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Hemagglutinins of Pneumococcic Antisera.*

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For a number of years it has been known that some pneumococcic strains possess the Forssman antigen and hence produce in the blood of immunized rabbits hemolysins for sheep blood and agglutinins for blood of Group A. According to a recent report[1] this antigen is present in all strains of pneumococci except those of Types IV, VIB, XI, and XXXI. Prominence was given to these facts only recently on account of the current increased use of therapeutic sera derived from rabbits.

We were led to examine the hemagglutinins and hemolysins of all antipneumococcic therapeutic sera available (rabbit and horse), especially in view of recent reports which indicate that horse sera containing strong-titered agglutinins for human blood were responsible for occasional fatal acute hemolytic reactions.[2, 3, cf. 4] These accidents were due to injection of horse serum Type XIV.

Our own experiments are based on tests done with therapeutic sera administered to patients at the Harlem Hospital. The sera were tested in dilution of 1:50 for hemolysins of sheep blood and for agglutination of human blood of Groups O, A_2, A_1, and B. Hemolysins for sheep blood were tested by adding 1 drop of 50% suspension of washed sheep-cells to a mixture of 0.5 cc of serum-dilution and 0.5 cc guinea-pig complement 1:10. Tests for agglutination were made by mixing in small tubes 2 drops of a 2% washed blood suspension and 2 or 3 drops of serum dilution; readings were made after the tests stood for one hour at room temperature.

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1 Powell, G. H., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 248.

2 Bullowa, J. G. M., *The Management of the Pneumonias*, New York, Oxford University Press, 1937, p. 316.

3 Finland, M., and Curnen, E. C., *Science*, 1938, **87**, 417.

4 Hoagland, C. L., Beeson, P. B., and Goebel, W. F., *Science*, 1938, **88**, 261.

Our results indicate that antipneumococcic rabbit sera of numerous types contain hemolysins for sheep blood and agglutinins with specific action on human blood of Group A. Of 90 rabbit sera of various types, more than half of them—49—contain distinct agglutinins for blood of Group A. The reactions were distinctly stronger on cells of subgroup A_1 than on A_2 so that in each case a suitable dilution could be found which acted only on A_1 and not on A_2. The incidence of hemolysins for sheep blood was somewhat slightly higher, 54 out of the 90 sera showing complete hemolysis in 1:50. The maximal hemolytic titer was 1:800; the maximal agglutinin titer for A_1 was 1:1000.

Both agglutinins and hemolysins are specifically absorbable by homologous pneumococci. As was to be expected from our knowledge of the specificity of the Forssman antigen the hemolysins for sheep blood and the agglutinins for human blood of Group A are 2 qualitatively distinct antibodies as could be shown by suitable cross-absorption experiments. In this respect our observation is not in agreement with that of Finland and Curnen.[3]

None of the antipneumococcic horse sera contains hemolysins for sheep blood, but in a number of them, particularly those of Type XIV, agglutinins for human blood of all groups could be demonstrated. The strongest reactions were found in 3 different specimens of Type XIV antisera, but weaker reactions were found in one specimen each of Type VII antiserum, and a bivalent serum, V and VII. The hemagglutinins in Type XIV antisera were specifically absorbable by large quantities of the homologous organism, a result which is in agreement with that of Finland and Curnen.[3]

The weaker hemagglutinins in the Type VII antiserum could be shown to be a property of the normal serum, since the reactions on human blood could not be absorbed after contact with the homologous organisms.

The surprising element in the results with the Type XIV antipneumococcic horse sera is the fact that these agglutinins reacted somewhat more intensely on bloods of Groups O and A_2 than on blood of Group A_1, in sharp contrast to the rabbit sera. This could be confirmed by suitable absorptions which show that such sera, after repeated absorption with blood of Group A_1, still acted distinctly on bloods of groups O and A_2 (see Table I).

Similar effects, but less differentiating were obtained with each of several different specimens of Type XIV antipneumococcic horse sera absorbed with blood A_1 so that it is safe to state that on the

TABLE I.
Type XIV Antipneumococcic Horse Serum Diluted 1:20 Absorbed 4 Times with One-half Volume of Washed Sediment of Group A_1 Blood and Tested for Action on Bloods of O, A_2, and A_1.

O				A_2		A_1			
1	2	3	4	5	6	7	8	9	10
+±	+±	++	+±	+	+±	±	0	tr	0

whole such horse sera are probably not superior to antibodies from other sources which act on bloods O and A_2.[5, 6]

Since these therapeutic sera are concentrated and antibodies specific for O and A_2 may be a property of normal animal sera, it was necessary to establish whether or not the specific action on O and A_2 is due to the immunization with pneumococcus XIV. Suitable experiments showed that this organism specifically absorbed the agglutinins prepared by preliminary absorption with blood A_1.

Further evidence as to the specificity of the reaction on bloods of groups O and A_2 is shown in hemolytic tests. Some, but not all, Type XIV antipneumococcic horse sera, when mixed in sufficiently high dilution (1:50 to 1:400) with suitable amounts of guinea-pig complement were found to hemolyze incompletely but distinctly bloods of Group O and to a lesser degree blood A_2 and not bloods A_1 or B. In these instances the hemolytic reactions were accompanied by agglutination. To demonstrate specific hemolysis it was necessary to select guinea pigs whose sera contained no normal hemolysins or agglutinins for bloods of Groups O and A. No hemolysis was observed when fresh human serum was used as a source of complement.

These results recall the observations made on the varying antigenic response of the Forssman antigen of the Shiga bacillus in the rabbit (non-Forssman type) and goat (Forssman type, like the horse). Thus, the Shiga bacillus produces in the rabbit hemolysins for sheep blood (and presumably agglutinins for blood of Group A), but in the goat (Eisler[7]) the same organism induces a response which so far as the hemagglutinins are concerned cannot be differentiated from that observed in Type XIV antipneumococcal horse serum, *i.e.*, agglutinins for human blood of all groups, and in addition another agglutinin specific for O and A_2.[6] It is also conceivable

[5] Landsteiner, K., and Levine, P., *J. Immunol.*, 1929, **17**, 1.

[6] Landsteiner, K., and Levine, P., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 309.

[7] Eisler, M., *Z. f. Immunitatsf.*, 1930, **67**, 38.

that an explanation for the strikingly varying behavior of antisera derived from the horse and goat in contrast to those from the rabbit may be found in a difference in their essential lipoids (Horsfall and Goodner).[8]

In view of the observations recorded, it is pertinent to inquire about the blood group of the individuals who died from the administration of the therapeutic horse sera. Unfortunately, these data are not available.

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Pneumonia in White Mice Produced by a Pleuro-Pneumonia-Like Micro-Organism.*

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During the past year, while working with tissues and exudates from patients with rheumatic fever or rheumatoid arthritis, a pleuro-pneumonia-like micro-organism has been encountered in our laboratory mice.

In each instance, normal young white mice of the same breed were inoculated intranasally under ether anesthesia with 0.05 cc of 10-20% tyrode or saline suspensions of human pathologic tissues. However, exudates were introduced without dilution. Serial mouse-passage was carried on at intervals of 4-6 days, using 10-20% lung-suspensions. Blind passages were done in a parallel fashion. Usually by the fourth passage, purple areas of pneumonic consolidation were clearly visible in one or more lobes. In one instance, the pneumonia appeared as early as the second passage. Further passage slowly increased the virulence, morbidity and mortality. Even after months of passage, however, the mortality never increased beyond 20-30%, with death usually occurring on the fourth or fifth day.

Culture of the ground lungs uniformly grew innumerable colonies

8 Horsfall, F. L., and Goodner, K., *J. Immunol.*, 1936, **31**, 135.

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