August 17, 1945.

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Dear Peter:

I am returning herewith a big stack of papers which I received from you over a month ago. I regret very much that this delay has occurred, but on reading over your letter and Sabin's questions, it seemed to me that it would be a rather large undertaking to attempt an answer. This material arrived just at the beginning of the encephalitis and poliomyelitis season and everything had to be put away in order to undertake the field problems as they arose. In addition to this, I have a very heavy summer teaching schedule. However, about a week ago I received instructions by telephone from Washington that I would be going into the Pacific Area. A few days of rush preparations were made, and now I sit tight waiting to leave. This gives me a breathing spell, and I have undertaken this job that has been postponed for so long.

After spending an afternoon reading and re-reading all the letters and suggestions, I find that my comments can be relatively short. However, I am not very optimistic regarding their usefulness. It seems to me that this matter is a bit too complicated to handle satisfactorily by mail. There are very obviously certain differences of opinion which make it very difficult for three persons to arrive at a compromise which all will approve. I feel that one of the difficulties is based on the fact of a difference in experience, or difference of viewpoint. Albert's extensive experience with the neutralization tests - at least those experiences that I have seen reported in this file - has dealt primarily with the level of antibody response to vaccination, and to the testing of hyperimmune animal sera. You have been very modest, and have quoted very little of your enormous experience with the neutralization tests. The more recent work coming from your laboratory, with which I am acquainted, has dealt however, largely with animal sera. My own experience has been restricted largely to diagnostic problems. This experience has been limited almost entirely to the neutralization test, but recently we have begun to use the complement fixation test to a slight degree.

I also will use Albert's questions and answers as a point for discussion.

1. No comment
2. I approve
3. Answer
   (a) "For diagnostic purposes with the neurotropic viruses, we are chiefly concerned with whether or not a given serum has neutralizing antibodies, and not with the amount of antibody present. I do not believe that we are prepared to make a diagnosis on different levels of antibody in acute and convalescent specimens"

I cannot agree with the above in any way. When Albert passed through here, I mentioned this fact to him and as far as I could understand him, he denied
Dr. Peter K. Olitsky

thinking in any such terms himself. From certain endemic encephalitis areas - and these are the areas from which we can expect to receive the largest number of specimens for diagnosis - anywhere from 10 to 50 per cent of the sera received contain antibodies, many within the range considered significant by Albert, in the first serum specimen submitted. Only on the basis of comparing the titer of two or more sera can a diagnosis be made by the neutralization test. Any test to be recommended by this Commission for the performance of diagnostic laboratories will be - I presume - for the purpose of diagnosis. We must therefore, be prepared to make a recommendation for determining the difference in the levels of antibody in acute and convalescent specimens.

4. Answer

(a) No single test will take care of the problem of diagnosis discussed above unless one throws economy to the winds and performs a set of serial titrations on all acute and convalescent sera received. The two dilution test recommended by Albert does not satisfactorily demonstrate a difference in antibody level between the acute and convalescent specimens, unless both specimens have been stored by freezing immediately after separating the serum from the clot, and it is obvious that specimens are not routinely received preserved in such a manner. For the sake of economy, the screen test is used on the convalescent specimens. Single convalescent specimens need confirmation, and if there are two sera, they need comparative titration. Since the same set of serial dilutions of virus can be used for all types of test, I fail to understand why this is complicated.

(b) I do not anticipate that all laboratories doing routine work will be required to titrate each virus preparation on 100 to 200 mice in order to determine the factor. If a standard virus and a standard strain of mice are used, no one else need repeat the preliminary work required to determine these factors.

(c) The question of the level of sensitivity of any serological test arises no matter what type of test or disease is being discussed. However, in general, an attempt has always been made to devise the most sensitive test possible which will give the least number of non-specific positives. My reasons for using the level of sensitivity selected are based on experience and experiment of such a nature that to present the data in writing would require an enormous amount of time and work. I am unable at this time to undertake such a job.

5. A. "In my opinion such comparisons are not necessary for routine diagnostic purposes, with the neurotropic viruses, since all that needs to be established is whether a serum is positive or negative."

My comments above also apply to this. I cannot agree with this at all.

Regarding the "protection which is afforded by undiluted sera and rapidly lost by dilution", I do not have any definite opinion. I have wondered, and have expressed this possibility that part of it might be non-specific. I merely suggest it as a problem that needs an answer.

6. I approve.
Dr. Peter K. Olitsky

Obviously if we agree, some compromise must be reached for the present.

In summary then, of Albert's test and Albert's questions, I feel that the 2 dilution test will not be useful in a diagnosis of the neurotropic virus infections, and that a satisfactory type of titration must be used when two sera are to be compared once the second serum has been demonstrated to contain antibodies. For the sake of economy—when time is not the principal question—a preliminary test of the second serum to determine whether it contains antibody or not is a reasonable procedure. In most instances time is not very important for we are unable to attempt diagnosis until after the patient has recovered. In dealing with the diagnosis of an epidemic, this is another question. Regarding the level of antibody required to be significant, I believe the question rests largely upon the reproducibility of results, and the expected range of variation from test to test, rather than upon the necessary antibody level as determined by the intracerebral test. I have observed the irregularities in a series of tests on the same virus that have been submitted by Albert. These are greater than ordinarily occur in my laboratory, and there are a number of differences in technique which I believe account for the difference in results. Not the least among these differences is the use of 10 fold and 5 fold dilutions by Albert and by me respectively. The amount of virus suspension placed in each ampule to be frozen, and a number of other differences.

In regard to the dilution factor, if the original article by Reed and Meunch is reviewed it will be noted that they used 2 fold dilutions and that death short of 100 per cent mortality was spread over 5 dilutions. We find in using 5 fold dilutions to determine a 50 per cent end point that deaths are usually spread over 2 or 3 dilutions, quite frequently only 2 dilutions. If we use the 10 fold dilution, quite often we have no deaths in one dilution, and all deaths in the next, and usually may have only one dilution with incomplete survival or death. This is certainly not a very accurate means of titration. Along this same line, it seems to me again relatively crude to use a 2 dilution test, with a 10 fold difference between the dilutions and consider all mice in both dilutions on the same basis when counting the number dead or alive. If all mice are to be considered equally, why not use one dilution—and use more mice.

I would like to make one important correction on the section that I previously submitted on the neutralization test. On page 6, the last line which now reads: "50 per cent rabbit serum saline is used as a diluant." After Albert raised the question regarding the use of broth as a diluant, it seemed to me as long as one was using a 10 per cent serum, there was no objection to using saline to replace the broth, and at the time I wrote this, we had begun to use saline. However, our results have been very unsatisfactory. The one lot of material which we prepared in saline and serum has not held its titer nearly as well as that which was put up with broth and serum. I refer now to the ampouled material which was 50 per cent serum and saline, instead of 50 per cent serum and broth. We noted a fairly rapid fall-off in titer in this material over a period of a few months, and finally discarded it. Also, we found that our dilutions prepared for the neutralization test made with a combination of serum and saline—10 per cent serum and saline—were not as stable as those formerly used and our variations from test to test were greater. I have therefore discontinue the use of saline and am again using broth. We have encountered no trouble at any time with broth.

I have made a few other changes in this manuscript, and have initialed them. These are principally of a grammatical nature.

I wish to say that I agree with your answer to question 4 0, in that the premise for his criteria is too rigid.
In regard to priorities of ideas: if it is felt essential that this enter into the recommended procedures, there is no question in my mind but what the observation by Albert that dilution greatly affects the neutralizing index was made prior to any of mine. Since I was not present at the 1942 meeting, I did not know of this and had not seen the data until just now - so far as I can remember. When these were discussed at a later meeting I believe that it was pointed out that Isabelle Morgan had been conducting experiments of this nature for some time. I believe that Loring Whitman and I were the first to point out that for problems of diagnosis this had a definite application, and that satisfactory diagnostic procedures could not be worked out until something more was known about these factors - or at least that they must be taken into consideration, when diagnosis was the question. Would it not be possible to omit all names in this discussion, merely making reference to work by members of the Commission and others associated or helping in the study of the problems?

As a personal note, to pass on a little news of interest, we have isolated a virus from a human case of encephalitis from Kern County this year, which we are having real difficulty in identifying. It is definitely not identified yet, and if it is one of the well recognized viruses which occur here, it is behaving in a most unusual fashion.

Please forgive this long, rambling discussion. I hope that I will be back to attend the meeting this fall and have a chance to see you personally.

Sincerely yours,

W. McD. Hammon, M. D.
Associate Professor of Epidemiology.