of a standard solution of polysaccharide. Examples of this kind are found, when it is necessary to find the rate of diffusion of the polysaccharide through a membrane.

Case II. When homologous rabbit-precipitin is also present. In studying the mechanism of the precipitin-reaction, it is often necessary to estimate the concentration of the polysaccharide in the presence of an excess of rabbit's precipitin. An experiment was performed to ascertain whether this method can be used in such a case.

The results (Table I) show that the presence of immune rabbit's precipitin, when not in great excess, may not alter the complement-fixation titer. Traces of the polysaccharide in the isolated precipitin used in this experiment were removed by precipitation of the antibody at half saturation of ammonium sulfate and dialysis against saline solution.

Case III. When horse-precipitin is present. Horse-precipitin inhibits the complement-fixation reaction and tends to give low results. The inhibitory effect can be overcome by using a higher concentration of rabbit's serum.

Table II shows that when the immune rabbit's serum was diluted only 5-fold (I, II, III) the inhibitory effect of the horse-precipitin was not observable. On the other hand, when the same serum was diluted 25-fold, the effect of the same amount of horse-precipitin was decidedly noticeable.

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Studies on the Mechanism of the Precipitin-Reaction. I. Behavior of Immune Precipitate Towards Washing.

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Several differences in the immunological activities of Type I anti-pneumococcal rabbit and horse sera have been reported.[1] However, little is known about the difference, if any, in the manner of union between the polysaccharide and its precipitin in the sera of these two animals. In this report, we shall show that such a difference exists. When the immune precipitates were washed with

[1] Goodner, K., and Horsfall, F. L., *J. Exp. Med.*, 1936, **62**, 485.

normal saline solution, and the amounts of polysaccharide present in the combined washings were estimated by the method described in the preceding paper, it was found that practically all the polysaccharide in the immune-rabbit precipitate could be accounted for in the washings. On the other hand, no appreciable amount of polysaccharide could be removed from the immune-horse precipitate.

Twenty cc. of Type I pneumococcal polysaccharide (0.1 mg. per cc.) were added to 20 cc. of homologous horse or rabbit antiserum. The mixture was incubated at 37°C. for half an hour. The supernate was poured off and was found to contain an excess of antibody. The precipitate was washed with four 50-cc. portions of 0.85% NaCl solution, dissolved in 5 cc. of water and 5 cc. of N/140 NaOH, and the alkaline solution was poured into 400 cc. of 0.85% NaCl solution (designated as P). The polysaccharide-content of the P-fraction and the combined washings (W) was determined by the complement-fixation method. The results are shown in Table I.

TABLE I.
Polysaccharide Contents of the Washed Immune Precipitate and Washings.

Animal origin		Total polysaccharide found mg.	Total protein mg.
Horse	P	2.0	40
	W	none	20
Rabbit	P	0.05	50
	W	1.8	25

In another experiment, the washed immune-rabbit precipitate containing 50 mg. protein was found to contain 0.025 mg. polysaccharide. The precipitate was suspended in 20 cc. of water and dissolved with 5 cc. of N/70 NaOH. The clear alkaline solution was brought to slight turbidity with N/70 HCl and allowed to stand in ice-water overnight. On the following morning the solution was neutralized, solid NaCl was added to 0.85%, and it was centrifuged. The supernatant fluid which contained 25% of the original protein was found to have a higher titer in agglutination, precipitation with homologous polysaccharide, and mouse-protection, than the combined washings. We have found that the addition of 0.025 mg. polysaccharide to 50 mg. of immunologically pure precipitin[2] caused no visible precipitation.

We have stated elsewhere[2] that the low immunological activity of the antibody-polysaccharide complex is due to some alteration in the antibody-protein itself and not merely to the combination of the

[2] Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 139.

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.