April 21, 1932.

Dr. Simon Flexner,
Rockefeller Institute for Medical Research,
66th Street and York Avenue,
New York City.

Dear Dr. Flexner:

While I can not presume to eludidate Ward's paper for you, as you suggest, I did hope you would be willing to hear my criticism of it, based on the quantitative studies Kendall and I have made.

In calculating that 200 cc. of Type I. antiserum would take care of 500 gm. of S, or the amount in 12,500 liters of culture, Ward has forgotten entirely that S will be "functionally" in excess as soon as it exceeds the amount which can combine chemically with the antibody of the serum. Our work has shown that when S begins to be in excess its ratio to combined antibody is 1:60. Now a Type I. antiserum of 1000 mouse protective units contains about 7 mg. of specifically precipitable protein per cc. (Ref. 8 in Ward's first April paper) and 200 cc. would contain 1400 mg. Therefore, as soon as $\frac{1400}{60}$ or 23 mg. of S. had combined with the antibody the serum would be useless and the rest of any S present would be actually in excess and free to "function" antibacterially. Since this amount corresponds roughly to that in one-half liter of culture, not 12,500 l., it is easy to understand why large amounts of serum must be given, and given early, to be of any value. And since virulent Type III. organisms produce at least 2.5 times as much S as Type I. does, 200 cc. of an equally good III antiserum would neutralize the S produced by only 200 cc. of culture, so that the inadequacy of Type III antiserum is thus very satisfactorily accounted for, as well.

As for the bactericidal effect of serum absorbed with polysaccharide, I cannot believe that 1% per cent of free anti-carbohydrate can be left behind, as we have quantitative measurements indicating that the amount must

be almost infinitesimal. A possible explanation is that the small amount of AS_2 (completely neutralized antibody) remaining in solution can combine at the surface of the PnIII with S (we have given evidence for an AS_3 compound) and thus prepare the organism for phagocytosis by the blood used in Ward's test. So-called protective antibodies remaining after absorption with S could be accounted for in thessame way.

As for the enhanced antibacterididal effect of a Pn III filtrate over that of a corresponding amount of pure S III, Ward's data do appear to show a 100-fold, not a 1000-fold increase, and while the cause of this may lie in a more reactive intermediate product, a part of the discrepancy, at least, may be traceable to too great reliance on the quantitative interpretation of a complex qualitative test which may, in the end, turn out to be an artifact.

Nina and I enjoyed the tea enormously and had hoped to tell you and Mrs. Flexner so at last night's very thrilling concert.

With many thanks for your patience in letting me send this criticism,

Sincerely,

MH-dg