February 3, 1959

Dr. Albert B. Sabin  
Children's Hospital Research Foundation  
Elland Ave. and Bethesda  
Cincinnati 29, Ohio

Dear Dr. Sabin:

I must apologize for not communicating with you before this time in regard to our mutual interest in a B-virus vaccine. Unfortunately, personnel changes and a variety of other complications during the past 6 months have resulted in a delay in our plans.

I am enclosing a short report which covers some inactivation studies that were done during the past 3 months and the results of a second vaccine, labeled vaccine #5, which also show some promise as an immunizing agent.

We have been most concerned about the determination of safety in the inactivated materials and at the present time are using rather extensive tissue culture tests.

We hope to have another vaccine prepared in the near future and I am wondering whether you would like to receive some of this material for tests in your laboratory.

With best personal regards.

Sincerely,

J. E. Prier, Director  
Biological Development

mc  
Enc.
TO: Those listed below
FROM: J. E. Prier
SUBJECT: B-Virus Vaccine

On 7/9/58 a memorandum was issued discussing the production of a B-virus vaccine that indicated some protection could be produced in rabbits. It has been recognized that prolonged inactivation with formalin at 37°C would destroy the antigenicity of B-virus. Consequently, it was decided to attempt inactivation at 4°C, thus assuring retention of antigenicity, at least as regards the adverse effects of higher temperatures. Rabbit cell cultures were used in all cases according to methods and procedures obtained from Dr. Albert B. Sabin.

Figure 1 shows the results of testing such a vaccine in rabbits. Two strains of virus were used for challenge, one the Sabin strain from which the vaccine was made and the other the Nagler strain obtained from Dr. F. P. Nagler, of the Dept. of National Health and Welfare, Ottawa, Canada. Protection was evidenced against both strains.

A cold inactivation procedure presents a problem in assuring that virulence of the material is completely destroyed. The inactivation curve shown in Figure 2 seems to indicate a rapid inactivation with a theoretical crossing of the base line in less than 4 days. More detailed inactivation studies, however, are presented in Figures 3 and 4. A concentration of 1-1000 formalin causes an approximate straight-line decrease to a virus concentration of $10^1$ at 48 hours. From this point on, however, live virus persists at a detectable level for varying periods of time (Figs. 3 and 4). Safety tests in 1 liter Blake bottles have indicated the presence of live virus for longer than 14 days, although such tests have been negative when done on the 26th day of inactivation.

Copies to:

Mr. Barclay
Dr. Bittenbender
Dr. Davidson
Mr. Frankhouser
Dr. Hegarty
Mr. LeBeau

Dr. Malsberger
Mr. Newman
Mr. Rotundo
Mr. Schuchardt
Mr. Sillaman
Mr. Sullivan
The rate of inactivation was increased by the use of 1-500 formalin (Fig. 3), but this material was not antigenic when tested in rabbits. Similarly, the rate was increased when a short period of 37°C inactivation was introduced in the process (Fig. 4). Material prepared in this manner has not been evaluated in rabbits.

J. E. P.

mc
Enc.
Fig. 1.--Rabbit Immunization with B-Virus Vaccine #5

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge Virus</th>
<th>Survival Ratio</th>
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<tbody>
<tr>
<td>I</td>
<td>Sabin BI</td>
<td>8/10</td>
</tr>
<tr>
<td>II</td>
<td>Sabin BI Controls</td>
<td>0/5</td>
</tr>
<tr>
<td>III</td>
<td>Sabin BI</td>
<td>9/9</td>
</tr>
<tr>
<td>IV</td>
<td>Controls</td>
<td>0/5</td>
</tr>
</tbody>
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Schedule - 5 ml vaccine 6/13/58
5 ml vaccine 6/27/58
Challenge 1000 TCD - 7/10/58 in 1 ml at 5 sites
Effect of Addition of Formalin During Inactivation of B-Virus (Vaccine No. 8) at 4°C.

Initial concentration of formalin = 1:1000; concentration in sample B increased to 1:500 at 8 hours.

Graph showing the effect of formalin concentration over time.
Comparison of 4°C Inactivation (Curve A) and 22°C 22-hour Inactivation at 37°C, beginning at 8 hours (Curve B).