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The Occurrence of Anti-Rh$_0$ Blocking Antibodies in Anti-Rh′ Serums.

ROY T. FISK AND PATRICE MORROW.

From the Collis P. and Howard Huntington Memorial Hospital, Pasadena, and the Department of Bacteriology, School of Medicine, University of Southern California, Los Angeles, Cal.

Wiener[1] has reported a new test for isoimmunization to the Rh agglutinogen. So-called "blocking antibodies" were detected in serum specimens from Rh-negative individuals even though agglutinins were absent according to the usual methods of testing. The demonstration of Rh isoimmunization is of sufficient importance to make the blocking antibody test of practical value in diagnostic laboratory work and the performance of this test should not be difficult for those familiar with Rh typing procedures. In the blocking test, agglutination by anti-Rh serums is inhibited by preliminary treatment of the cells with the unknown serum. Normal serums do not interfere with the action of anti-Rh agglutinating serums under the same conditions.

In the present study the blocking antibody test was of value in proving isoimmunization in three cases of erythroblastosis where serum specimens from the Rh-negative mothers showed no Rh agglutinins. The purpose of this report, however, is to call attention to the occurrence of Rh$_0$ blocking antibodies in Rh subtype testing serums. Rh agglutinins developed in Rh-negative persons are known to differ in specificity in accord with differences in antigenic structure of the Rh agglutinogen and the subtypes of Rh have been classified by Wiener,[2] whose new nomenclature is used in this discussion. Two anti-Rh serums were employed in experiments to demonstrate the presence of blocking antibodies in subtype antiserums. The antiserums, "MF" (Group A) and "Lo" (Group B) were obtained from Rh-negative mothers who had delivered erythroblastotic infants; both produced strong clumping and conformed to the anti-Rh′ subtype in giving about 70% positive reactions with cell suspensions selected at random. Eleven Group O, Rh-positive bloods which gave negative reactions with the two serums were employed. The cells were washed once in saline and used in 2% suspensions which were added in amounts of 1 drop to 1 drop of the antiserums and normal control serum. After preliminary incubation of 30 minutes at 37°C, the tubes were examined for clumping and a drop of anti-Rh$_0$ testing fluid was added. The tubes were then reincubated and examined for agglutination.

Results of the tests, which are summarized in Table I, showed the presence of anti-Rh$_0$ antibodies in both of the anti-Rh′ serums which were active for all of the blood suspensions employed. The normal control serum which was used in the same manner did not inhibit clumping.

Wiener[1] has commented on the coexistence of agglutinating and blocking antibodies active in the presence of the same subtypes of agglutinogen. In the present study, blocking

TABLE I.
Blocking Effect of Anti-Rh′ Serums on Anti-Rh$_0$ Agglutinins.

Serum diluted 1-2 1 drop	2% cell suspensions 1 drop	Anti-Rh$_0$ serum diluted 1-2 1 drop
Anti-Rh′, "MF" Anti-Rh′, "Lo"	Tubes incubated 30 minutes at 37°C; 11 specimens tested; no clumping noted.	Tubes reincubated; complete or marked inhibition of agglutination with all specimens.
Normal, Rh negative		Strong clumping with all specimens.

[1] Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 173.

[2] Wiener, A. S., *Science*, 1944, **99**, 532.

antibodies (anti-Rh_o) which were active with Rh_2 cells were demonstrated in antiserums containing agglutinins (anti-Rh′) for a different Rh subtype (Rh_1). This observation suggests further study on the specificity and origin of blocking antibodies in respect to subtypes of Rh agglutinogen.*

* Since the completion of this study our attention has been called to a publication by Race[3] in which blocking antibodies of standard anti-Rh specificity were demonstrated in anti-Rh′ serums. This observation was also made by Wiener[4] whose findings are awaiting publication.

[3] Race, R. R., *Nature*, 1944, **153**, 771.

[4] Wiener, A. S., *Science*, in press.

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The Toxic Effect of Influenza Virus in the Rabbit Eye.*

C. A. EVANS AND E. R. RICKARD. (Introduced by R. G. Green.)

From the Department of Bacteriology and Immunology, University of Minnesota, and the Influenza Laboratory, Minnesota Department of Health.

Injection of type A and B influenza virus into the rabbit eye has been found to cause corneal opacity without multiplication of the virus.

Materials and Methods. The PR8 strain of influenza type A and the Lee strain of influenza type B virus were used in the form of allantoic fluid collected from infected chick embryos 48 hours after inoculation and preserved by freezing on CO_2 ice. The 50% mortality[1] titer of the PR8[2] strain in mice was $10^{-5.5}$ and the 50% infectivity titer in eggs[3] was $10^{-8.0}$ The corresponding titers for the Lee[4] strain were $10^{-3.7}$ in mice and $10^{-7.8}$ in eggs. Specific antisera were obtained from ferrets 10 days after intranasal inoculation with either the PR8 or the Lee strain of virus.

Young adult domestic rabbits, in nearly all instances of the albino variety, were inoculated under ether anesthesia by insertion of a 26-gauge needle through the cornea near the limbus. After withdrawal of slightly more than 0.2 cc of aqueous humor, approximately 0.2 cc of the inoculum was injected.

Observations. In the course of several experiments 15 rabbit eyes were inoculated with the PR8 virus and 17 with the Lee virus as described. Without exception there developed a haziness of the cornea which progressed to a complete opacity. As a rule the cornea remained clear for 48 hours, except for the transient alteration due to trauma at the point of inoculation. The reaction was definite on the third day and attained a maximum on the fourth or fifth day, at which time the cornea was thickened and obviously edematous. In a few instances, the reaction was more rapid, some haziness being apparent at 24 hours and the opacity being complete at 72 hours. With the exception of moderate conjunctival injection and congestion of the iris, lesions were not noted in other parts of the eye than the cornea.

In 10 eyes inoculated with normal allantoic fluid and 4 with mixtures of normal allantoic fluid and normal or immune ferret sera no lesions were observed. Infected allantoic fluid inactivated by formalin 1:1000 for 12 hours or by temperature of 56°C for five minutes did not produce lesions. Likewise Seitz filtrates of infected fluids caused no pathological changes.

Lesions were only produced by the inoculation of large amounts of active virus. When the inocula were diluted 10 times in horse serum-broth typical opacity resulted; how-

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[1] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **38**, 59.

[2] Francis, T., Jr., *Science*, 1934, **80**, 457.

[3] Hirst, G. K,. *J. Immunol.*, 1942, **48**, 285.

[4] Francis, T., Jr., *Science*, 1940, **92**, 405.