FAILURE TO NEUTRALIZE THE POLIOMYELITIS VIRUS
WITH SERA OF ADULT MACACUS RHECUS AND OF
YOUNG FEMALE RHECUS TREATED WITH
ANTERIOR PITUITARY EXTRACTS*

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The usual conception of the epidemiology of poliomyelitis is that
man is relatively resistant to the disease, that the virus is commonly
transmitted from and to the nasopharynx and is more widely distrib-
uted than is evidenced by clinical records, and that the general
population is largely immunized by these factors of host resistance
and parasite distribution. Difficulty of direct experimental proof
of these principles lies in the fact that the virus is not cultivable by
bacteriological methods and hence is dependent upon animal inocula-
tion for its demonstration. By the use of this method, however, the
virus has been recovered in non-clinical cases in a few instances (1).
Several more indirect types of evidence are available to support the
classic conception, but the one we are here concerned with is the power
of the serum of certain adults without history of poliomyelitis to
neutralize the virus, a property which has been assumed to be due,
by analogy with other diseases, to subclinical infections with the
causative agent.

The view that such an assumption is not entirely tenable has been
expressed by Jungeblut and Engle, who suggest that "resistance to
poliomyelitis . . . is predominantly a function of normal physi-
ological maturation and to a large extent seems to develop independ-
ently of previous contact with the specific antigen" (2a). This

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theory is based on their ability to demonstrate the neutralization of virus with the sera of certain adult *Macacus rhesus* and of immature female *rhesus* caused to menstruate by injections of anterior pituitary extracts (*2a, b*). These monkeys had had no exposure to poliomyelitis virus, and hence the occasional neutralization was attributed to endocrine factors and maturation. More recently, the same authors have detailed the experiments with adult monkeys of both sexes and with young females treated with a variety of gonad-stimulating principles. They report (*2c*) that a certain proportion of each group was shown to possess neutralizing serum and some were resistant to infection by the cerebral route.

We have been concerned with this problem of specificity in poliomyelitis immunity and have attempted to obtain experimental data in support of the theory of physiological ripening as a factor in resistance to this disease. The present communication reports the results of tests on the sera of adult *rhesus* monkeys and of young female monkeys menstruating after treatment with extracts of the anterior pituitary. The technic of testing the serum was as previously described (*3*), employing 3 parts of serum to 1 part of 1.25 per cent centrifugated monkey virus cord (PMV strain). Monkeys fatally injected with these mixtures were diagnosed as dying of experimental poliomyelitis on the bases of fever, paralysis, and histopathology of the cord. Virus and serum control monkeys were included in the several experiments, each of the former dying of poliomyelitis and each of the latter surviving without symptoms.

**EXPERIMENTAL**

The first neutralization tests were carried out on the sera of adult male and female monkeys. These animals were judged to be mature or submature on the basis of their height, weight, and dental and sexual development. The dentition was either completely permanent or early permanent in type. The males possessed completely descended testicles and the females were passing through more or less regular, but definite, menstrual cycles. Most of these animals had been in the department animal quarters for a year or more (prior to the first test) and were used for various bacteriological purposes; the majority of them had been injected with mixtures of serum and poliomyelitis virus but had never shown any evidence of this disease.
### TABLE I

Tests of Serum of Adult Male Monkeys for Property of Neutralizing Poliomyelitis Virus

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Dentition</th>
<th>Sexual Development</th>
<th>Dates Bled</th>
<th>Remarks</th>
<th>Serum Neutralization of Poliomyelitis Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.1</td>
<td>67</td>
<td>Early permanent</td>
<td>Testes fully descended</td>
<td>June 9, 1932</td>
<td>These animals had received human nervous tissue 6 mos. before test</td>
<td>Negative</td>
</tr>
<tr>
<td>B</td>
<td>4.9</td>
<td>71</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Mar. 22, 1933</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.0</td>
<td>73</td>
<td>Complete permanent</td>
<td>&quot;</td>
<td>Mar. 22, 1933</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6.5</td>
<td>74</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 9, 1932</td>
<td>These animals had received an intracerebral injection of a serum-virus mixture nearly 2 yrs. before first bleeding; have been used in studies on intestinal flora for the last 3 yrs.</td>
<td>Negative</td>
</tr>
<tr>
<td>E</td>
<td>6.2</td>
<td>71</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 9, 1932</td>
<td>&quot;</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>6.8</td>
<td>72</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 9, 1932</td>
<td>&quot;</td>
<td>Negative</td>
</tr>
<tr>
<td>G</td>
<td>5.6</td>
<td>74</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 9, 1932</td>
<td>Had been kept with fatally injected poliomyelitis monkeys for 15 mos.</td>
<td>Negative</td>
</tr>
<tr>
<td>H</td>
<td>5.3</td>
<td>72</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 7, 1932</td>
<td>Normal monkey</td>
<td>Negative</td>
</tr>
<tr>
<td>I</td>
<td>8.3</td>
<td>Not determined</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Apr. 18, 1933</td>
<td>Father of male born Mar. 17, 1933</td>
<td>Negative</td>
</tr>
<tr>
<td>J</td>
<td>5.0</td>
<td>71</td>
<td>&quot;</td>
<td>&quot;</td>
<td>June 9, 1932</td>
<td>Immunized by cutaneous inoculation of virus cord 1 yr. before</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Measured from crown to heel.

**545**
TABLE II
Tests of Serum of Adult Female Monkeys for Property of Neutralizing Poliomyelitis Virus

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Description</th>
<th>Dates bled</th>
<th>Remarks</th>
<th>Serum neutralization of poliomyelitis virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>4.9</td>
<td>70</td>
<td>Complete permanent</td>
<td>Specimen taken 2nd day of vaginal bleeding, edema and hyperemia of sex skin; no gross vaginal bleeding, no edema or hyperemia of sex skin; no gross vaginal bleeding</td>
<td>June 9, 1932, Jan. 18, 1933, Mar. 8, 1933</td>
<td>Had been kept with fatally injected poliomyelitis monkeys for 15 mos.</td>
</tr>
<tr>
<td>L</td>
<td>6.0</td>
<td>Not determined</td>
<td>&quot; &quot;</td>
<td>Specimen taken 9 days after onset of menstruation</td>
<td>Apr. 18, 1933</td>
<td>Mother of male born Mar. 17, 1933; bled 32 days postpartum</td>
</tr>
<tr>
<td>M</td>
<td>3.2</td>
<td>63</td>
<td>Early permanent</td>
<td>Specimen taken 9 days after onset of menstruation</td>
<td>Mar. 18, 1933</td>
<td></td>
</tr>
</tbody>
</table>
| N  | 3.7 | 68 | Early permanent | Specimen taken 10 days before onset of menstruation  
|    |     |    |                | Edema and hyperemia of sex skin; vaginal bleeding 6 days after specimen taken  
|    |     |    |                | Faint hyperemia of sex skin; specimen taken 14 days after onset of menstruation  
|    |     |    |                | Faint hyperemia of sex skin; vaginal bleeding 8 days after specimen taken  
|    |     |    |                | Slight hyperemia of sex skin; specimen taken 1st day of vaginal bleeding  
|    |     |    |                | Faint hyperemia of sex skin; specimen taken 9 days after onset of menstruation  
|    |     |    |                | June 9, 1932  
|    |     |    |                | Jan. 18, 1933  
|    |     |    |                | Feb. 7, 1933  
|    |     |    |                | Repeatedly inoculated cutaneously with normal monkey cord 1 yr. before  
|    |     |    |                | Feb. 28, 1933  
|    |     |    |                | Mar. 8, 1933  
|    |     |    |                | Mar. 17, 1933  
|    |     |    |                | Negative  
|    |     |    |                | "  
|    |     |    |                | "  
|    |     |    |                | "  
|    |     |    |                | "  

* Measured from crown to heel.
Twelve samples of undiluted serum from nine adult males were tested. Six of these specimens were obtained in June, 1932, and were uniformly devoid of neutralizing power. Since it was proposed by Jungeblut (4) that a seasonal factor might enter the problem, three of the animals still available were bled again in March, 1933. Serum was also obtained at this time from three other adult males. These six undiluted sera likewise failed to inactivate the virus (for details of experiment, see Table I). The serum of a tenth adult male immunized intracutaneously with virus cord, served as a control in this and subsequent experiments and invariably neutralized the virus.

A similar lack of virucidal property was observed in the sera of four adult females. Of the eleven specimens tested, two were obtained in June, 1932, the others from January to April, 1933. Ten samples, drawn before, during, or after menstruation, failed completely to neutralize the virus when used undiluted. The eleventh sample, taken from a female 32 days postpartum, gave equivocal results: in the first test, it inactivated the virus; but when titration was attempted with the serum undiluted and diluted 1:5, it failed to neutralize in both concentrations (Table II).

These experiments did not reveal a correlation between the virucidal capacity of monkey serum and maturity, with the possible exception of the instance just mentioned. Furthermore, physiological fluctuations in endocrine balance, as exemplified by the menstrual cycle, failed to induce the appearance of virus-neutralizing substances in the serum of the animals tested. The opportunity of determining the resistance of the adult monkeys to infection by cerebral injection was not afforded.

We attempted to confirm Jungeblut and Engle's observation that immature monkeys treated with a gonad-stimulating principle of the anterior pituitary occasionally yielded serum possessing virucidal properties (2 b, c). Our experimental animals for this purpose were young female rhesus monkeys weighing between 2 and 2.5 kilos and possessing entirely deciduous teeth. Their sexual development appeared to be in a completely immature state.

The gonad-stimulating substance (5) used in these experiments was a clinical preparation made by Mrs. Zonja Wallen-Lawrence of the Department of Physiological Chemistry and Pharmacology. It was
prepared from whole sheep pituitary powder by a method which will be reported from that laboratory. Anterior pituitary extract was administered to ten monkeys intramuscularly in equal daily amounts (except in two instances) over periods varying from 5 to 13 days. As treatment progressed the area in the region of the buttocks, called the sex skin, became hyperemic and edematous. By means of rectal

examination, an increase in the size of the ovaries and the uterus was noted, while vaginal lavages, which during a control period prior to treatment were negative, revealed mucus and a rapidly increasing number of leucocytes and epithelial cells. Upon cessation of treatment the intensity of the hyperemia and edema in the sex skin diminished and gross uterine bleeding occurred. The ovaries and uterus also decreased in size at this time.

### TABLE III

Tests of Serum of Young Female Monkeys Treated with Anterior Pituitary Extracts for Property Neutralizing Poliomyelitis Virus

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Description*</th>
<th>Anterior pituitary extract injected</th>
<th>Number of injections intramuscularly</th>
<th>Dates of menstruation during period of observation</th>
<th>Serum specimen taken after onset of menstruation</th>
<th>Serum neutralization of poliomyelitis virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0</td>
<td>55</td>
<td>13</td>
<td>Mar. 3–12</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>58</td>
<td>13</td>
<td>Apr. 19–28</td>
<td>10</td>
<td>“</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>60</td>
<td>13</td>
<td>Mar. 1–20</td>
<td>11</td>
<td>“</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>57</td>
<td>13</td>
<td>May 16–26</td>
<td>9</td>
<td>“</td>
</tr>
<tr>
<td>5</td>
<td>1.9</td>
<td>57</td>
<td>13</td>
<td>“ 14–26</td>
<td>9</td>
<td>“</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>55</td>
<td>13</td>
<td>“ 16–20</td>
<td>9</td>
<td>“</td>
</tr>
<tr>
<td>7</td>
<td>2.2</td>
<td>56</td>
<td>13</td>
<td>“ 22–28</td>
<td>9</td>
<td>“</td>
</tr>
<tr>
<td>8</td>
<td>2.3</td>
<td>55</td>
<td>13</td>
<td>“ 22–28</td>
<td>9</td>
<td>“</td>
</tr>
<tr>
<td>9</td>
<td>2.0</td>
<td>56</td>
<td>13</td>
<td>“ 22–31</td>
<td>9</td>
<td>“</td>
</tr>
</tbody>
</table>

* Completely deciduous dentition in all monkeys of this group.
† Measured from crown to heel.
‡ As determined by microscopic examination of vaginal washings.
Blood for the neutralization tests was drawn from 7 to 11 days after the onset of uterine bleeding, in conformity with the interval used by Jungeblut and Engle (2 a). Fourteen specimens of serum from the ten females were tested undiluted. Nine of these animals received intracerebrally a mixture of their own serum plus virus. The serum of the tenth female, which succumbed to intercurrent infection in the interim between bleeding and the test, was examined in an unused monkey (as were the remaining four specimens). None of the fourteen sera neutralized the virus (Table III).

At the conclusion of the experiment the sexual tract of each monkey was studied. The ovaries were juvenile in appearance and in none was there any evidence of a corpus luteum, nor was there any other indication of ovulation. In every case the uterus appeared undeveloped and the mucous membranes presented the condition typical of puberty. The uterine mucous membrane was 1 mm. or less in thickness in all the animals except Monkey 3, in which it was 2 mm. thick. There was no sign of pseudopregnancy anywhere in the uterine mucosa. Nothing resembling sexual maturity was found in any of these animals.

From these findings—the hyperemia and edema of the sex skin followed by a menses-like bleeding which occurs normally in M. rhesus at the time of puberty, together with the degree of development in the sexual organs—it is concluded that the administration of anterior pituitary extract caused in our monkeys a precocious sexual development to a state similar to that found at puberty, but which cannot be interpreted as sexual maturity. Such a condition induced neither a virucidal capacity of the serum nor a systemic resistance to the virus of poliomyelitis.

**COMMENT**

The technic of the neutralization test employed in these experiments is somewhat different from that generally used elsewhere, in that the serum-virus ratio is 3:1 and the amount of virus is reduced to the supernatant of a centrifugalized 1.25 per cent emulsion of monkey virus cord. While this technic may seem to favor the determination of serum virucidal property, our results in examining human sera have been entirely comparable to those of other laboratories. In fact,
the results of our testing Chinese sera (6) were the same as those obtained by Jungeblut (7) in examining specimens of identical origin and lot. Our dose of virus, on the other hand, is well above the minimal infective dose, so that no false positive results have occurred. The significance of the negative serum tests reported in this paper is more apparent, in view of these remarks on technic.

The only possible evidence in these experiments for the influence of a physiological factor in resistance to the experimental disease was the result of testing the serum of an adult female 32 days postpartum. The same specimen neutralized the virus in one test but not in a second, which may be ascribed either to a low virucidal content perhaps referable to the previous pregnancy or to a peculiar resistance on the part of the young test monkey.

It is interesting to note that two adult monkeys, male and female, included in these series, did not acquire a serum virucidal power through intimate contact over a period of 15 months as cage mates with numerous monkeys succumbing to injections of virus.

The possibility that a general bodily resistance to infection with poliomyelitis virus might have been produced in the immature animals treated with anterior pituitary extract was taken into consideration. The serum-virus mixtures were injected into the brain of the animal that had furnished the serum, so that factors other than the possible virucidal capacity of the serum might exert their influence. In this group, too, every monkey succumbed to infection.

Our inability to effect neutralization with the sera of adult male and female monkeys and of young monkeys induced to menstruate by artificial means lends experimental evidence to the view that the virucidal property of serum is not attributable only to maturity or to certain fluctuations in the physiological state of the individual.

**SUMMARY AND CONCLUSIONS**

1. Twelve specimens of serum from nine adult male monkeys failed to neutralize the virus of poliomyelitis.

2. Ten samples of serum obtained from three adult female monkeys at various phases of the menstrual cycle likewise proved incapable of neutralizing the virus. An eleventh serum, drawn from a fourth female 32 days postpartum, gave irregular results. It neutralized
once and failed to do so on second test. This is the only suggestion in our experiments that a physiological factor may play a part in poliomyelitis immunity.

3. Fourteen sera from ten immature monkeys caused to menstruate by treatment with anterior pituitary extract were devoid of virucidal property. This treatment failed also to induce a systemic resistance to intracerebral injections of virus in the nine monkeys of the same group available for test.

4. We were unable to demonstrate in our monkeys a correlation between virucidal capacity of the serum and maturity or physiological variations as exemplified by menstruation.

We wish to thank Mrs. Zonja Wallen-Lawrence for the anterior pituitary extracts, Dr. I. Schour of the University of Illinois College of Dentistry for the determination of dentition, and Dr. George W. Bartelmez of the University of Chicago for the interpretation of the generative tract findings in the young treated monkeys.

REFERENCES