REINFORCEMENT OF CONVALESCENT ANTIPOLIO-
MYELITIC SERUM IN THE MONKEY

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(Received for publication, October 1, 1930)

Flexner and Lewis (1), in connection with their studies of the pro-
tective and therapeutic powers of convalescent serum in experimental
poliomyelitis, employed the method of reinoculation of virus in
recovered monkeys to increase the strength or potency of this serum.
The procedure was largely empirical, as no actual quantitative estima-
tions of neutralizing action before and after the reinoculations were
made. The impression gained, however, was that reinoculation did
increase the potency of the serum.

Monkey immune serum as now employed for experimental purposes
may be considered to be of two sorts: convalescent serum in the true
sense, and serum derived from monkeys actively immunized with
virus introduced by various routes without symptoms of infection
appearing at any time. In practical experiments, little or no distinc-
tion has been made between these two kinds of immune sera, and no
exact quantitative neutralization comparisons have been carried out.
Stewart and Rhoads (2) have, however, shown that monkey serum may
be virus neutralizing in vitro when the actively immunized animals
yielding it are incapable of withstanding an intracerebral injection
of a highly potent virus. This experimental discrepancy between the
in vivo and in vitro inactivating power of immune sera is instructive in
that it not only indicates quantitative variations in immune power,
but suggests that human beings also may yield neutralizing serum
without themselves being completely or enduringly protected against
the pathogenic action of a highly infectious virus strain.

In order to decide whether or not quantitative differences in various
immune sera actually existed, a simple experiment was carried out.
Monkeys which had recovered from typical poliomyelitis were repeat-
edly reinoculated with large amounts of active poliomyelitis virus,
and the neutralizing value of serum obtained before and after the treatments was determined. The details of the experiment are as follows:

Reinforcement.—Six Macacus rhesus monkeys, which had survived typical attacks of experimental poliomyelitis and still showed residual paralyses 15 to 19 months after the original inoculation, were selected. Each was bled 20 cc., and the serum from the individual bleedings was separated in the usual manner. The several sera were pooled, and the resulting mixture was stored, unpreserved by Chemicals, at 4°C. The “reinforcing” injections were begun immediately after the first bleeding and conducted in the following manner. A 5 per cent suspension of glycerolated nervous tissue of the “pooled mixed” virus strain was employed in physiological saline solution. The intradermal route of inoculation was selected, since Stewart and Rhoads (2) had shown that route to be more effective than subcutaneous inoculation for giving rise to an active immunity in monkeys. 15 cc. of the material was introduced in each set of injections by forming multiple superficial blebs. The treatments were repeated 10 times at 3 day intervals. Thus a total of 150 cc. of virus was given in 30 days. After a rest period of 1 month, the animals were bled, and the serum was separated, pooled, and stored as before. The monkeys were carefully observed to detect any evidence of recurring symptoms during the injections, and none whatever was seen. The technique of reinforcement is summarized in Table I.

Neutralization before Reinforcement.—In determining the effectiveness of the serum, the usual in vitro technique was employed. The fresh Berkefeld filtrate virus was mixed with the serum to be tested, kept an hour at 20°C., and inoculated intracerebrally into normal monkeys of approximately the same size.
Tables II and III are consistent in showing that, given a constant potent virus filtrate used in amounts of 0.12 cc., the pooled convalescent monkey serum before reinforcement was ineffective in quantities less than 0.75 to 1 cc. A sample of pooled human convalescent serum in a volume of 0.1 cc. neutralized effectively in one test (Table III).

Although the meaning of the test is not at once clear, it is well worth recording that in two instances a single sample of a human serum, taken from a child 8 years old who had never shown clinical evidence of poliomyelitis, effected neutralization in the proportion of serum 0.1 cc. and virus filtrate 0.12 cc. This observation is in conformity with earlier experiments of Anderson and Frost (3), in which supposedly normal human serum was found to be inactivating, and with recent tests by Aycock and Kramer (4). The latter attribute the inactivating power of the serum to nonclinical mass immunization to the virus of poliomyelitis.

Neutralization after Reinforcement.—Table IV, which includes three separate tests made on different dates, presents clear evidence that...
the pooled reinforced serum contained greater quantities of neutraliz-
ing antibodies than did the original pooled convalescent serum from
the same monkeys. The fact is even more striking in that, so far as
the tests were carried, there is clear indication that the reinforced
serum possessed neutralizing value equal to that of pooled con-
valescent human serum. A discrepancy will be noted between the
series of animals inoculated March 5, 1930, and the experiment
summarized in Table III; in the former instance 0.5 and 0.25 cc. of

### TABLE III

*Neutralizing Value of Monkey Convalescent Serum before Reinforcement*

<table>
<thead>
<tr>
<th>Date</th>
<th>No.</th>
<th>Serum Type</th>
<th>Amount</th>
<th>Virus Strain</th>
<th>Amount Filtrate</th>
<th>Preliminary treatment</th>
<th>Result</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/30</td>
<td>1</td>
<td>Monkey convalescent serum before</td>
<td>0.5</td>
<td>M.V.</td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis</td>
<td>14 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reinforcement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/30</td>
<td>2</td>
<td>&quot;</td>
<td>0.75</td>
<td>&quot;</td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>No symptoms</td>
<td>7 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;</td>
<td>1.0</td>
<td>&quot;</td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>10/30</td>
<td>3</td>
<td>Pool human convalescent</td>
<td>0.1</td>
<td>&quot;</td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>10/30</td>
<td>5</td>
<td>Control</td>
<td>0.1</td>
<td>&quot;</td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis</td>
<td></td>
</tr>
</tbody>
</table>

non-reinforced serum protected against 0.12 cc. of virus filtrate,
although 0.1 cc. failed to do so. The probable reason for this differ-
ence is to be found in the virus filtrate. A degree of inconstancy is
encountered even in dealing with the most highly potent virus strains,
for which adequate explanation is not at hand. The filtrate prepared
from an occasional monkey, sacrificed promptly after the appearance
of paralytic symptoms, proves somewhat less active than the rule.
Whether the fault is due to the quantity of virus units in the nervous
system of a particular animal, or is influenced adversely by the
operation of extracting it, is not known. On the whole, however,
this series of experiments was remarkably regular.

TABLE IV
Comparative Neutralizing Power of Monkey Convalescent Serum before and after Reinforcement

<table>
<thead>
<tr>
<th>Date</th>
<th>No.</th>
<th>Serum Type</th>
<th>Amount</th>
<th>Strain</th>
<th>Amount Dilution</th>
<th>Route</th>
<th>Preliminary Treatment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/13</td>
<td>1</td>
<td>Reinforced monkey convalescent</td>
<td>0.5</td>
<td>M.V.</td>
<td>0.12 Icer.</td>
<td>1 hour 20°</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>2/13</td>
<td>2</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>2/13</td>
<td>3</td>
<td>Control</td>
<td>—</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis 5 days</td>
<td></td>
</tr>
<tr>
<td>3/20</td>
<td>4</td>
<td>Before reinforcement</td>
<td>0.5</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis 11 days</td>
<td></td>
</tr>
<tr>
<td>3/20</td>
<td>5</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis 6 days</td>
<td></td>
</tr>
<tr>
<td>3/20</td>
<td>6</td>
<td>After reinforcement</td>
<td>0.5</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>3/20</td>
<td>7</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>3/20</td>
<td>8</td>
<td>Control</td>
<td>—</td>
<td></td>
<td>0.12</td>
<td>—</td>
<td>Typical poliomyelitis 12 days</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>9</td>
<td>Before reinforcement</td>
<td>0.5</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>10</td>
<td></td>
<td>0.25</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>11</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>Typical poliomyelitis 9 days</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>12</td>
<td>After reinforcement</td>
<td>0.5</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>No symptoms</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>13</td>
<td></td>
<td>0.25</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>3/5</td>
<td>14</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.12</td>
<td>1 hour 20°</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
</tbody>
</table>

There is a practical side to these observations. Hereafter in testing the prophylactic and therapeutic value of convalescent monkey serum for the purpose of securing indications of the value of convalescent
human serum, either as a prophylactic or therapeutic measure, it may be desirable to employ not merely convalescent, but reinforced convalescent monkey serum. If serum from actively immunized monkeys is also employed, preliminary tests of neutralizing power are desirable. Undoubtedly certain discrepancies and failures of experiments are traceable to the use of weak convalescent monkey serum instead of the stronger human convalescent serum. For experiments on monkeys the homologous reinforced monkey serum may be desirable.

SUMMARY

A comparison has been made of the neutralizing value of pooled convalescent monkey serum for the filtered virus of poliomyelitis, before and after a series of reinforcement injections of the same virus strain.

The strength of the pooled convalescent serum is increased by the reinforcing procedure.

The original monkey convalescent serum had a neutralization value much below that of a pooled human convalescent serum. By reinforcement the neutralization value of the monkey serum was brought approximately to that of the human serum.

One sample of serum from a supposedly normal child of 8 years exhibited a neutralizing value approximately equal to that of a pooled human convalescent serum and the reinforced pooled monkey serum.

BIBLIOGRAPHY