April 5, 1958

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 28 March and all the interesting new information it contained. I was very interested to learn about your new results and I would like to comment on several of the points that you have raised:

1) What is the significance of the fact that after feeding of the type 2 virus 3 weeks after the type 3 virus alimentary infection was demonstrated in only 85% and an antibody response in 100%? Since I have never observed an antibody response without being able to demonstrate virus in the stools, I am still wondering whether the method that you used for testing virus in the stools may miss the excretion of smaller amounts of virus - either because the number of tubes that are inoculated with stool extract is too small or because the presence of any kind of serum in the tissue culture medium prevents the demonstration of minute amounts of virus. Nevertheless if in 15% of individuals (that is those who develop antibody without demonstrable virus in the stools by your method) the multiplication of virus is so low it may perhaps either be due to the fact that the type 3 virus is interfering with more extensive multiplication or that they represent individuals who have had a previous natural infection with the virus without having antibody that is demonstrable by the usual procedures. In either case I would go along with your suggestion that a longer interval between the feeding of type 3 and type 2 virus may be desirable.

2) What is the significance of the fact that when type 3 virus was fed 3 weeks after the type 1 alimentary infection was demonstrated in 92% and an antibody response in only 80% of individuals without preexisting antibody? Here my own experience would suggest 2 possible explanations: first, the appearance in some individuals who had demonstrable multiplication of the virus I have found to be due to the fact that the
antibody response to type 3 is not infrequently chiefly of the low avidity type and even that in low titer - this occurs not only after feeding of attenuated strains but also after natural infection. Furthermore, the antibody response to type 3 in a small proportion of individuals has been found to be fleeting so that the titer at 6 to 8 weeks may be very much lower than at 2 to 4 weeks. Of course happens to be one of those individuals in whom type 3 virus multiplied in concentrations of \(10^3\) to \(10^5\) TCD\(_{50}\) per gram of stool for a period of 20 days and yet 8 weeks later had no demonstrable type 3 antibody by either the pH method or the cytopathogenic test. I have had occasion to test a few volunteers in whom this had occurred and found that the alimentary tract is resistant to reinfection in such individuals despite the fact that the antibody had decreased to below the demonstrable level.

Now, as regards the failure to detect either multiplication or antibody response in 8% of those who received the type 3 virus I would again think first of all of the possibility that they may not have been individuals without previous experience with type 3 virus and secondly of the possibility that there was interference from the type 1 virus in these individuals.

Although you have not encountered any failures in "takes" of the type 1 virus, you will recall that in my July 1957 JAMA paper I reported at least 3 such individuals.

3) When you spoke of the high percentage of immune individuals in whom a rise in antibody occurred after feeding of virus you did not state whether that occurred with all 3 types or more especially with type 3. Again referring you to my JAMA 1957 paper you will observe the marked differences that I found for naturally immune type 3 individuals as compared with type 1 and 2, but once again I must state here that I have never observed a rise in antibody titer without being able to find at least minimal multiplication of virus in the alimentary tract.

4) I was indeed surprised at your report that when you inoculated 2 monkeys intramuscularly with the undiluted tissue culture fluid of the type 3 virus that you, yourself, had passaged - that one of them had developed paralysis. I have never observed paralysis in monkeys after intramuscular injection of very large doses of the type 3 attenuated virus and with the particular lot made from the seed virus that was used to prepare the type 3 vaccine I had inoculated 15 cynomolgus monkeys into the right deltoid with 2 ml of undiluted culture fluid which contained \(10^8\) TCD\(_{50}\) of virus. None of the 15 monkeys showed any paralysis and in 14 there were no lesions whatever in 50 levels of spinal cord from each monkey. In one of the 15 monkeys there were focal infiltrated lesions
in two levels of an anterior horn of lumbar cord without any changes in the roots that might have indicated neuronal destruction. It would certainly be important not only to inject a larger number of monkeys with the particular culture fluid that you used originally but also to repeat the whole thing to see what you get. Until that is done I am afraid I have no explanation for your observations.

I was delighted to read of the plans for a trial of the attenuated poliovirus vaccine in one of the smaller isles of the Dutch Antilles. You may be interested to know that a large scale trial has been in progress in children in Mexico since the end of February. According to a letter that I received several days ago 2800 children had already received the type 1 virus beginning at the end of February 1958 and of these about 2500 have already received the type 3 virus. It is possible that you may see Professor Smorodintsev of Leningrad at the WHO European meeting who will tell you of the work that he has already done and is planning in the Leningrad area. Of particular interest are his studies on serial passage of the attenuated viruses from the alimentary tract of one child through one passage in monkey kidney tissue culture to the alimentary tract of another child, etc., etc. When he last wrote me, more than a month ago, he had already carried out 5 consecutive passages with the type 1, and 3 consecutive passages with the type 2 and type 3 viruses without observing any greater change in the neurotropism in the excreted virus than you and I have found after a single passage in the alimentary tract.

I am sending you herewith copies of two papers which have been submitted for publication and which may be of interest to you particularly in connection with the discussions at the WHO meeting of European virologists. One of these is entitled: "The Role of ECHO Viruses in Human Disease", and the other: "ECHO 9 Virus Disease: Virologically Controlled Clinical and Epidemiologic Observations During 1957 Epidemic in Milwaukee".

With best wishes and kindest personal regards.

Sincerely yours,

Albert B. Sabin, M. D.

ABSlmeh

Enclosures.