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Protective Value of Immune Rabbit Serum and Its Globulin Fraction Against Experimental Murine Infection with *Hemophilus pertussis.**

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The intranasal inoculation of young mice with virulent *Hemophilus pertussis* produces an experimental disease against which the value of immunizing agents may be tested. By this method the protective capacity of hyperimmune human serum (lyophile) has been demonstrated.[1]

The present report describes the results obtained by the use of immune rabbit serum and of a globulin fraction prepared from it. Rabbits were immunized by the intravenous injection of suspensions of living *H. pertussis.* Three injections of 0.1, 0.3, and 0.5 cc of a standard suspension containing approximately 10 billion organisms per cc were given on successive days. After a rest period of a week, a second course was given. Trial bleedings were made 10 days after the last injection. When the agglutinating titer of the serum was 1:2560 or greater, the rabbits were bled from the heart, and the serum was separated and stored at 4°C.

The globulin fraction was prepared in the manner described by Heidelberger, Turner and Soo Hoo.[2] To 40 cc of serum were added 40 cc of sodium-sulfate solution, saturated at 37°C. The resulting precipitate was separated from the supernate by repeated centrifugation, and washed in sodium-sulfate solution, 60% saturated at 37°C. For the present experiments, it was deemed advisable to free the globulin from sodium sulfate, which was accordingly removed by dialysis against 0.85% sodium-chloride solution at 4-6°C. Asepsis was maintained throughout. The volume after dialysis was 26 cc. A small amount of insoluble matter was removed by centrifugation and the solution, now nearly clear and colorless, was diluted to 40 cc.

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1 Bradford, W. L., and Wold, M., *Am. J. Dis. Child.*, in press.

2 Heidelberger, M., Turner, J. C., and Soo Hoo, C. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 734.

Tested against a suspension of *H. pertussis* containing 5 billion organisms per cc, the agglutinating titers of the various fractions were: Original immune rabbit serum, 1:5120; globulin fraction, 1:5120; supernate from original precipitation, 0; combined washings of precipitate, 1:10. The dilutions were directly comparable. It is obvious that, within the limits of accuracy of the determination of agglutinin, all of the agglutinating antibody was precipitated by 50% saturated sodium sulfate (37°C) and that none was lost in washing and dialyzing.

The nitrogen contents of the original serum and globulin fraction were respectively 10.6 and 3.6 mg per cc indicating that the latter contained only one-third of the protein originally present.

The method of testing sera for protective capacity has been described[1] in detail. Briefly, it is as follows: *H. pertussis* is grown on Bordet medium for 72 hours. The growth is scraped off and homogenized in 0.85% sodium-chloride solution. The turbidity of the suspension is then adjusted to match that of a standard suspension of *H. pertussis* containing 10 billion organisms per cc. Three-weeks-old Swiss mice are lightly anesthetized in a jar containing cotton saturated with a mixture of 1 part of chloroform and 2 parts of ether. From a 1 cc tuberculin syringe fitted with a short, blunt-tipped, 24-gauge needle, 0.05 cc of the standardized suspension of organisms is dropped into the nares of each mouse. Immediately after infection, 0.3 cc of the solution being tested for protective capacity is injected intraäbdominally into each mouse. This dose is repeated on each of the 2 following days. Control groups receive a comparable amount of saline.

As the mice die, they are necropsied and the degree of consolidation of the lungs is scored by a method described previously.[1] Cultures are made from the lungs and spleen. All mice surviving on the tenth day after infection are killed and examined.

The data of the first experiment, recorded in Table I, indicate the degree of protection to be expected and demonstrate that the result is statistically valid. A total of 120 infected mice were divided into 3 sub-groups of 40 each. The first group was treated in the manner already described with immune rabbit serum; the second, with normal rabbit serum; and the third, with saline. The protection afforded by the immune serum was reflected by all 3 criteria employed to measure it, *viz.*, mortality, pathological and bacteriological findings, although the bacteriological results will hardly bear statistical scrutiny. It is obvious that normal rabbit serum exerted no more protective action than did saline. It is of interest that Mishulow with

TABLE I.

Protective Value of Immune Rabbit Serum Against Experimental Murine Infection with *Hemophilus pertussis.*

Material tested	Mortality						Bacteriology				Pathology					
	No. mice	No. survived	Difference in % of survivors in the 2 groups	Standard error of difference*	Difference / Standard Error		No. mice showing positive lung cultures	Difference in % of positive lungs in the 2 groups	Standard error of difference*	Difference / Standard Error	No. of mice with "none or slight" lung lesions (0-5)	No. of mice with "moderate" lung lesions (6-14)	No. of mice with "severe" lung lesions (15-19)	Difference in % of the 2 groups showing "none or slight" lung lesions	Standard error of difference*	Difference / Standard Error
Immune rabbit serum	40	31	57.5	11.2	5.1		30	22.5	7.7	2.9	21	18	1	40	10.5	3.8
Normal rabbit serum	40	8					39				5	25	10			
Saline	40	10					32				0	24	16			

Infecting dosage of organisms = 500 million.

*Calculated by the formula $\sqrt{P_o Q_o \left(\frac{1}{N} + \frac{1}{N'}\right)}$ where P_o is average chance of death; Q_o is the average chance of survival; N is the number of mice in one group; and N′ is the number of mice in the other group. Topley and Wilson, *Principles of Bacteriology and Immunity,* second edition, Wm. Wood & Co., Philadelphia, Pa., p. 777, 1936.

TABLE II.

Comparison of Protective Power of Immune Rabbit Serum and of a Globulin Fraction Prepared from it Against Experimental Murine Infection with *Hemophilus pertussis*.

Infecting dosage of organisms	Ratio of No. of mice surviving to the No. infected			Incidence of positive lung-cultures			Incidence of No. of mice showing "none or slight" lung-lesions		
	Immune rabbit serum	Globulin fraction	Controls	Immune rabbit serum	Globulin fraction	Controls	Immune rabbit serum	Globulin fraction	Controls
1,000 million	6/6*	3/6	—	1/6	1/6	—	5/6	2/6	—
500 "	2/6	4/6*	0/6	3/6	4/6	6/6	2/6	1/6	0/6
250 "	5/6	4/6	0/6	3/6	5/6	6/6	4/6	2/6	0/6
125 "	6/6	4/6	0/6	2/6	3/6	6/6	5/6	5/6	0/6
62.5 "	5/6	5/6	0/6	2/6	1/6	6/6	5/6	5/6	0/6
31.25 "			1/6*			6/6			0/6
Total	24/30	20/30	1/30	11/30	14/30	30/30	21/30	15/30	0/30

* Endpoint of the "50% mortality dosage." In the control group this is less than 31.25 million organisms while in the immune-serum and in the globulin-fraction groups, it is calculated as 1000 million and 500 million respectively. This represents protection against at least 32 MLD for the immune serum and 16 MLD for the globulin fraction.

her co-workers,[3] using an entirely different technic, have recently reported results quite comparable to these. In her experiments, the immune serum was administered intramuscularly, while infection was produced by intraäbdominal injection of the organisms suspended in a solution of mucin.

The second experiment was designed to evaluate the protective action of a pool of immune rabbit serum (not the same one used in the first experiment) in terms of the number of MLD of the organism used, and to compare the protective capacity of the serum with that of the partially purified globulin fraction prepared from it. Accordingly, the technic of the protective test was altered by inoculating sub-groups of 6 mice each with serial 2-fold dilutions of bacterial suspension, as outlined in Table II. It was thus possible to estimate the endpoint of 50% mortality by the method of Reed and Muench.[4] The results, recorded in Table II, corroborate those of the first experiment on all points. In addition, it was estimated that the serum protected against at least 32 MLD, whereas the globulin fraction protected against 16 MLD. By all 3 of the criteria employed, there was a slight but not statistically significant difference in favor of the whole serum. One may conclude, therefore, that but little loss of protective antibody occurred during the process of fractionation. It should be noted that the titration-curve (mortality *vs.* dosage) was much less steep in the presence of antibody than it has been in control titrations where the mortality rate usually passes from 80-100% to 0-20% within a two-fold change of dosage. Such a flattening of the curve decreases the accuracy of estimation of the endpoint.

Summary. Immune rabbit serum, injected intraäbdominally into mice, protects them against experimental infection produced by the intranasal inoculation of *Hemophilus pertussis.* By precipitation in 50% saturated sodium-sulfate solution (37°C), a globulin fraction was prepared which had essentially the same protective capacity and agglutinating titer as the original serum, but only one-third of its content of protein.

[3] Mishulow, L., Klein, I. F., Liss, M. M., and Leifer, L., *J. Immunol.*, 1939, **37**, 17.

[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.