cc. of the immune serum were injected intravenously. At intervals of 6, 9, and 24 hours fluid was withdrawn by lumbar puncture and employed for neutralization tests. The 6 and 9 hour specimens were combined so that the tests were actually made with samples of cerebrospinal fluid taken 6 and 9 hours and 24 hours after the horse serum was introduced. The control tests were made with normal horse serum. The procedure, as far as the actual neutralization is concerned, was identical with that of the present experiments. The results show that normal horse serum is devoid of neutralizing power for the virus; that at the expiration of 6 to 9 hours sufficient amount of the antibodies introduced into the blood had already passed into the cerebrospinal fluid to effect neutralization of the virus; and that the fluid withdrawn after 24 hours might no longer neutralize the virus perfectly.

These experiments were regarded as having a certain significance in respect to the specific therapy of poliomyelitis. Flexner and Lewis\(^2\) and Flexner and Amoss\(^3\) had already shown that the introduction of immune monkey or immune

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\(^1\) Flexner, S., and Amoss, H. L., J. Exp. Med., 1917, xxv, 499.
\(^3\) Flexner, S., and Amoss, H. L., J. Exp. Med., 1914, xx, 249; 1917, xxv, 525.
human serum into the meninges of monkeys prevented experimental infection with active poliomyelitic virus. Netter and his associates first applied the principles of this observation to the treatment of cases of poliomyelitis in man. Later others, notably Amoss and Chesney, reported series of cases treated in this manner with immune (convalescent) human serum. The promising results obtained led to the employment by others of normal sera. Thus Sophian injected normal horse serum and Zingher normal human serum intraspinally in cases of acute poliomyelitis. Their results were not definite, and yet they have been regarded as favorable in some instances.

Since normal serum exhibits no neutralizing action on the poliomyelitic virus in vitro, the possibility exists that the normal sera may serve merely to divert the neutralizing substances from the blood into the meninges by increasing the permeability of the meningeal-choroidal complex. It is now known that these bodies are detectable in the circulating blood of man in some instances as early as the 3rd day of an attack of poliomyelitis. Hence the possibility exists that cases reacting favorably to intraspinal injections of horse serum may have endured long enough at the time of injection to be benefited by the diversion of immune bodies indicated.

Under these circumstances the diversion would take place not in a passively but in an actively immune person. It seemed desirable, therefore, to make actual tests upon actively immune monkeys. A number of animals which had recovered from attacks of experimental poliomyelitis were available. They had all subsequently been injected with virus suspensions to increase (reinforce) their immunity.

EXPERIMENTAL.

The specific experiments made to ascertain the presence of neutralizing substances in the cerebrospinal fluid were preceded by a series of

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tests to determine whether active complement is actually required to accomplish the destruction of the poliomyelitic virus in vitro. Diverse opinions on this point prevail. Our previous experiments had led us to believe fresh complement not essential. On the other hand, irregularities not always readily explained sometimes arise in the course of the neutralization tests. As sometimes fresh and sometimes stored sera have been employed, the lack of uniformity has been attributed to the variation in the complement. As Experiment 1 indicates, inactive sera are perfectly neutralizing. The irregularities probably are to be accounted for rather by the quality of the virus, for a serum which contains in a given volume sufficient antibodies to neutralize a unit of virus of one degree of activity may fail to neutralize this unit of a more intense or active virus. Hence it is imperative to cover all tests on immunity in relation to poliomyelitis with adequate control observations. While the virus once adapted to monkeys by successive passages acquires and retains for a long time a marked virulence, yet quantitative fluctuations occur from time to time which are not predictable and heighten or depress the activity.

Experiment 1. Relation of Neutralization to Active Complement.—For this experiment a glycerolated virus (spinal cord and medulla) was employed. A 5 per cent suspension in isotonic saline solution was prepared, centrifuged, and filtered through a Berkefeld candle. Macacus rhesus A was inoculated intracerebrally with 2.6 cc. of a mixture of 2.4 cc. of fresh normal monkey serum and 0.2 cc. of virus filtrate. The mixture of filtrate and serum had been kept at 37°C. for 2 hours and in the refrigerator (4°C.) over night. 6 days after the inoculation the animal was ataxic, showed head tremor, and moved about slowly. Death took place on the 7th day. The autopsy showed the characteristic lesions of poliomyelitis. Macacus rhesus B received an intracerebral inoculation of the following: inactivated immune monkey serum 2 cc., inactivated normal serum 0.4 cc., virus filtrate 0.2 cc., mixed and treated as for Macacus rhesus A. No symptoms developed. Macacus rhesus C was a repetition of Macacus rhesus B in which active normal serum replaced the inactive. No symptoms appeared. For Monkey D an inactive human (convalescent) serum was employed in proportion of 2 cc. of serum to 0.2 cc. of virus filtrate. No symptoms developed.

This experiment yielded a clear and definite result. The neutralization of the virus is accomplished directly and without the inter-

Passage of Neutralizing Substance. This point has an interest in connection with the test with the cerebrospinal fluid which follows, since it shows that the presence of active complement can be disregarded.

Experiment 2. Passage of Neutralizing Substance into the Cerebrospinal Fluid.—This experiment was performed with cerebrospinal fluids withdrawn 6 and 9 hours respectively after the injection of 2 cc. of normal horse serum into the meninges of actively immune monkeys. The two fluids were combined and mixed with the virus filtrate in the manner of Experiment 1. The control tests were made with virus mixed with normal cerebrospinal fluid and with normal horse serum respectively. Macacus rhesus E (control) received an intracerebral inoculation of an incubated mixture of 1 cc. of normal cerebrospinal fluid, 0.3 cc. of isotonic saline solution, and 0.2 cc. of filtrate virus. On the 24th day ataxia, right facial paralysis, and weakness of extremities were present. The next day the extremities were paralyzed and death occurred. The autopsy showed lesions of poliomyelitis. Macacus rhesus F (control) received an intracerebral inoculation of an incubated mixture of 1 cc. of normal horse serum, 0.3 cc. of isotonic saline solution, and 0.2 cc. of filtrate virus. On the 6th day the extremities were paralyzed and a right facial paralysis existed. The animal was etherized and the autopsy showed marked lesions of poliomyelitis. Macacus rhesus G received an intracerebral inoculation of an incubated mixture of 0.2 cc. of virus filtrate and 1 cc. of the combined cerebrospinal fluid withdrawn from an actively immune monkey 6 and 9 hours respectively after the intraspinol injection of normal horse serum. 9 days later ataxia, right facial paralysis, tremor of head, and weak deltoids were present. The next day all the extremities were paralyzed and the animal was etherized. The autopsy showed lesions of poliomyelitis. Macacus rhesus H was an exact repetition of Monkey G. On the 12th day ataxia, paralysis of right arm, and weakness of many other muscles were noted. The paralysis extended somewhat and then gradually receded. Recovery with residual paralysis took place.

An immediate interpretation of this result would necessitate the conclusion that passage of neutralizing substances from the blood into the chemically inflamed meninges either did not take place in actively immune monkeys at all, or only inadequately in the period of 6 to 9 hours. Since in the passively immunized monkeys this period sufficed for the passage, some other explanation must be sought. Doubtless it is found in the discrepancy of the experimental procedure in the two series. In the case of the passively immunized monkey, the meningeal inflammation is induced about 16 hours before the immune serum is injected; hence a 9 hour specimen of cere-
brospinal fluid would be withdrawn about 25 hours after the horse serum was injected intraspinally. In the actively immune animals the fluid is withdrawn 9 hours after the horse serum is injected. In the one instance the inflammation is at its height, in the other in process of development when the cerebrospinal fluid is withdrawn. That this is the proper explanation is indicated by Experiment 3.

**Experiment 3. Passage of Neutralizing Substance into the Cerebrospinal Fluid.**—This experiment is a repetition and extension of Experiment 2. The manner of carrying it out was identical. Normal horse serum was injected intraspinally, and the periods at which the fluid was taken by lumbar puncture were 12, 24, and 48 hours. In the instance of the 48 hour withdrawal, a second injection of horse serum was made at the end of the first 24 hour period, in order to maintain the inflammation at a high level. Inoculations of monkeys with the cerebrospinal fluid and virus mixtures were made in duplicate, in order to cover any unforeseen variation in the results. *Macacus rhesus I* (control) received an intracerebral injection of an incubated mixture of 2 cc. of normal horse serum and 0.2 cc. of virus filtrate. On the 4th day the animal was excited; on the 5th, the extremities were all paralyzed and death resulted. The autopsy disclosed marked lesions of poliomyelitis. *Macacus rhesus J* and *J' each received an intracerebral inoculation of an incubated mixture of 0.2 cc. of virus filtrate and 1 cc. of cerebrospinal fluid withdrawn from an actively immune monkey 12 hours after an intraspinal injection of normal horse serum. No symptoms developed. *Macacus rhesus K* and *K' received similar injections of mixtures containing 24 hour specimens of cerebrospinal fluid and 0.2 cc. of virus filtrate. No symptoms developed. *Macacus rhesus L* and *L' received identical injections of mixtures containing 48 hour specimens of cerebrospinal fluid and 0.2 cc. of virus filtrate. No symptoms appeared.

This experiment is conclusive. It shows that beginning 12 hours after the normal horse serum is injected into the meninges of actively immune monkeys, and at a period when the inflammation induced may be regarded as marked, readily measurable quantities of the neutralizing antibodies were poured into the cerebrospinal fluid. This passage continues for 48 hours at least, that is considerably longer than in the passively immunized animals, as might have been predicted. Probably the passage would continue as long as the permeability of the meningeal-choroidal complex persisted. The results of this experiment indicate also that the explanation offered for the failure of Experiment 2 is probably the correct one.
The results of the experiments make clearer the manner in which recovery from poliomyelitis may be supposed to be brought about, and throw light on the probable value of a serum therapy. Immune bodies do not pass normally from the blood to the cerebrospinal fluid, which is, as it were, the lymph of the central nervous system (Mott). In poliomyelitis, however, the entire vascular system of the meninges and affected portions of the solid nervous organs, as well as the structures of the choroid plexus, are often so severely injured as to be rendered readily permeable to the protein of the plasma and hence to immune bodies contained in it. The latter should therefore begin to appear in the cerebrospinal fluid just as soon as they begin to accumulate in the blood, and from that fluid permeate to the interior of the central nervous organs. From the moment this transfer of antibodies begins, the neutralization of the virus present in the nervous tissues would also begin; and gradually or quickly in the non-fatal cases an arrest of multiplication of the virus would be effected. That the cessation of the extension of the paralysis occurs very quickly in some cases is a matter of common observation. The presence of the neutralizing antibodies seems to be wholly determined by an excessive permeability of the blood vessels of the nervous system, for once their integrity is restored and the cerebrospinal fluid has returned approximately to normal composition, neutralizing antibodies can no longer be detected there. By the time the acute process is at an end, the infection has run its course. There remains to be accomplished merely the restoration, as far as may be, of the organic and functional integrity of the injured structures.

CONCLUSIONS.

For the neutralization of the virus of poliomyelitis by antibodies, active complement is not required.

In carrying out immunity tests it is imperative to choose a virus of established grade of virulence and to make adequate control observations.

The neutralizing substances pass from the blood of actively immune monkeys into the cerebrospinal fluid when the permeability of the meningeal-choroidal complex is increased by an aseptic inflammation such as that induced by an intraspinal injection of horse serum.

The immunity bodies in effective neutralizing quantities can be detected in the cerebrospinal fluid as early as 12 hours and as late as 48 hours after the intraspinal injection of horse serum. Doubtless the passage continues as long as the inflammation persists.

This ability of the neutralizing substances to pass from the blood into the cerebrospinal fluid under conditions of inflammation doubtless plays an important part in arresting the multiplication of the virus on which the cessation and restoration of the poliomyelitic processes depend. The widespread involvement in the inflammatory conditions of the meninges, choroid plexus, and substance of the nervous organs, accompanied by severe lesions of the blood vessels in the last structures especially, opens the way widely for the passage of antibodies into the cerebrospinal fluid, whence all parts of the nervous tissues are reached, and also, probably, for direct transudation into the affected parts of the spinal cord and brain. The neutralization of the virus on which the continuance of the active pathological process depends is thus readily accomplished.

Under these circumstances the use of an alien specific immune serum to anticipate the action of the individual's own immunity products appears logical, while the employment of normal serum has no basis in experiment and would seem not to offer any therapeutic advantage whatever.