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Determination of Minute Amounts of Type I Pneumococcal Polysaccharide by the Complement-Fixation Method.

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A quantitative method for the determination of a precipitinogen is valuable in the study of the mechanism of the precipitative reaction.[1] Heidelberger[2] determined the concentration of a solution of the polysaccharide of Type III pneumococcus by analyzing the amount of antibody-nitrogen precipitable from a previously standardized homologous serum. Obviously, such a method requires considerable material and is not applicable to minute amounts of polysaccharide. In the course of our study of the mechanism of the precipitin-reaction of Type I pneumococcus it was necessary to devise a method for the determination of a very small amount of polysaccharide. Since the polysaccharide united with its homologous rabbit-precipitin can fix complement,[3] it seemed advisable to investigate the possibility of adapting this method to our needs. We report the details of this method which permits the estimation of as little as 0.00001 mg. of Type I pneumococcal polysaccharide even in the presence of homologous antibody.

The usual procedure of complement-fixation was followed: 0.5 cc. of Type I antipneumococcal rabbit serum plus 0.5 cc. of the homologous polysaccharide in various dilutions was incubated at 37° for 30 minutes. After the addition of 1.0 cc. of guinea pig's complement containing 2 units per cc. it was placed in an icebox overnight. The following morning, the tubes were warmed to room temperature and 0.5 cc. of hemolysin (2 units) and 0.5 cc. of 2% sheep-cell suspension were added. The mixture was incubated at 37°C. for one hour. In order to estimate the concentration of an unknown solution, a solution of known concentration of polysaccharide was used for comparison.

This method is particularly useful in the following 3 cases:

Case I. When there is no interfering substance. When the unknown contains only a minute amount of polysaccharide it can be easily estimated by comparing the titer of the unknown with that

[1] Hooker, S. B., and Boyd, W. C., *J. Gen. Physiol.*, 1935, **19**, 373.

[2] Heidelberger, M., *J. Exp. Med.*, 1932, **55**, 555.

[3] Goodner, K., and Horsfall, F. L., *J. Exp. Med.*, 1936, **64**, 201.

TABLE I.
Effect of Rabbit-precipitin on the Complement-fixation Titer.

Exp.	0.25 cc. IRS (1 + 2) +	Final dilution of polysaccharide 1:1,000,000	1:2,000,000	1:4,000,000	1:8,000,000	1:16,000,000	1:32,000,000
I	0.25 cc. saline	++++	++++	++++	++++	—	—
II	0.25 cc. RP (2 mg. protein/cc.)	++++	++++	++++	++++	—	—
III	0.25 cc. RP (0.5 mg. protein/cc.)	++++	++++	++++	++++	—	—

The components used in each experiment are given below:
Exp. I 0.5 cc. of polysaccharide + 0.25 cc. IRS + 0.25 cc. 0.85% NaCl.
II 0.5 " " " + 0.25 " IRS + 0.25 " of RP (2 mg. protein/cc.)
III 0.5 " " " + 0.25 " IRS + 0.25 " of RP (0.5 mg. protein/cc.)
Where IRS = immune rabbit serum.
RP = rabbit-precipitin.

TABLE II.
Effect of Horse-precipitin on the Complement-fixation Titer.

Exp.	0.25 cc. IRS (1 + 4) +	Final dilution of polysaccharide 1:1,600,000	1:32,000,000	1:64,000,000	1:128,000,000	1:256,000,000
I	0.25 cc. of 0.85% NaCl	++++	++++	++++	—	—
II	0.25 cc. HP (2 mg. protein/cc.)	++++	++++	++++	—	—
III	0.25 cc. HP 0.25 mg. protein/cc.) 0.25 cc. IRS (1:25)	++++	++++	++++	—	—
IV	0.25 cc. 0.85% NaCl	++++	++++	++++	++++	—
V	0.25 cc. HP (0.25 mg. protein/cc.)	++++	+++	—	—	—

The components used in the experiments are given below:

	cc. of polysaccharide solution		cc. of IRS		
I	0.5	+	0.25	(1:5)	+ 0.25 cc. 0.85% NaCl.
II	0.5	+	0.25	(1:5)	+ 0.25 cc. HP 0.25 mg. protein/cc.
III	0.5	+	0.25	(1:5)	+ 0.25 cc. HP 0.06 mg. " "
IV	0.5	+	0.25	(1:25)	+ 0.25 cc. 0.85% NaCl.
V	0.5	+	0.25	(1:25)	+ 0.25 cc. HP 0.25 mg. protein/cc.

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.