Dec. 5, 1956

Prof. Dr. J. D. Verlinde
Nederlands Instituut Voor Praeventieve Geneeskunde
Wassenaarseweg 56
Leiden, Holland

Dear Dr. Verlinde:

Thank you very much for your letter of 30 November describing new tests with single particle cultures of the Leiden 1956/31 strain. I need hardly point out to you that with strains that are already as highly attenuated for the spinal neurons of monkeys as is the Leiden 1956/32 strain, tests on 2 monkeys or even 4 monkeys are most misleading. Although my own tests with the subculture of the strain you were good enough to send me are not yet complete I hasten to send you the results obtained thus far.

You will note first of all a much higher titer of virus I obtained in the cultures that I prepared. On the basis of previous experience I would think that may be due to the fact that your medium is not Hanks¹ solution which, undoubtedly, quickly becomes acid and thus interferes with the propagation of this particular virus. Please note also how misleading the information about this strain could have been if I had used less than 20 monkeys in this original trial. The pattern exhibited by this spinal titration is not unlike that which I have observed with other strains even when the progeny of single triply purified plaques has been used. This naturally occurring strain behaves so much like the LSc virus that I thought it worth while to pick a certain number of plaques, purify them 3 times and then test them again to determine whether by chance the progeny of one of them may actually be so highly attenuated that it will uniformly fail to produce lesions as well as paralysis. The purification of the plaques is now under way and I shall let you know the results as soon as they are available.

You can appreciate, however, that under the circumstances even an orienting test could not be carried out with less than 15 monkeys for each plaque derivative (using 5 monkeys for each of 3 dilutions). Even then I have found that when such a preliminary test on 15 monkeys is entirely negative a repetition on another 15 monkeys may not give quite the same results. Accordingly, unless you are prepared to use the necessary number of monkeys for such tests it is very difficult to obtain results on which one can place some reliance.

With all good wishes and kindest personal regards.

Sincerely yours,

Albert B. Sabin, M. D.

ABS:meh
Preliminary Data on Leiden 1956/32 Strain of Type 1 Poliovirus

Cincinnati Subculture: - 0.5 ml of a Berkfeld V filtrate of the Leiden, KP 6 culture fluid, that was in the mails without refrigeration for 8 days, was added to each of three 3-ounce prescription bottles (area of cynomolgus kidney epithelial sheet is 7.5 cm x 3.5 cm). After adsorption at 37°C for 1 hour, 10 ml of medium was added to each bottle. The medium consists of 0.5% lactalbumin hydrolysate (neutralized with 4 ml of N/1 NaOH per 100 ml of 10% hydrolysate) in Earle's solution (pH 8.2), the final mixture being gassed with CO₂ to a pH of about 7.6. [Note: the epithelial sheet is grown out in a Hanks solution lactalbumin hydrolysate medium containing 2% calf serum; the fluid is changed at 4 days using the same medium but with twice the usual concentration of bicarbonate in Hanks' solution. The bottles are used at 7 days after planting and the fluid is poured off just before the virus is added for adsorption.] At 48 hours after addition of the virus, the cytopathogenic effect was complete with the cells off the glass, and the fluid was harvested.

Titer of First Cincinnati Subculture: - The virus was identified as Type 1 polio.

Roller tubes in roller drum - 10⁷.⁵ TCD₅₀/ml.
Plaque count in rubber-stoppered bottles - 10⁷.₉ P. F. U./ml.

Note: Based on the test in roller tubes, the titer of this fluid is 32 times higher than that reported from Leiden. The plaque procedure furthermore was 5 times more sensitive than the roller tubes in this case.

Spinal Paralytogenic Activity in Cynomolgus Monkeys: - Test is still in progress. Results based on 9 days of clinical observation. Histological examination to be made at about 20 days.

<table>
<thead>
<tr>
<th>Dose - 0.1 ml.</th>
<th>No. of monkeys</th>
<th>Days on which paralysis was observed in individual monkeys</th>
<th>Result 9th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>TCD₅₀</td>
<td>P. F. U.</td>
<td></td>
</tr>
<tr>
<td>Undil.</td>
<td>10⁶.⁵</td>
<td>10⁶.⁹</td>
<td>10</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>10⁵.⁵</td>
<td>10⁵.⁹</td>
<td>5</td>
</tr>
<tr>
<td>10⁻²</td>
<td>10⁴.⁵</td>
<td>10⁴.⁹</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: The paralysis was partial, limited to one leg and nonprogressive. Where the notation is "slight?" it means that without histologic examination one cannot be certain that it may not be a delayed traumatic effect.