August 14, 1952

Dr. Albert B. Sabin
Children's Hospital Research Foundation
Elland Avenue & Bethesda
Cincinnati, Ohio

Dear Dr. Sabin:

I have read carefully the manuscript entitled "Toxoplasmosis: Present Status of Clinical Manifestations in Man; Indications and Provisions for Routine Serologic Diagnosis" and feel that it is an excellent compilation of the information gathered at the conferences of the Committee on Toxoplasmosis. A couple of minor typographical errors were noted on pages 2 and 22. The following though are the major questions or comments we have:

Page 15. Perhaps there should be more specific information on how to collect the accessory factor. It is stated "to defibrinate the blood rapidly and quickly distribute the centrifuged serum in individual ampules." Should this be accomplished mechanically by the use of glass beads or applicator sticks? It was our understanding that it was best to allow the blood to clot in the refrigerator.

Page 17. It is stated "...the whole test should be completed within one hour after the peritoneal exudate is removed from the animal". If the preliminary test of the condition of the toxoplasma requires incubation of 20 minutes and the actual test 60 minutes, it cannot be accomplished in less than 1½ hours. Of course the preliminary test might be run on a small sample withdrawn from the peritoneal cavity before all of it is removed, but this procedure would not be practical since an adequate quantity of exudate might not be obtained from the tested animal. Using the preliminary test, it perhaps might be recommended that no longer than 1½ hours should elapse between the removal of the exudate from the animal and the adding of the methylene blue to the tubes after incubation. The examination of the mounts may require an additional hour or more.

Page 18. Although I know that some individuals use the "high dry" objective, we feel more confident when the oil immersion objective is employed. Laboratories just starting the test probably feel the need of the higher magnification more than the more experienced laboratories. Inasmuch as we use oil immersion routinely we seal each preparation with melted paraffin and petroleum jelly (50% of each). This procedure facilitates the use of oil, reduces the movement of the organisms, and prevents the mounts from drying.
Page 28. We are not convinced that the addition of merthiolate does not have an effect upon the dye test. We have not run enough tests to observe a definite pattern, but in control tests we have occasionally obtained an increase in titer with a 1:10,000 addition, and more frequently, at higher dilutions. Until this point is definitely cleared up, it would seem advisable not to recommend the addition of merthiolate but to insist on submitting sterile sera.

The statement on the "Diagnosis of Congenital Toxoplasmosis by Serologic Methods" appears to be very appropriate for the back of the laboratory report forms. However, it might be advisable to divide it into paragraphs, i.e., in accordance with the age of the patient.

We will certainly look forward to receiving the revision of this report. I am sure that everyone will benefit greatly from your clear presentation.

With best regards,

Sincerely yours,

[Signature]

M. M. Brooke, Sr. Scientist
In Charge
Parasitology & Mycology Section

Encls.
GC: Dr. Hogan