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Monsieur le Docteur SABIN
The Children's Hospital Research
Foundation
Elland ave. and Bethesda
CINCINNATI 29, OHIO
U.S.A.

Dear Doctor SABIN,

May I once again ask you to be kind enough to give me your opinion about some facts which seem at first sight very simple and evident and yet still cause arguments and misunderstandings?

I should like to have your opinion about the interpretation of neutralising antibody factors against polio viruses, and their signification for eventual production for a polio infection and also in order to judge the efficiency of polio virus vaccination.

The difference of opinion about the interpretation of significative titers of neutralizing antibodies, comes partly from the fact that, for a long time, many virologists made their research on these antibodies by putting into contact for one hour at laboratory temperature, a volume of serum (pure or diluted) and a volume of virus suspension containing $\pm 10$ TCID$_{50}$. It was then agreed that patient whose serum did not give in dilution 1/4 the neutralization of poliovirus could be considered as not containing any antibodies. It was also agreed that a rise of as least 4 times (that is the appearence of antibodies at a titre of $\geq 1/16$) could be considered as meaning the acquisition of a certain immunisation.

Following, in particular, S. GARD, the immunoinactivation method was used (I studied it in his laboratory where I worked in 1956). Then there was used a simplification that consisted in making the serum-virus contact at $37^\circ$ for 3 hours (with...
many non negligible differences that, I believe, are underestimated, for some do it by water bath and others in stove where the temperature of 37 ° is very slowly obtained in the mixture.

If such a method is used, it is more and more so antibody titres are obtained which are much higher and the difference may be of 2 or 3 dilutions (1/16 or even 1/32 instead of 1/4).

Thus, if it is agreed that a patient having a neutralizing antibody titre of ≤ 1/4 has no antibodies, assuring a valid protection, with the method of contact of one hour at laboratory temperature it should also be agreed that he has no antibodies either if it is found that the titre is ≤ 1/16 with the method of contact of 3 hours at 37 °. It should then be agreed that a vaccine has given a significative rise of antibody titre, if the titre passes from ≤ 1/16 to ≥ 1/64. Would you be kind enough to give me your opinion about this?

I should also like to know the conversion rate that you consider good to be demanded for patients who have taken a live polio vaccine. Should one require a 100% conversion for triple negative patients (that is to say ≤ 1/4 by contact one hour at laboratory temperature or ≤ 1/16 for 3 hours at 37 ° contact), or can one accept a lesser percentage and what should be the minimum tolerated?

May one also admit that in certain patients who already have a high titre of antibodies (≥ 1/64 with the method: 1 hour at laboratory temperature, ≥ 1/256 with the method: 3 hours at 37 °), the rise is not constant as if certain patients have reached a "ceiling" not forgetting that with the live vaccine they have the benefit of local immunity!

Finally, I should very much like to have your opinion about the interpretation which can be given to the results of certain methods of neutralization. Nearly all authors declare that they titre antibodies in "pure" serum when they put it into contact with 1 volume of "pure" serum and 1 volume of virus suspension containing +100 TCID or that they titre the antibodies in serum of 1/10 if they put into contact 1 volume of diluted serum at 1/10 and 1 volume of virus suspension.

May one say -as some authors do- that antibodies are titred in serums of 1/10 if one volume of pure serum is put into contact with 9 volumes of virus suspension or a virus suspension and cells (e.g. serum 0.1 + virus suspension 0.9)?

Some immunologists think that in this case, if the neutralization is made, it is not possible to say that the patient has antibodies in serum diluted at 1/10 but in "pure" serum. Such interpretation seems valid.
Of course, it is possible to find the answers to these questions more or less implicitly contained in the published works, but certain facts seem to me to be still badly defined.

Your opinion will be very useful to me, especially at the moment when I must make sure that the Institutes which produce the live vaccine prepared it properly and also that the efficiency controls of the vaccine are made and interpreted properly.

Please forgive me for causing your so much trouble and accept my grateful thanks in advance.

Yours sincerely,

Professeur R. SOHIER.