Dr. Albert B. Sabin  
The Children's Hospital  
Department of Pediatrics  
Cincinnati, Ohio.

Dear Dr. Sabin:

For the past year I have been doing laboratory and research work on infections in the Department of Ophthalmology of the University of Toronto Medical School. Part of this work has been a study of toxoplasmosis. The toxoplasma skin test, as outlined by Dr. J.K. Frenkel in the Proc. Soc. Exper. Biol. and Med., was prepared and we have been testing normal individuals and suspected cases of toxoplasmosis.

Up to the present, we have not encountered any cases of toxoplasmosis in the acute stages. We have done your methylene blue test (science, December 10, 1948) on two probable cases of arrested toxoplasmosis, a 26 year old woman and a 2 year old female. The titer end point in the former was 1:64 (the next dilution, 1:128, showed 40% unstained toxoplasma); in the latter case, the end point was 1:512 (the next dilution, 1:1024, showed 30% unstained).

In this brief experience several questions have arisen. We would be grateful if you would answer them for us.

What are the quantities of toxoplasma suspension and diluted serum that are placed in the tubes prior to incubation? How long can the tubes be stored in the refrigerator before doing the microscopic examinations of their contents? When our saturated alcoholic solution of methylene blue is added to the buffer solution, the pH becomes about 10.4. How does a less alkaline pH affect the microscopic examinations? Why must the serum be heated to 56 degrees C. for 30 minutes prior to testing? Is it to prevent too much "accessory factor" being present? If heparin is omitted in the diluting of the toxoplastic exudate with serum, and a coagulum in the lower part of the tube is noticed when about to make the microscopic preparations, what is the effect on the test?
Dr. Albert B. Sabin
June 6, 1949.

I would appreciate receiving a copy of your article on the methylene blue test and any of your previous articles on toxoplasmosis that you may have available.

Sincerely,

Morris Shusterman, M. D.
Fellow in Ophthalmology