

Inhibition of the α and β Isoagglutinins in Anti-RH Serums.

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Recently Oliver-González¹ described a polysaccharide isolated from the pig roundworm *Ascaris suum* which has the property of inhibiting the α and β agglutinins in human serums. Furthermore, Oliver-González and Montilla² reported that the action of the ascarid polysaccharide is limited to the α and β agglutinins with no effect on the anti-M, anti-N and cold agglutinins. These investigators therefore conclude that the polysaccharide may be used in the preparation of anti-Rh serums, where the α and β agglutinins need to be removed. Since the studies of Oliver-González and Montilla² on the treatment of anti-Rh serums with the ascarid polysaccharide, included only 2 human anti-Rh serums the work that follows was done to test the effect of this substance on a larger number of anti-Rh serums coming from various human sources.

Materials and Methods. The anti-Rh serums* used in this study were obtained from the following sources:

1. From a boy suffering from osteomyelitis. This patient was successfully given 8 blood transfusions before the appearance of atypical agglutinins.

2. From a case of subacute bacterial endocarditis. This patient developed demonstrable anti-Rh antibodies after 2 blood transfusions.

3. From a patient with bleeding peptic ulcer immunized through 6 blood transfusions.

- 4 and 5. From women who delivered infants that developed acute hemolytic anemia of the newborn.

The method used for the inhibition of isoagglutinins follows the one described by

Oliver-González and Montilla.² To small volumes of serums enough polysaccharide was added to make a 4% suspension. Such treated serums were incubated for 30 min at 37°C and left overnight in the icebox. The centrifuged and clear supernatants were then used as the test serums. With some serums further treatment with polysaccharide was found to be necessary.

The Rh positive and the Rh negative cells used in these experiments have been classified as such after testing them with anti-RH₀, anti-Rh' and anti-Rh'' serums. These test serums determine the 8 Rh types described recently by Wiener³ and which are designated as follows: Rh negative, Rh₁, Rh₂, Rh₁Rh₂, Rh₀, Rh', Rh'' and Rh'Rh''.

Agglutination tests were done by the test tube technic recommended by Levine⁴ that is more sensitive in detecting weak reactions such as those due to the Rh factor. One drop of anti-Rh serum and one drop of 2% red blood cell suspension in saline were mixed in a small test tube and incubated for one half hr at 37°C. The quality of the sediment was observed then centrifuged and by means of a mirror read macroscopically while shaking gently. A drop placed on a slide was also read microscopically for evidence of clumping. Tubes showing agglutination of the erythrocytes were considered positive.

Results and Summary. As may be seen in Table I, in the majority of serums the polysaccharide inhibited the α and β agglutinins when this was added to the serums to a concentration of 4%. The anti-Rh agglutinins remained unaltered. In serum No. 4, however, addition of polysaccharide to make a concentration of 12% was necessary in order to inhibit the β agglutinins. In serum No. 5 the β agglutinins could not be removed from the

¹ Oliver-González, J., *J. Infect. Dis.*, 1944, **74**, 81.

² Oliver-González, J., and Montilla E., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 169.

* These test serums were obtained through the courtesy of Dr. Alexander S. Wiener of the Brooklyn Jewish Hospital, New York City, N. Y.

³ Wiener, A. S., *Science*, 1944, **99**, 532.

⁴ Levine, Ph., Katzin, E. M., and Burnham, L., *Proc. Soc. Exp. Biol. and Med.*, 1944, **45**, 346.

TABLE I.
Effect of Ascarid Polysaccharide on the α and β Isoagglutinins in Various Anti-Rh Human Serums.

Serum No.	Agglutinins in serum	Conc. of polysaccharide in serum %	Group A		Untreated serums Erythrocytes tested					
			Rh+	Rh—	Group B		Group AB		Group O	
			Rh+	Rh—	Rh+	Rh—	Rh+	Rh—	Rh+	Rh—
1	β		+	—	+	+	+	+	+	—
2	β		+	—	+	+	+	+	+	—
3	α and β		+	+	+	+	+	+	+	—
4	α " β		+	+	+	+	+	+	+	—
5	α " β		+	+	+	+	+	+	+	—
6	α		+	+	+	—	+	+	+	—
Serums treated with ascarid polysaccharide										
1	β	4	+	—	+	—	+	—	+	—
2	β	4	+	—	+	—	+	—	+	—
3	α and β	4	+	—	+	—	+	—	+	—
4	α " β	12	+	—	+	—	+	—	+	—
5	α " β	16	+	—	+	+	+	+	+	—
6	α	4	+	—	+	—	+	—	+	—

serum even after the addition of the polysaccharide to a concentration of 16%. Difficulty in removing the natural β isoagglutinins from some anti-Rh serums has been also encountered by Levine⁵ when the specific substances A and B were added to serums. Work is in progress to determine the relations that might exist between these non-absorbable natural isoanti-

bodies and those resulting from isoimmunization to the different Rh types.

The results of this study also confirm and extend the findings of Oliver-González and Montilla indicating that the polysaccharide from *Ascaris suum* may be used to inhibit the α and β isoagglutinins in human serums so that they can be used to detect Rh positive and Rh negative cells.

⁵ Levine, Ph., *Arch. Path.*, 1944, **37**, 83.

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Characteristics of Diphtheria Bacilli Found in Baltimore Since November, 1942.*

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In Baltimore from time to time during the past 22 years systematic examinations have been made of diphtheria in its clinical, epidemiological, immunological and bacteriological aspects. Reports have been published concern-

ing these various matters, including data on the distribution of diphtheria bacilli, fluctuations in ratio of virulent to avirulent strains, changes in carrier rate, the occurrence of various biological types such as the so-called gravis and mitis varieties, and miscellaneous facts relating to the natural history of diphtheria.^{1,2} It is the purpose of the present paper to present new data accruing from

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† Of 169 virulent strains in this survey in which colony form was noted 92 formed minute colonies while 77 were large colony formers. Not all minute colony formers are minimus type organisms (Table II).

¹ Frobisher, M., Jr., *Am. J. Hyg.*, 1938, **28**, 13.

² Phair, J. J., and Smith, M. R., *Am. J. Hyg.*, 1942, **35**, 47.