An interesting phenomenon of "acute tolerance" was also observed. That is, the second injection of the day caused much milder symptoms than the first injection. Although not yet well worked out, it seems that the optimal interval between injections for demonstrating this phenomenon is from 2 to 4 hours.

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The Complement Fixation Test in Yellow Fever.*

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Yellow fever offers opportunity for study of the immune reactions of a virus disease under circumstances which eliminate error due to collateral antigens. According to Schultz[1] collateral antigens have been a frequent source of error in previous studies of the complement fixation reaction in virus diseases. Material quite free from collateral antigens can be obtained in yellow fever as antigen for the complement fixation test.

Aragao[2] has reported unfavorably upon the complement fixation test in yellow fever with antigens prepared from phenolated tissues. On the supposition that the antigenic substances in tissues affected by virus diseases might be within the cells, Ciuca[3] has made use of a process described as septic maceration in order to liberate the cellular contents. He reports success in differentiating between the 3 principal types of foot and mouth disease by complement fixation with antigens prepared in this way. The method of preparing the antigen for our tests is based on a procedure followed by Hindle[4] in making vaccine from yellow fever tissues. He produced rupture of the cells by causing a sudden change in the osmotic pressure of the fluid in which the tissues were suspended.

In preparing antigen, pieces of liver and spleen were taken at autopsy from monkeys which had died from experimental yellow fever. This material was ground thoroughly with sterile sand and

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

[1] Schultz, E. W., *J. Immunol.*, 1928, xv, 229.

[2] Aragao, H. de B., *Compt. Rend. Soc. Biol.*, 1928, xlix, 1341.

[3] Ciuca, A., *J. Hyg.*, 1929, xxviii, 22.

[4] Hindle, E., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1929, xxii, 405.

soaked over night in the refrigerator in half its weight of 9.0% sodium chloride solution. The mixture was then suddenly diluted to the physiological concentration of salt by the addition of distilled water. The suspension of tissue thus prepared was centrifuged and passed through Berkefeld "V" filters. The filtrate constituted the antigen. For purposes of control an antigen was similarly prepared from tissues of a normal monkey.

In performing the complement fixation tests a simple standard procedure was used, employing an anti-sheep hemolytic system and 0.5 cc. quantities of each reagent. The sera to be tested were used undiluted after 30 minutes inactivation at 56° C. Fixation was allowed to proceed for 30 minutes at 37° C.

The results of these tests are summarized in the table. The human yellow fever sera listed as "recovered" or "immune" had all been tested for their power to protect monkeys against yellow fever and found to prevent death after the injection of at least 10 lethal doses of virus. Four sera, which were from persons supposed to have had yellow fever but which had failed to protect monkeys, have not been listed as "recovered" or "immune", but are included with the presumably normal sera. One of these, however, gave a positive reaction.

Except in this instance, sera which had failed to protect monkeys also failed to give complement fixation. On the other hand, all sera which protected monkeys gave positive fixation except one, which had failed to prevent fever in a monkey but had prevented death.

In addition to the tests for which results are given in the table, 38 retests were made with 14 of the same series and in all but one instance the results were in agreement with those obtained in the first tests of the same specimens.

In over 100 other tests made prior to those reported in the table, various antigens were tried, namely, urine from monkeys dead of yellow fever, alcoholic and aqueous extracts of their organs, and sera drawn from monkeys at the first elevation of temperature due to yellow fever. The results were found to be less reliable than those obtained later with the saline antigen.

Our results were in agreement with those of Aragao[2] in that yellow fever sera obtained during the first day of fever did not give strong or frequent reactions and that the sera of yellow fever convalescents did not fix complement in the presence of syphilis antigens.

Although the antigens used were made from tissues of monkeys inoculated with yellow fever virus from West Africa, positive complement fixation reactions were obtained with human sera from

TABLE I.
Result of Complement Fixation Tests in Yellow Fever.

Sources of sera tested	Antigen Used.					
	Saline extract of liver and spleen of yellow fever monkeys.			Saline extract of liver and spleen of normal monkey.		
	Positive	Negative	% correct	Positive	Negative	% correct
Normal men and monkeys	3	38	93	1	28	97
Recovered and immune men and monkeys	18	5	78	3	9	75
Convalescents from other diseases*	1†	19	95	0	18	100

* Syphilis 14, dengue 4, poliomyelitis 2 (1 human, 1 monkey).
† Syphilis.

South America and with the sera of monkeys which had been inoculated with virus from Brazil.

The method described appears to offer a means of identifying at least a useful percentage of the convalescents from yellow fever in a community. As far as can be determined by the tests which could be made with the small number of sera available, the reaction seems to be specific. Further work needs to be done in the search for more sensitive antigens, and the usefulness of the improved test should be determined by numerous trials under field conditions.

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Hemoglobin Standards in Normal Men.

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In an earlier report on normal blood standards,[1] a Newcomer hemoglobinometer, restandardized on the basis of 10 hemoglobin determinations made by the Van Slyke oxygen capacity method, was employed. Further standardization of this Newcomer instrument has led to the conclusion that the original correction curve was incorrect.

Additional hemoglobin determinations by the Newcomer and Van

[1] Wintrobe, M. M., and Miller, M. W., *Arch. Int. Med.*, 1929, xliii, 96.