Dr. A. Giovanardi
Istituto Di Igiene
Via Francesca Sforza 35
Milano, Italy

Dear Dr. Giovanardi:

April 15, 1957

I was very glad to receive your letter of April 9th and can inform you that the National Foundation for Infantile Paralysis has approved my distribution of aliquots of the large lots of highly attenuated polioviruses which I tested in 1957.

The standard dose that I used was 0.1 ml of a $10^{-1}$ dilution of the culture fluid (prepared either in Hanks' or Earle's solution). The 0.1 ml dose was added to a teaspoonful of cherry syrup immediately before administration, although boiled milk would probably serve the purpose equally well. Accordingly 0.1 ml of the undiluted culture fluid is sufficient for 100 individuals. On April 28th I am leaving Cincinnati for Europe. I shall stop in Amsterdam for a day at which time I shall also deliver aliquots of these large lots to Professor Verlinde at Leiden for tests that he expects to carry out in Holland. For your confidential information requests for aliquots of these large lots have also been received from Mexico, South Africa, and the U.S.S.R. where certain experimental studies will be carried out by highly qualified investigators. It is my intention to ask Dr. Verlinde to send you packed in dry ice 20 ml of each type by air express to Milan. If time permits I shall attend to this myself while I am in Leiden on April 30th.

In reply to your specific questions I should like to say the following:

1) The material that you will receive belongs to the same lots that I tested in January 1957 and subsequently on 100 adult volunteers as well as on 10 individuals in my own family and one of our neighbors - which includes 5 children aged 5 years to 11 years, all of them without antibody for any of the 3 types of polio.

2) The vaccine must be kept in the frozen state - we have stored it in a deep freeze at about -20°C - and it is suitable as long as the titer of the virus is maintained. If by any chance there should be a delay in the shipment that is being sent to you and
the fluid has thawed all you need do is to titer it in tissue
culture and then refreeze. I have had an experience in
which that has happened and found that the titer of the
culture fluid was actually unchanged.

3) Our tests on the simultaneous administration of more than
one type of virus indicate that that is not a good way to do
it. Accordingly, the procedure that I described in my last
paper in the JAMA (December 29, 1956) of administering
each type separately at 3-week intervals is the one of choice.
Because of the dominance of one type over another the procedure
which I have found optimum is to administer type 1 first, followed
by type 3 and finally by type 2. This is the procedure that I have
followed in my own and a neighbor's family - the type 1 virus was
given on March 17th and the type 3 virus was given on April 7th
and I expect to give the type 2 virus on April 28th.

I have carried out detailed studies on the duration and quantity
of virus excretion and have found it to vary considerably in different
individuals. However, as I pointed out in my JAMA paper of December
1956 when the different viruses are administered at 3-week intervals in
the order listed above the new type begins to multiply within a few days
even when the preceding type was still being excreted at the end of the
3-week period. The type of tests that you carry out depend entirely on
your own interests. It is possible that in the groups that you study Echo
viruses may be present in the intestinal tract before the administration
of the poliovirus and thus it would be of interest to have at least one stool
specimen prior to the administration of the poliovirus. Our tests on the
time of appearance of neutralizing antibodies indicate that when the pH
test is used for measuring antibody they can be detected within 7 to 10
days after feeding. When the cytopathogenic test for antibody in roller
tubes is used the first antibody may not be detected until later and in the
case of type 1 and type 3 in a few individuals the antibody is either of
very low titer or can hardly be detected.

I am leaving tomorrow morning for about a week but after that
I hope to prepare a brief description of the preparation and safety tests
on the culture fluids that will be sent you. In the meantime I am enclosing
two tables which I should like to ask you to substitute for the ones that accompanied the manuscript that I sent you on March 29, 1957.

With all good wishes and kindest personal regards.

Sincerely yours,

Albert B. Sabin, M. D.

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