AN EXPERIMENTAL TEST OF NUZUM’S ANTIPOLIOMYELITIC SERUM.

By Harold L. Amoss, M.D., and Frederick Eberson, Ph.D.
(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 22 to 24.

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In a recent paper we reported a series of experiments with Rosenow’s antipoliomyelitic serum, which is prepared in the horse by injections of streptococci derived from cases of poliomyelitis. The present paper deals with a similar test of a serum prepared in the horse by Nuzum also by inoculating streptococci. The two sera—Rosenow’s and Nuzum’s—while apparently similar, are prepared in somewhat different manner. Thus Nuzum has had the exceptional and on the whole extraordinary experience of cultivating streptococci from the cerebrospinal fluid removed by lumbar puncture from 90 per cent of the cases of epidemic poliomyelitis studied, and he employs these spinal strains in the immunization process. Nuzum’s reported results stand alone; no other bacteriologist employing a strict technique has found bacteria either on microscopic examination or in cultures in the cerebrospinal fluid. Perhaps the largest single experience reported is that of Abramson, who published results of the bacteriological study of more than 1,200 fluids, all being negative for bacteria aside from a few instances of obvious contaminations.

However, it is imperative to approach the question of immunological relation of certain streptococci to poliomyelitis without prejudice; and as Rosenow and Nuzum have reported the successful application of their sera, not only in experiments on monkeys, but also in treating human cases of the disease, it is essential that confirmatory and, as far as possible, decisive tests be carried out by others.

We believe that we have performed a decisive test with Rosenow’s serum with the result of showing that under the conditions of the experiment it is devoid of protective power when injected intraspinally against experimental infection of monkeys with an active virus of poliomyelitis introduced intravenously. That the conditions of the experiments were not too severe is indicated by the

fact that perfect protection was secured from the serum of monkeys which had recovered from an experimental poliomyelitic infection.

Nuzum has developed a somewhat more elaborate therapeutic technique than Rosenow. Thus he bases his contention that his serum is therapeutically active on three sets of tests: (a) the neutralization of the virus in vitro; (b) the neutralization of the virus in vivo by intraspinal injections of the serum; (c) the neutralization of the virus in vivo by combined intraspinal, intravenous, and intramuscular injections of the serum. Hence, in order to repeat and check Nuzum's experiments, the three modes of administering the serum should be followed.

Through the kindness of Dr. Nuzum we were supplied with 50 cc. of his serum. This proved sufficient for one set of experiments, one monkey being used for each kind of test. The series of tests is therefore a small one; and had the results not been absolutely consistent would have had no great value. But as the results were consistent, and as the control tests with the serum of recovered monkeys were equally definite, no real doubt need be entertained regarding their significance.

EXPERIMENTAL.

The essential condition in dealing experimentally with the virus of poliomyelitis is to possess a specimen of regular and high potency; otherwise an element of irregularity is introduced which involves many separate inoculations. Moreover, the virus should be so active as to produce infection uniformly when small doses of the Berkefeld filtrate are injected intracerebrally. A virus which acts only when relatively large doses of the unfiltered suspension are injected is certain to produce infection irregularly and, in addition, may give confusing results, owing to traumatic and other forms of injury to the brain substance through which paralysis, weakness, and other disturbing effects are produced.

Neutralization in Vitro.

When one minimum lethal dose of virus filtrate is incubated in the presence or absence of complement with 2 cc. of serum from a

monkey which has had experimental poliomyelitis and recovered, the
virus is rendered non-infective for a monkey on intracerebral inocula-
tion. Normal serum does not possess this power.

Three experiments were carried out in precisely the same manner,
comparing Nuzum and Willy's serum with normal horse serum and
immune monkey serum. To 2 cc. of the serum to be tested there was
added 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of
glycerolated active poliomyelitic monkey cord. The mixture was
incubated for 2 hours at 37°C. and placed at 4°C. for 16 hours, and
then injected intracerebrally into a normal monkey.

*Monkey A (Macacus rhesus), Control.*—Apr. 12, 1918. Injected intracerebrally
the incubated mixture resulting from the addition of 0.2 cc. of virus filtrate to 2
cc. of normal horse serum. Apr. 16. Excited and slow. Apr. 17, a.m., pro-
strate, moribund; p.m., died.

Autopsy showed characteristic changes of poliomyelitis in the brain and cord.

*Monkey B (Macacus rhesus).*—Apr. 12, 1918. Injected intracerebrally the
incubated mixture resulting from the addition of 0.2 cc. of virus filtrate to 2 cc.
of Nuzum and Willy’s serum. Apr. 19, a.m., marked ataxia, both arms para-
lized (Fig. 1); p.m., prostrate, etherized.

Microscopic examination of the nervous tissues showed typical diffuse and ex-
tensive poliomyelitic lesions in medulla and spinal cord; marked perivascular in-
filtration (Fig. 2), necrosis, neurophagocytosis of the ganglion cells, and focal
hemorrhages in the anterior horn of the cord.

*Monkey C (Macacus rhesus), Immune Control.*—Apr. 12, 1918. Injected in-
tracerebrally the incubated mixture resulting from the addition of 0.2 cc. of
virus filtrate to 2 cc. of serum drawn from a monkey which had been paralyzed
from experimental poliomyelitis and recovered. The monkey remained well.

The results show that Nuzum and Willy’s serum, under the con-
ditions of the experiment, possesses no more neutralizing power for
poliomyelitic virus in vitro than does normal horse serum.

*Neutralization in Vivo.*

Nuzum and Willy’s serum was subjected in the following experi-
ments to the same test applied to Rosenow’s antipoliomyelitic serum.
This consists in the injection of a large dose of the virus intravenously
into a monkey, and the repeated intraspinal injection of the serum to
be tested. Flexner and Amoss\textsuperscript{10, 11} have shown that under these conditions a serum possessing no neutralizing properties sets up inflammatory changes, thus opening the way for the virus to pass from the blood stream into close relation with the meninges and cause infection. Immune serum, on the other hand, while causing the same inflammatory changes, neutralizes the virus as it passes through and prevents infection.

*Monkey D (Macacus rhesus), Control.*—Apr. 9, 1918, 5 p.m. Injected intraspinally 2 cc. of normal human serum. Apr. 10, 11 a.m. Injected intravenously 50 cc. of a centrifuged 5 per cent suspension of fresh poliomyelitic cord in isotonic salt solution. 11.10 a.m. Injected intraspinally 2 cc. of normal human serum. Apr. 11, 12 m. Intraspinal injection of 2 cc. of pooled serum from monkeys recovered from experimental poliomyelitis. The dose of serum was repeated on the 2 following days. The monkey remained well.

*Monkey E (Macacus rhesus).*—Apr. 9, 1918, 4.30 p.m. Injected intraspinally 2 cc. of Nuzum and Willy's antipoliomyelitic serum. Apr. 10, 11.15 a.m. Injected intravenously 50 cc. of a centrifuged 5 per cent suspension of fresh poliomyelitic cord in isotonic salt solution. 11.25 a.m. Injected intraspinally 2 cc. of Nuzum and Willy's serum. The intraspinal injection of 2 cc. of the serum was repeated daily on the 3 succeeding days. Apr. 16. Slow; excitable. Apr. 17. The same. Apr. 18. Slow; tires easily. Apr. 19. Prostrate (Fig. 3). 3.10 p. m. Died.

Autopsy and microscopic examination of the tissues showed lesions of poliomyelitis, chiefly in the medulla and cervical enlargement of the cord. They consisted of the usual perivascular infiltrations, which were of moderate severity, and of focal infiltrations of cells with polymorphous and fragmented nuclei. The focal infiltrations were sometimes near and sometimes away from blood vessels and were frequently in association with hemorrhages of considerable size. Moreover, nerve cell degeneration and neurophagocytosis had been progressing actively in the medulla (Fig. 4) and in the cervical portion of the cord. These lesions indicate a severe poliomyelitis. The number of focal hemorrhages (Fig. 4) and their size are unusual in experimental poliomyelitis, although they are commonly found in human poliomyelitic nervous tissues.

The experiments show that while the immune monkey serum completely neutralizes the virus as it passes through into the meninges and thus prevents infection, the serum of Nuzum and Willy possesses no such power, but acts in the same manner as normal horse serum.

\textsuperscript{10} Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

\textsuperscript{11} Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.
Curative Experiments.

Nuzum and Willy make the claim that their serum possesses curative properties against the virus in experimental poliomyelitis in monkeys. In order to test this property an experiment was made comparable with their recorded test with Monkey 25 of their series. Their dose of virus given intracerebrally was 1 cc. of a 5 per cent suspension of poliomyelitic cord. In the experiment about to be recorded only 0.5 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic monkey cord was used. Thus the test in our hands was apparently less severe; however, the result is decisive.

Monkey F (Macacus rhesus).—Apr. 11, 1918, 2.55 p.m. Injected intracerebrally under ether anesthesia 0.5 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord. 3 p.m. Injected intravenously 15 cc. and intraspinally 2.5 cc. of Nuzum and Willy's serum. Apr. 13. Injected intramuscularly, left gluteal region, 10 cc. of the serum. Apr. 14. Injected 10 cc. of the serum intramuscularly. Apr. 16. Ataxic; arms and legs weak; coarse tremor present. Apr. 17, 8 a.m. Found dead.

Autopsy and microscopic examination of the brain and cord showed typical diffuse lesions of poliomyelitis in the spinal cord and medulla. (Fig. 5.) There were extensive necrosis of the gray matter in the cord and marked typical perivascular infiltration in the intervertebral ganglia, with hyaline degeneration and neurophagocytosis of the ganglion cells. The medulla showed neurophagocytosis of nerve cells, focal infiltration of cells, of which many were polymorphonuclear, due probably to the injection of horse serum.

DISCUSSION.

The significance of the experiments is self-evident. Thus, while the serum of the recovered monkeys completely neutralizes the virus of poliomyelitis in vitro or as it passes from the blood into the meninges after an aseptic inflammation has been induced in the latter, the serum prepared in the horse by Nuzum and Willy by means of injections of streptococci exhibited no neutralizing power whatever as far as could be detected. Indeed, the indications are that the latter serum acts in the manner of normal horse serum and as such rather promotes than prevents experimental poliomyelitic infection.

This, the main question raised by the experiments, having been answered, several subsidiary questions may now be considered. The
first one of the latter relates to the character of the experimental
evidence on which Rosenow\textsuperscript{12} and Nuzum\textsuperscript{6} base their claim that the
antistreptococcic serum is neutralizing for the virus of poliomyelitis.
It is significant that Rosenow\textsuperscript{12} uses the expression "appears to have
developed neutralizing, protective and curative power against the
virus of poliomyelitis." The tests in monkeys can be made so
definite that there need be no room left for doubt as to what the results
imply. There are few pathogenic organisms which may be made to
yield laboratory results as clear-cut as the virus of poliomyelitis.

The basis of Nuzum's conclusion is also not definite. He claims for
his serum not merely neutralizing, protective power, but curative prop-
erties and the ability to immunize monkeys passively against sub-
sequent intracerebral inoculation of active virus suspension. Al-
together the number of criteria which enter into his calculations makes
it difficult to arrive at a decision. The source of this uncertainty
may reside in the quality of the virus which Nuzum employed. As
he injected 1 cc. of an unfiltered emulsion and even then failed in some
instances to secure infection, the potency must have been low. As we
have already pointed out, a virus with low virulence gives such ir-
regular results as to make accurate deductions difficult or impossible
to draw. In our opinion, therefore, Nuzum's experimental evidence
is inconclusive.

Both Rosenow\textsuperscript{12, 13} and Nuzum\textsuperscript{6} have treated human cases of polio-
myelitis with their antistreptococcic sera. The mode of application
differs. Thus Rosenow injects the serum intravenously, and Nuzum
uses the combined intraspinal and intravenous injection and sometimes
intramuscular injections. The question as to the value of the evi-
dence which is provided by the treatment of human cases of poliomy-
elitis in the preparalytic stages, so called, in which, apparently, the
therapeutic results are supposed to be striking, cannot now be
answered. Our knowledge of the evolution of the preparalytic cases
is still in its infancy. The wide employment of lumbar puncture, in
the last 2 years, for diagnostic purposes, is giving us for the first time
data on which eventual conclusions may come to be based. It would

\textsuperscript{13} Rosenow, E. C., \textit{J. Infect. Dis.}, 1918, xxii, 379.
be premature to undertake to interpret those data now. On the other hand, little evidence has been adduced that the antistreptococcic serum injections have affected the mortality in the frankly paralytic cases, halted the advancing paralysis, or brought about a more rapid and complete retrogression of the existing paralyses. At the best, therefore, the case for the serum, as far as human therapy goes, should be considered unproved.

There is, however, another subsidiary aspect of the subject. Streptococcus infection as a concomitant or terminal or agonal infection in epidemic poliomyelitis would now seem not to be uncommon. It is not yet clear just when the invasion of streptococci into the nervous system takes place. The fact that the cerebrospinal fluid, withdrawn under sterile conditions, practically never contains the streptococci, would seem to indicate that the antemortem invasion is small or agonal. The point is very difficult to decide in human cases. On the other hand, it is one that should be easily decided experimentally. This Smillie¹⁴ has attempted to do. Streptococci may be cultivated from the brain¹⁵ and cord and other viscera¹⁵ of monkeys which have succumbed to experimental poliomyelitis. When the spinal cord and medulla carrying streptococci are placed in 50 per cent sterile glycerol, the streptococci survive for weeks. On reinoculating this material intracerebrally into monkeys, the streptococci, if still alive, either merely survive or multiply somewhat alongside the poliomyelitic virus. Hence they can be recovered from the inoculated brain tissue. But if inoculation is made with a filtered virus free of streptococci, then, as Smillie has found, the presence or absence of those organisms is determined in great part by the stage of the disease and the state of the animal at the time the postmortem examination is performed and cultures are taken. An animal etherized when paralyzed and still strong tends not to show the presence of streptococci, while a moribund or already dead animal often does show their presence. In

other words, Smillie's view, which has still to be confirmed, would place the streptococcal invasion in poliomyelitis among the agonal and not the concomitant infections.

It is on the basis of a possible concomitant streptococcus infection of real importance for the termination of the disease that the employment of an antistreptococcic serum in poliomyelitis may still be urged, once it is proved that it has no specific action on the virus of poliomyelitis. Even for this purpose we should need far more knowledge than we now possess to justify its employment.

The value, in general, of antistreptococcic sera in combating streptococcus infections is unproved. Streptococci have not been cultivated from cases of poliomyelitis in man during life. Many, chiefly negative, results have been obtained with cultures from the cerebrospinal fluid; and it is in our opinion desirable to make many cultures from the blood before resorting to the intravenous injection of antistreptococcic serum on a large scale on the assumption of a streptococcus infection playing an essential part in the pathology of poliomyelitis.

CONCLUSIONS.

The antistreptococcic serum of Nuzum and Willy has failed to show in the monkey neutralizing or therapeutic power when applied by their methods against small doses of the virus of poliomyelitis. Under the same conditions the serum of monkeys recovered from experimental poliomyelitis proved neutralizing and protective.

The experimental and other evidence adduced by those who regard the streptococcus as playing an essential part in the pathology of epidemic poliomyelitis and the antistreptococcic sera as exhibiting therapeutic properties for man and monkeys is regarded as imperfect and inconclusive.

EXPLANATION OF PLATES.

PLATE 22.

Fig. 1. Monkey B. 7 days after the intracerebral injection of the incubated mixture of 0.2 cc. of virus filtrate and 2 cc. of Nuzum and Willy's serum.

Fig. 2. Monkey B. Cervical enlargement of the cord showing perivascular infiltration. × 165.
PLATE 23.

FIG. 3. Monkey E. 9 days after the intravenous injection of virus. This monkey was treated with five intraspinal injections of 2 cc. each of Nuzum and Willy’s serum.

FIG. 4. Monkey E. Medulla, showing perivascular infiltration, hemorrhage, and neurophagocytosis. × 192.

PLATE 24.

FIG. 5. Monkey F. Medulla, showing congestion, necrosis of gray matter, cell degeneration, and neurophagocytosis. × 240.
Fig. 1.

Fig. 2.

(Amos and Eberson: Nuzum's antipolioyelitic serum.)
Fig. 3.

Fig. 4.

(Amos and Ehren: Nurum's antipoliomyelitic serum.)
(Amoss and Eberston: Nuzum's antipoliomyelitic serum.)