COMMENTS ON PROPOSED COMPLEMENT FIXATION TECHNIQUES

p. 13 a) Since reaction in dilution less than 1:4 is not considered very significant, may it not be advisable to start with this dilution, i.e., dilute serum 1:4 with saline and heat at 60° C. for 20 minutes?

b) Pickels' angle-head centrifuge not commonly available; would not ordinary angle centrifuge at about 4,000 rpm for 1 hour do?

p. 14 a) If these are to be directions, then technique for irradiation should be given or reference quoted.

b) I believe that more detailed statement is indicated about titrating complement in presence of antigens and sera, under conditions of the test, prior to setting up the final mixtures.

c) Nothing is said about preliminary incubation of the hemolytic system.

The way it is worded now it may be interpreted that the sheep cells and amboceptor are added separately. Perhaps this is so intended, but since it is not customary, some clarifying statement may be indicated.

p. 15 a) If the technique used by Havens et al is recommended as an alternate, it should be so stated.

b) Again if these are directions, not enough detail is given for preparation of control positive sera; e.g., it is not stated that guinea pig brain must be used for inoculating guinea pigs, mouse brain for mice, etc. Some description of preparation of suspensions for inoculation, dosage, schedule, etc. is also indicated.

p. 16 a) Indicate dilution at which hyperimmune serum is heated.

b) There is some question as to whether mouse brain is the best antigen for ICM, and whether it should be recommended, simply because of uniformity of
antigens. Guinea pig spleen seems to be preferred by other laboratories.

p. 17 a) Since presumably these are directions for routine practical use, and since only avirulent antigens are recommended, it is worth repeating that more detailed directions for their preparation are indicated and that it is advisable to have them prepared and standardized either commercially or in the Army Medical School for distribution to other laboratories.