ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS IN MONKEYS*

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Although the active immunization of monkeys against poliomyelitis has been attempted many times with attenuated and chemically inactivated poliomyelitis virus (Landsteiner and Levaditi (1), Kraus (2), Zappert et al. (3), Abramson and Gerber (4)), success has been achieved only with living virus (Flexner and Lewis (5), Aycock and Kagan (6), Stewart and Rhoads (7), Rhoads (8)). However, the danger of infection occurring during the course of treatment is ever present, (Thomson (9), Aycock and Kagan (6)). Therefore, the purpose of this work was to attempt active immunization with active virus, obtained from monkeys prostrate with poliomyelitis, in from 6 to 8 days, together with sufficient human convalescent serum to add to the safety of the method.

Flexner and Lewis (5) were the first to confer active immunity against poliomyelitis to monkeys. They used subcutaneous injections of active poliomyelitis virus emulsion. Later Aycock and Kagan (6) used the intradermal route with success, while Stewart and Rhoads (7), in their experiments, found the intracutaneous injection superior to the subcutaneous for immunization.

A combination of immune serum and virus was used by Romer and Joseph (10, 11) and by Thompson (12), but the only serious attempt to produce active immunization with such material was made by Rhoads (13). He used equal parts of 5 per cent virus emulsion and immune serum, that had been in contact an hour. Several series of animals were treated subcutaneously and intradermally, either with two large injections, or multiple small inoculations.

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The purposes of the following experiments were: (1) the production of active immunity against poliomyelitis by the least possible number of injections; (2) the determination of the minimal quantity of serum required to protect an animal against the dose of virus given; and (3) the determination of the optimum method and time of administration of the serum.

**Technique**

At each inoculation the material was injected in one pîqûre. Except where otherwise stated, the virus was injected into the skin, although some of it infiltrated more deeply. Serum was administered subcutaneously in one pîqûre.

Throughout these experiments, active poliomyelitis virus was used both for skin and intracerebral inoculation. Glycerinated cord was used, which was obtained from animals prostrate in 6 to 8 days after inoculation with "Fl. mixed virus." This virus was obtained from The Rockefeller Institute, where it was developed from the passage of pooled specimens of M.A. and K. virus (14). At intervals the potency was checked in this laboratory, and it was found that 0.01 cc. of a 5 percent glycerinated suspension produced prostration in from 10 to 12 days. Pooled human convalescent serum, which had been collected some 9 months previously, and kept at 4°C, was used. Its neutralizing power had been established in monkeys. Throughout the course of vaccination, the animals were observed for mild symptoms of the disease.

Inasmuch as Romer and Joseph (10) claimed that it took 26 days for immunity to develop, tests were not carried out until more than a month after the last injection. Control animals received a quantity of serum equal to the largest volume given to any of the experimental animals. In this way the retention of any passive immunity was controlled.

In testing the immunity, the so called "in vitro" test was used, by which is meant the ability of a serum from the test animals to neutralize a given quantity of virus. Stewart and Rhoads (7) found this procedure to be more delicate than the direct intracerebral inoculation of the experimental monkey with the virus. It was carried out in the usual way. Sufficient serum, taken from the treated animals, was added to the virus to make 1 cc., and after mixing well, incubation was carried out at 37°C, for 2 hours. The mixture was then kept overnight at 4°C, and injected intracerebrally into another animal.

**EXPERIMENTAL**

*Experiment I.*—A series of five monkeys received the equivalent of 20 to 30 cc. of 5 per cent active poliomyelitis virus emulsion, in one or two injections. Actually 10 per cent, or 20 per cent suspension was given to facilitate the injection of such large quantities of virus. In Table I the equivalents of 5 per cent emulsion have been calculated in each case to make the figures comparable with others in
<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>No. of Injections</th>
<th>Total Virus %</th>
<th>Serum</th>
<th>Course of Immunisation</th>
<th>Symptoms During Immunisation</th>
<th>Amount of Virus 5% Serum</th>
<th>Result</th>
<th>Amount of Virus 5% Serum</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-0</td>
<td>2 cc.</td>
<td>10</td>
<td>None</td>
<td>2 cc. of 20% virus intradermally</td>
<td>None</td>
<td>0.05 0.95</td>
<td>Immune</td>
<td>0.1 0.9</td>
<td>8 days, weakness arms. 9 days — prostrate</td>
</tr>
<tr>
<td>5-4</td>
<td>7 cc.</td>
<td>6</td>
<td>None</td>
<td>6 cc. of 20% virus intradermally</td>
<td>None</td>
<td>0.05 0.95</td>
<td>Immune*</td>
<td>0.1 0.9</td>
<td>9 days — weak. 10 days — prostrate</td>
</tr>
<tr>
<td>5-7</td>
<td>9 cc.</td>
<td>4</td>
<td>None</td>
<td>4 cc. of 20% virus intradermally, plus 6 cc. of serum subcutaneously</td>
<td>None</td>
<td>0.05 0.95</td>
<td>Symptoms at 18 days, with weakness of right leg. All cleared up</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5-8</td>
<td>6 cc.</td>
<td>5</td>
<td>None</td>
<td>5 cc. of 20% virus intradermally, plus 3 cc. serum subcutaneously</td>
<td>None</td>
<td>0.05 0.95</td>
<td>Immune</td>
<td>0.1 0.9</td>
<td>10 days, both arms paralyzed. 11 days, prostrate</td>
</tr>
<tr>
<td>1-18</td>
<td>3 cc.</td>
<td>12</td>
<td>None</td>
<td>12 cc. of 10% virus intradermally</td>
<td>None</td>
<td>0.05 0.95</td>
<td>Immune</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2-1</td>
<td>10</td>
<td>Control</td>
<td>—</td>
<td>Control</td>
<td>—</td>
<td>0.05 0.95</td>
<td>Prostate — 8 days</td>
<td>0.02 0.98</td>
<td>Prostate 9 days</td>
</tr>
</tbody>
</table>

* Died of intercurrent infection at end of 6 weeks.
‡ Tested by in vivo tests with 0.05 cc. virus intracerebrally, developed paralysis 9th day. Necropsied.
§ No. 1-18 died of intercurrent infection at end of 9 weeks.
Results. — A study of Table I will show the antibody production of these animals against 0.05 cc. of 5 per cent active poliomyelitis cord emulsion (Neutralization Test 1). The sera of four out of five animals neutralized the virus. When the virus and the serum mixture of each of these was injected into another monkey, no symptoms occurred. The animal which received the serum from Animal 5-7 developed weakness of the right leg, which cleared up within 2 weeks. Three of the animals, Nos. 5-0, 5-4, and 5-8, whose serum was tested against 0.1 cc. of the same virus emulsions, failed to neutralize that amount (Neutralization Test 2). Monkey 5-7 was rendered prostrate on the 9th day after a direct intracerebral inoculation of 0.05 cc. of the same virus.

Four out of five animals failed to respond to 0.05 cc. of a 5 per cent suspension of active virus, which rendered the control prostrate in 8 days. It can be presumed that the fifth animal partially resisted the virus, for when the virus and serum mixture was injected into a monkey, only transient symptoms occurred after an incubation period of 18 days.

Monkey 1-18 was not tested against 0.1 cc. of 5 per cent virus. The remaining three were unable to resist that amount of cord suspension, as shown by Neutralization Test 2 in Table I.

The experience with Monkey 5-7 illustrates what has already been cited in the literature (Aycock and Kagan (6) and Rhoads (8)), namely, that the neutralization test is more sensitive for the demonstration of immune bodies than direct intracerebral inoculation.

According to the above experiments, virus together with human convalescent serum can produce immunity. It was important, therefore, to ascertain the minimal quantity of serum necessary to protect an animal against the disease during vaccination, without interfering with the immunizing power of the virus. Therefore, with a fixed amount of virus, varying quantities of immune serum were used.

Experiment II. — 1 gm. of spinal cord, equivalent to 20 cc. of a 5 per cent suspension, was emulsified in 8 cc. of distilled water, and administered to each of six monkeys intradermally (Table II). The first received only the virus intradermally. The second and third received the virus with 2.5 cc. and 5 cc. of serum respectively, subcutaneously. The fourth animal had the virus and 6 cc. of serum. The last two were not injected with the virus and serum at the same time.
received 4 cc. of serum 4 days earlier than the virus, while the other had 6 cc. of serum 3 days subsequent to the virus inoculation.

**Results.**—The first two animals succumbed to poliomyelitis within 9 days, while the third, which had been given 5 cc. of serum, fell ill on the 12th day, and was prostrate the 19th day. The fourth animal, which had received 6 cc. of serum, resisted the disease. The fifth animal, which had received 4 cc. of serum before the virus, succumbed to the disease on the 6th day, while the sixth monkey, which had been given 5 cc. of serum 3 days after the virus, remained well.

The above experiment indicates that, by this method of administration, 6 cc. of immune serum is required to protect a monkey against a gram of virus given intradermally. This is in contradiction to the results of Rhoads (8), who injected 16 cc. of virus emulsion (0.8 gm. of cord) intradermally into each of a series of four monkeys. None of his animals developed the disease. This discrepancy may be accounted for by the manner of injection of the virus, as many piqures by Rhoads, and as one by us. Thus, in this work, more virus infiltrated the subcutaneous tissues (on account of the size of the dose given), thereby allowing more rapid absorption and more likelihood of infection.

The proportions of virus and serum that proved innocuous when administered simultaneously, were 6 cc. of serum to each gram of virus.

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**TABLE II**

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Virus amount</th>
<th>Serum amount</th>
<th>Combination used</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Virus intradermally</td>
<td>8—paralysis 1 arm. 9—died</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2.5</td>
<td>Virus intradermally plus serum subcutaneously</td>
<td>8—paralysis right arm. 9—died</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>5</td>
<td>Virus intradermally plus serum subcutaneously</td>
<td>12—weakness right arm. 19—prostrate</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>6</td>
<td>Virus intradermally plus serum subcutaneously</td>
<td>No paralysis</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>4</td>
<td>Serum subcutaneously. In 4 days—virus intradermally</td>
<td>4—weakness left arm. 5—prostrate. 6—died</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>5</td>
<td>Virus intradermally. In 3 days—serum subcutaneously</td>
<td>No paralysis</td>
</tr>
</tbody>
</table>

 Died at end of 1 month of tuberculosis—no lesions of poliomyelitis.
ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS

The next experiment was to test the safety of the same proportions when the virus was given first, followed some days later by the serum (as indicated by the experience with Monkey 6 in our Table II), or when the serum was given first, followed later by the virus.

Experiment III.—Two monkeys (Table III) were used for this experiment. The first received 1 gm. of virus, followed in 3 days by 6 cc. of serum, while the other was given 6 cc. of serum, and 3 days later, a gram of virus.

Results.—Neither animal developed symptoms. Therefore, since we used a highly active virus and whereas, as in the case of Monkey 1-36, the virus was allowed to act 3 days before the serum, the administration of 6 cc. of serum with a gram of virus may be considered innocuous under the conditions outlined.

The next step was to determine the effects produced when the above method of administering these materials was reversed. Therefore,

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Virus</th>
<th>Serum</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-36</td>
<td>1 gm.</td>
<td>6 cc.</td>
<td>1 gm. virus given intradermally. In 3 days 6 cc. serum subcutaneously</td>
<td>No paralysis</td>
</tr>
<tr>
<td>1-34</td>
<td>1</td>
<td>6</td>
<td>6 cc. serum subcutaneously. In 3 days 1 gm. virus intradermally</td>
<td>No paralysis</td>
</tr>
</tbody>
</table>

 Died intercurrent infection at the end of 4 weeks. Histological sections ruled out poliomyelitis, as did a monkey transmission of cord.

the infective substance was injected subcutaneously, and the serum was given intradermally, presuming thereby that the virus was absorbed more rapidly than the serum.

Experiment IV.—One monkey was given a gram of virus subcutaneously, and 6 cc. of serum intradermally.

Result.—In 5 days the animal was paralyzed. Therefore, the quantities of virus and serum that had proved innocuous, using the former intradermally, and the latter subcutaneously, were infective when the virus was given subcutaneously, and its serum intradermally.

Having determined that 6 cc. of serum given subcutaneously rendered the intradermal inoculation of 1 gm. of virus innocuous, the next step was to test the immunizing power of virus and serum in these amounts.
Experiment V.—Three animals, each of which was injected intradermally with a
gram of virus made up to 8 cc. of emulsion, received 6 cc. of serum subcutaneously.
The first received the serum 3 days before the virus inoculation, the second at the
same time, while the third received serum 3 days after the infective material.
Again, the neutralization test was used, the serum of the treated animals being
tested against 0.05 cc. of virus emulsion. Not until 6 weeks after the last injection
was the test made, thereby guarding against any residual passive immunity from
the serum.

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Process of immunization</th>
<th>Virus %</th>
<th>Serum</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-34</td>
<td>6 cc. serum subcutaneously. In 3 days 1 gm. virus intracutaneously</td>
<td>0.05</td>
<td>0.95</td>
<td>Partial protection. Incubation 12 days, paralysis hind legs, and later partial of forearms. Survived. Recovering</td>
</tr>
<tr>
<td>1-35</td>
<td>1 gm. virus intradermally. 6 cc. serum subcutaneously</td>
<td>0.05</td>
<td>0.95</td>
<td>Immune</td>
</tr>
<tr>
<td># 1-36</td>
<td>1 gm. virus intradermally. In 3 days 6 cc. serum subcutaneously</td>
<td>0.05</td>
<td>0.95</td>
<td>Immune</td>
</tr>
<tr>
<td>1-42</td>
<td>Control</td>
<td>0.02</td>
<td>0</td>
<td>9 days weakness right arm. 12 days—prostrate</td>
</tr>
<tr>
<td>1-43</td>
<td>Control</td>
<td>0.02</td>
<td>0</td>
<td>8 days weakness right arm. 10 days—prostrate</td>
</tr>
<tr>
<td>1-45</td>
<td>Control</td>
<td>0.02</td>
<td>0</td>
<td>12 days prostrate</td>
</tr>
</tbody>
</table>

Neutralization tests carried out 5 weeks after completion of course of vaccination.

# Died at the end of 4 weeks of intercurrent infection.

Results.—(Table IV.) The serum of Animal 1-34, which received serum first and the virus later, gave doubtful protection. The test animal developed complete paralysis of the hind limbs, and partial of the upper extremities, but survived, and is recovering. The other two monkeys resisted the virus. The controls succumbed in from 10 to 12 days to 0.02 cc. of this virus emulsion.

In this small series, serum given subcutaneously with or after the injection of virus, was more effective than when the serum was given first. Moreover, a gram of virus with 6 cc. of serum administered in either of the more effective ways (i.e., virus and serum simultaneously,
or serum 3 days after virus) induced sufficient immunity to resist two and one-half times the dose of virus that paralyzed the control animals. With specimens of this virus, infection had been produced with doses as small as 0.01 cc. of a 5 per cent suspension.

Immunity is a relative thing, and none is probably so great that it cannot be broken down by a large amount of virus. Indeed, Aycock and Kagan (6) have reproduced poliomyelitis in animals by using, at a second injection, large amounts of virus. Therefore, an immunity to several lethal doses may be considered definite, and perhaps useful.

In the two series, immunization was carried out on eight animals. Six of these resisted 0.05 cc. of 5 per cent virus emulsion, and the other two partially resisted as indicated by the prolonged incubation period and the milder attack in the test animals, as compared with the controls. Using larger amounts of virus, Rhoads (13) did not obtain as complete immunity, for only half of his monkeys resisted 0.01 cc. of a 5 per cent filtrate. However, he used more serum in proportion to virus, and in addition combined them. In this way he had complete neutralization as checked by intracerebral test.

Todd (15), Andrewes (16), and Long and Olitsky (17), using vaccine virus, and Schultz et al. (18) and Olitsky et al. (19) poliomyelitis virus, have shown that neutralization of the virus with immune serum does not destroy the virus. Yet, the fact that virus can be recovered from a combination with its serum, is no indication that such a mixture always dissociates sufficiently in the body to immunize efficiently. Only when the serum is not in excess is this possible. This has been pointed out by Zinsser and Tang (20), who produced active immunization against herpes virus with virus emulsion and immune serum. They concluded as follows: "Active immunity can be attained only when some degree of reaction to the living virus has occurred. Rabbits which survived neutralized serum-virus mixtures did not acquire immunity." Similarly Rhoads (21) used vaccine virus with immune serum, in rabbits, in such quantities as were innocuous intradermally. He found that an excessive amount of immune serum rendered the mixtures ineffective. Therefore, by using less serum than Rhoads, and yet sufficient to render the procedure safe, a greater degree of immunization has been obtained by us.
CONCLUSIONS
1. A combination of poliomyelitis virus and specific human serum is effective for the production of active immunity.
2. For each gram of active virus given intradermally as an emulsion, 6 cc. of human immune serum, injected subcutaneously, was required in our experiments to protect a monkey from paralysis. Some degree of active immunity was induced.
3. Immunity, without symptoms of the disease, was secured when the serum was given at the time of inoculation, or within 3 days preceding or following inoculation of the virus.
4. For the production of immunity, virus, preceded by serum administration, is probably less effective than when it is given simultaneously with, or before, the injection of serum.
5. The virus neutralization test is more sensitive than the direct intracerebral test for determining the production of immunity.

BIBLIOGRAPHY
2. Kraus, R., Z. Immunitätsforsch., 1911, 9, 117.
15. Todd, C., Brit. J. Exp. Path., 1928, 9, 244.