antibody with the polysaccharide. If the antibody had not been altered during precipitation, the protein in the washings of immune-horse precipitate which contained no polysaccharide should have the same activity as pure precipitin. But this is not the case. Furthermore, it is difficult to conceive that the immune-rabbit precipitate after losing nearly all its polysaccharide should still retain the original low solubility and low activity of the precipitate. The increase in solubility and immunological activities of the washed precipitate can be brought about not by the removal of the polysaccharide but by treating with alkali under conditions suitable for the reversion of denatured protein.

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Fractionation of Antibody in Type I Antipneumococcal Serum by Addition of Ammonium Sulfate.

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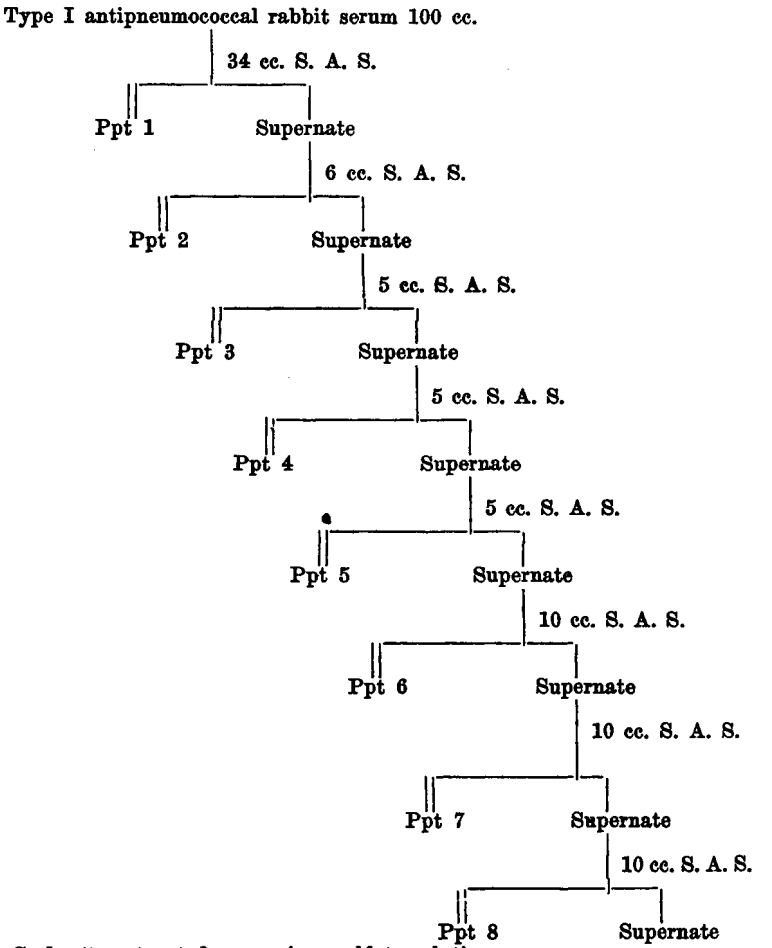
It has been reported[1] that the antibody of Type I pneumococcus in horse or rabbit serum is separable into fractions with different immunological and physical properties and that this separation can be effected by absorption with Type I pneumococcal polysaccharide. In this paper we shall present evidence that the antibody of Type I pneumococcus is separable into fractions by precipitation with ammonium sulphate in such a manner that only a small portion of the antibody comes down in each successive precipitation.

The results of a typical experiment are given below:

To 100 cc. (12.0 mg. N/cc.) of Type I antipneumococcal rabbit serum were added 34 cc. of saturated ammonium sulfate solution. To hasten flocculation the suspension was warmed to about 37°C. It was centrifuged, and the supernate was poured into another tube to which another portion of saturated ammonium sulfate solution was added according to the scheme given in the accompanying diagram. The precipitates were dissolved in water, dialyzed against 0.005 M acetate buffer at pH 5.0, and centrifuged. Both the supernate and the precipitate were adjusted to the usual saline concentration, and the immunological activities were tested. The percentage of protein-nitrogen present in each fraction is given in Table I.

[1] Chow, B. F., *Proc. of the Chinese Physiol. Soc.*, 1937, 18.



S. A. S. denote saturated ammonium sulfate solution.
Ppt denote precipitates.

TABLE I.

	Insoluble at pH = 5.0		Soluble at pH = 5.0	
No.	Mg. N found	% of original	Mg. N found	% of original
1	1.06	0.88	74.8	6.16
2	0.33	0.28	238.4	19.80
3	0.17	0.14	105.0	8.75
4	0.56	0.47	57.6	4.75
5	0.81	0.67	23.6	1.96
6	0.90	0.75	19.8	1.65
7	0.41	0.33	23.6	1.96
8	0.48	0.40	23.4	1.95

TABLE II.

No.	Mg. N protecting mice from 1,000,000 MLD of Type I pneumococcus	A/2	A/8	A/16	A/32	A/64	A/128
				Agglutination			
1	0.011	++++	++++	++++	++++	++	+
2	0.012	++++	++++	++++	++++	++	—
3	0.006	++++	++++	++++	++++	+++	+
4	0.004	++++	++++	++++	++++	+	—
5	0.006	++++	++++	++++	+	—	—
6	0.006	++++	++++	++++	+	—	—
7	0.012	++++	++++	++	+	—	—
8	0.110	++++	++++	+	—	—	—
	A = 0.4 mg. N/cc.						
				Precipitation			
		Final dilution of original polysaccharide					
		1:4,000		1:16,000		1:64,000	
1	0.011	++++		++++		++++	
2	0.012	++++		++++		++++	
3	0.006	++++		++++		++++	
4	0.004	++++		++++		++++	
5	0.006	++++		++++		++++	
6	0.006	++++		++++		++	
7	0.012	++++		+		—	
8	0.110	—		—		—	
		Final dilution of polysaccharide* hydrolyzed by acid					
		1:4,000		1:16,000		1:64,000	
1	0.011	++++		++++		++++	
2	0.012	++++		++++		++++	
3	0.006	++++		+		—	
4	0.004	++++		+		—	
5	0.006	+++		+		—	
6	0.006	—		+		—	
7	0.012	—		—		—	
8	0.110	—		—		—	
		Final dilution of polysaccharide* hydrolyzed by both acid and alkali					
		1:4,000		1:16,000		1:64,000	
1	0.011	++++		++++		++++	
2	0.012	++++		++++		+	
3	0.006	+++		+		—	
4	0.004	—		++		—	
5	0.006	—		—		—	
6	0.006	—		—		—	
7	0.012	—		—		—	
8	0.110						

*The hydrolysis of the original polysaccharide was carried out in the same manner previously[6] described.

The insoluble portion represents only a very small fraction of the serum-globulin, and was only partially soluble in saline solution. Therefore its immunological activities were not studied.

[6] Chow, B. F., *J. Exp. Med.*, 1936, **64**, 843.

Although all these 8 fractions soluble at pH 5.0 could agglutinate Type I pneumococci, they differed not only quantitatively but qualitatively in their reactivity with the homologous polysaccharide and its hydrolytic products. The results of qualitative agglutination and precipitation of these fractions (Table II) reveal the following facts: First, all 8 fractions contain antibodies, for they agglutinate Type I pneumococci and protect mice from an otherwise lethal dose of the organism. Secondly, fraction 8 corresponds to the antibody[2] obtained by the agglutination of Type I antipneumococcal rabbit serum previously absorbed with the homologous polysaccharide. It possesses both agglutinative and protective power but fails to react with the homologous polysaccharide. Thirdly, hydrolysis of the original polysaccharide by acid or both acid and alkali destroys the reactivity with certain fractions of the antibody. These fractions (*e. g.*, Nos. 5 and 6) obtained by the addition of ammonium sulfate gave a heavy precipitate with the original polysaccharide, but only a slight precipitate with the product hydrolyzed by acid and practically none with the product hydrolyzed by both acid and alkali.

The extensive study on the fractionation of normal serum-globulin with salts by Sørenson[3] and other workers supports the current view that it is an easily dissociable complex consisting of the so-called euglobulin and pseudoglobulin in varying proportions. Numerous studies on the distribution of antibodies in antipneumococcal sera have been made by fractionation with ammonium sulfate. However, the estimation of antibody in each fraction is usually made by mouse-protective tests[4] or by the precipitative reaction[5] on the assumption that the same antibody is present in each fraction. This has been found not to be the case in the present study.

9610 P

A 2,4-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid and the Estimation of Vitamin C.

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When dehydroascorbic acid is treated with a saturated solution

2 Sørensen, S. P. L., *Compt. rend. trav. lab.*, Carlsberg, 1923-25, **15**, No. 11.

3 Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 163.

4 Felton, L. F., and Kaufmann, G. J., *Immunology*, 1933, **25**, 165.

5 Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **34**, 651.