MEMORANDUM

May 8, 1961

TO: Members, Enterovirus Committee

FROM: Executive Secretary

SUBJECT: Production of Immune Sera in Horses with the Polio Viruses.

I am enclosing for your information two interesting letters on some experiments conducted on the production of immune sera in horses using polio virus antigens.

One letter is from Dr. Robert N. Hull, Research Associate in Charge, Tissue Culture Research, Biological Research Division, The Lilly Research Laboratories, Indianapolis, Indiana. The other letter is from Dr. Alan P. Goffe, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, England.

Enclosures

MKH:co
April 25, 1961

Marvin M. Harris, Ph.D.
Chief, Human Virology Program
National Cancer Institute
National Institutes of Health
Bethesda 14, Maryland

Dear Dr. Harris:

This is in reply to your letter of April 17th concerning the production of antiserum in horses.

Our experience in this area amounts to one experiment done in 1955. Three horses were immunized with monovalent poliomyelitis vaccine; one horse for each serological type. The actual immunization was done in our Greenfield Laboratories and the records for same are filed away in a vault. Without digging these out, it is my recollection that the horses received intramuscular doses of 250 ml of vaccine, but I don't remember exactly the spacing between doses nor the exact time of bleeding. The results of the immunization I have in my own files, however, and these are seen in the following table.

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>Pre</th>
<th>Post 1</th>
<th>Post 2</th>
<th>Post 3</th>
<th>Post 4</th>
<th>Cross Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dose 1</td>
<td>doses 2</td>
<td>doses 3</td>
<td>doses 4</td>
<td>types I, II, III</td>
</tr>
<tr>
<td>6657 - I*</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;16</td>
<td>16</td>
<td>256</td>
<td>16 4</td>
</tr>
<tr>
<td>6658 - II*</td>
<td>&lt;4</td>
<td>16</td>
<td>16</td>
<td>64</td>
<td>256</td>
<td>4  4</td>
</tr>
<tr>
<td>6659 - III*</td>
<td>&lt;4</td>
<td>16</td>
<td>64</td>
<td>256</td>
<td>1024</td>
<td>32 8 4</td>
</tr>
</tbody>
</table>

As you will note, the horses received a total of four doses which would equal one liter of vaccine in all. Most likely the bleedings in each instance were obtained one week after the last dose. The response following four doses was fairly good, but as you will note in the last three columns, there was some cross-reactions amongst the three types of virus. At the time of the experiment, we were trying to prepare type specific antiserum and because of these cross-reactions abandoned the use of horses for this purpose.

In addition to checking the horses sera for poliovirus antibody, we also checked two of the horses for antibody to the simian viruses which were classified at that time. One horse was found to have antibody against S.V.5 (1:32) and to S.V.12 (1:8). The other horse had low level antibody against S.V.4 and S.V.6 but greater than 1:4 for S.V.5. Since it was necessary for us to have poliovirus typing sera which was free of simian virus antibodies, these also were discouraging findings.
April 25, 1961

If it would be important to you to have more information about the actual immunization of the horses, we could dig out these old records. If there is any other way I can be of help, please do not hesitate to contact me.

Sincerely yours,

/s/ Robert N. Hull, Ph.D.
Research Associate
In Chg., Tissue Culture Res.
Biological Research Division

RNM:SLG
(co 5/2/61)
M. M. Harris, Ph.D.
Chief, Human Virology Programme
National Cancer Institute
Public Health Service
Bethesda 14
Maryland, U.S.A.

Dear Dr. Harris:

Thank you for your letter of April 17th. Our experience of making enterovirus antisera has been limited to two batches of horses hyper-immunised with Sabin's attenuated polioviruses.

To remain within the framework of the prescribed test requirements the strains were first passaged in a human cell line; the HEP-2 cell was found to be the best among those we tried. In order to reduce interspecies antibody stimulation each batch of HEP-2 cells was initiated on Horse serum medium. No special adaptation of our cell line, normally grown on calf serum, was found necessary. The immunizing antigen was grown by a stage system based on Westwood (Brit. J. exp. Path., XL/2/1960) and further concentrated ten-fold by one cycle in the Spinco. Deposition was effected by 30,000 r.p.m. for 3 hours in a gelatin medium. Single lots of antigen, one for each strain, were prepared in this way and stored frozen.

We attempted to compare straight fluid antigen with the same in mineral oil adjuvant (Arlacel/Bayol), both given by the intramuscular route in 20 ml. volumes. This was not entirely successful as we had to reduce the volume of adjuvant mixture. Volumes of more than 10 ml. given at a single site were found to give severe local reactions with systemic disturbance. Sera from test bleedings were titrated against 10^7 TCD50 of the homologous strain. Peak antibody production appeared to be between 2 1/2 and 3 1/2 months after initiating injections when serum diluted 1:320 neutralised over 7 logs of virus. Horses receiving adjuvant mixture at 4-week intervals had similar levels to those receiving double the volume of fluid antigen at 2-week intervals. The adjuvant technique, therefore, allows one to economise on antigen and numbers of injections, but does not seem per se to produce better serum.
Our present procedure, based on this experience, is to give concentrated fluid antigen, in 20 ml. volumes, at 2-week intervals, by the intramuscular route; test bleedings are collected at the intermediate 2-week intervals. Currently, 2 months after the first injection of the course, antibody titres of 1:16 against 7.5 logs of virus have been achieved. We shall wait till they top the 1:100 level, then put in an intravenous booster and bleed out.

I would emphasize the need to take serial bleedings to locate the period of maximal antibody level; quite a dramatic fall-off in titre occurred within 3 weeks of the peak in some of our horses.

I am sure that a great deal more needs to be found out about production of antiviral sera in horses and other large mammals. Adjuvants have their place too; we have abandoned them in deference to the wishes of our veterinarians and after the experience with the severe local reactions. However, as I said before, we were able to show that small volumes distributed in multiple sites were well tolerated. It is traditional here that all injections are given in the neck muscles only, a procedure which seems to me rather a waste of horse; so I think there is still work to be done on other sites and increased volume of inocula.

I hope these few notes will be of use to you and the Enterovirus Committee. I shall look forward to hearing your own experience in the fullness of time.

Yours sincerely,

/s/ Alan P. Goffe

CC: Dr. Sabin

(co 5/4/61)