control titers. Unfortunately the rate of bacterial growth likewise was reduced by the iodoacetic acid and it is therefore not possible to interpret the results as being due to the inactivation of precursor by iodoacetic acid. They could equally well arise from the effect of iodoacetic acid on bacterial growth with resultant lowering of the number of precursor-producing units available.

To summarize, it has been shown that concentrations of iodoacetic acid ranging from M/2,000 to M/10,000 serve to abolish the phage-augmenting capacity of activated staphylococci (organisms containing phage precursor) at 5°C and pH 6.1, without killing them.

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Quantitative Determination of Pneumococcal Capsular Polysaccharide by Photronreflectometric Titration of Precipitation with Excess Antibody.*

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Several recent publications[1, 2] have again dealt with the occurrence of capsular polysaccharide (SSS) in the blood of pneumococcal pneumonia patients and the serious prognosis usually associated with this finding. A number of observers in the past[3–10] have called attention to the relationship of SSS to the virulence of pneumococci and

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to its ability to "neutralize" immune bodies specifically. Despite the well-developed knowledge of the effect of SSS and its clinical significance, little is known of the conditions governing its production by pneumococci either *in vivo* or *in vitro*. A simple, accurate method for the quantitative determination of the substance is essential to the pursuit of such studies and additional studies on the effect of sulfonamide compounds on the elaboration of SSS. Heidelberger and Kendall[11] utilized a standardized solution of antibody which had been titrated with varying dilutions of known SSS to obtain a curve of precipitates determined by micro-Kjeldahl procedures, precautions being taken to work with purified SSS and to remain within the zone of antibody excess. This procedure is not easily adapted to the studies planned. Hooker and Boyd[12] observed that, in regions of considerable antibody excess, times of flocculation varied inversely and in the same ratio as changes in concentration of antigen. By applying this formulation they were able to estimate small quantities of precipitinogen present in solutions of unknown concentration. Recently, Libby[13] developed the photronreflectometer which he described as useful for the detection of unknown solutions of SSS by titrating a standard serum with varying dilutions of the unknown until predetermined equivalents of unit and galvanometer reading are obtained.

In the present investigation a commercially prepared type III pneumococcal rabbit antiserum (Lederle), having approximately 4,000 units per ml, or 3.75 mg of precipitable nitrogen per ml was diluted with ether-phenol saline solution to a concentration of 25 units per ml or .023 mg precipitable N per ml. By testing supernates of a series of reactions between capsular polysaccharide and this antibody solution, the equivalence point concentration of capsular polysaccharide was found to be .004 mg. A dried preparation of purified polysaccharide III‡ was accurately weighed§ and volumetrically diluted to 1 to 100,000 (.01 mg per ml). Volumetrically accurate dilutions ranging between .0005 and .005 mg per ml, and differing by .0005 mg concentrations, were then made, using a saline diluent. Using volumetric pipettes, 1 ml of each of these dilutions

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‡ Kindly provided by Dr. W. G. Malcolm and by Dr. Rachel Brown.

§ For the weighing of the samples we are indebted to Dr. Joseph B. Niederl and to Victor Niederl of the microanalytical laboratories at Washington Square College of New York University.

TABLE I.

Turbidity readings of reaction between antipneumococcal rabbit serum III 473h273h (1-160 dilution, .023 mg precipitable N per ml) and SSS III (R. Brown t-16) in saline solution. Incubated 20 minutes at 37°C. Sensitivity of photronreflectometer at 15.

Photronreflectometer readings	Mg capsular polysaccharide [per ml saline]																	
	.005		.0045		.004		.0035		.0030		.0025		.0020		.0015		.001		.0005	
Dry blank reading	2.5	3.0	2.0	2.5	2.5	2.0	3.0	2.0	2.0	3.0	3.0	2.5	2.5	3.0	2.5	2.0	2.5	2.5	3.0	2.5
Reading after 20 min	30.0	31.0	29.5	31.0	31.5	30.5	31.5	29.5	29.0	30.0	28.5	28.0	24.0	24.5	15.0	15.5	12.5	12.5	8.0	7.5
Corrected reading	27.5	28.0	27.5	28.5	29.0	28.5	28.5	27.5	27.0	27.0	25.5	25.5	21.5	21.5	12.5	13.5	10.0	10.0	5.0	5.0
Avg reading (nearest .5)	28.0		28.0		29.0		28.0		27.0		25.5		21.5		13.0		10.0		5.0	

was mixed with 1 ml of the standard serum‖ in refraction cells, incubated in a water bath (totally immersed) at 37°C for 20 minutes,¶ and the resulting turbidities read, in duplicate, in the photronreflectometer. Table I gives the readings obtained in such a determination, performed in duplicate. Readings checked, on repetition, with a high degree of accuracy, variations of not greater than 0.5 to 1.0 galvanometric deflection being observed for each point determined. Fig. 1 is a curve prepared from 5 such determinations. The concentration of an unknown solution can be determined by reference of experimental titrations to this curve. In titrating an unknown solution, precaution is always taken to assure the use of a dilution which will be in the range of antibody excess with the standardized serum, *i. e.*, in the zone to the left of "equivalence point concentrations" in Fig. 1 (this can also be checked by testing the supernate of the tube after the reaction is brought to completion by refrigeration and centrifugation). The method seems well-adapted to compara-

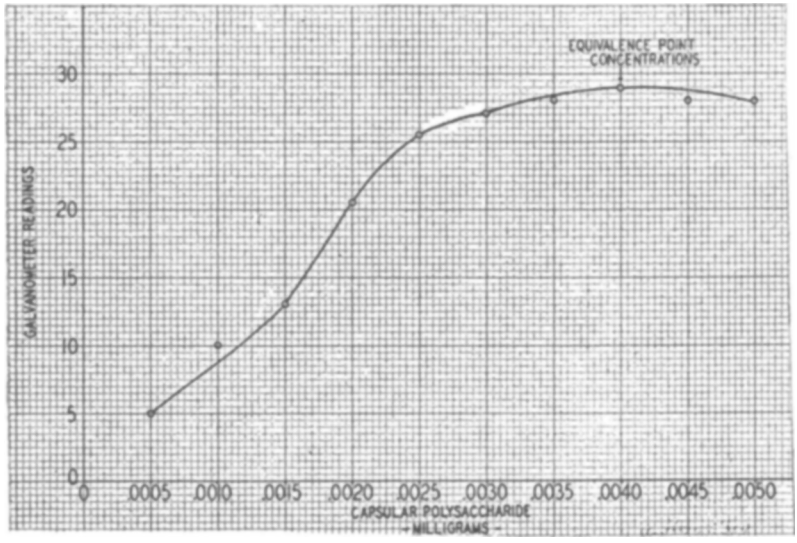

FIG. 1.

Smoothed graph of photronreflectometric readings (average of 5 determinations) obtained by titrations of varying quantities of capsular polysaccharide III (Dr. Brown T-16) with rabbit antipneumococcal serum III (containing .023 mg precipitable N per ml).

‖ The serum was not absorbed with "C" substance or somatic nucleoprotein; however, filtrates of cultures of heterologous pneumococci (4 and 21) failed to react with the serum.

¶ Preliminary experiments (unpublished) by Dr. Ely Perlman, of this laboratory, indicated this to be a satisfactory concentration.

tive determinations for a single preparation of SSS. Since it may be expected that a given strain of pneumococcus produces a uniform SSS, *in vitro,* comparative determinations of SSS produced by such a single strain under differing conditions of growth, using the same serum, would appear to be valid. In such instances, the standard curve should be prepared with dilutions of SSS made in broth.

Summary. A standard solution of antipneumococcal rabbit antibody type III was titrated with varying dilutions of capsular polysaccharide, in the zone of antibody excess. Photronreflectometric readings were made after 20 minutes of incubation at 37°C and a standard curve made. Unknown concentrations of SSS can be determined by identical titrations of varying dilutions with reference to the standard curve, provided a single system is utilized.

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A Micromortar Especially Adapted to Virus Studies in Insects.*

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During efforts to improve quantitative methods of studying yellow fever virus in arthropods, it became evident that a single insect, such as a mosquito, can not be triturated in the ordinary mortar without the loss of much of the material, particularly when only a little liquid is added. The use of a small mortar that would also serve as a centrifuge-tube would reduce the loss and simplify the technic.

A device designed to achieve these ends was made of Pyrex glass; the two parts were first formed as accurately as possible in a flame; then the pestle was ground on a wheel to an approximate fit with the apposing surface of the mortar. The grinding surface was then formed at the bottom of the tube by seating the pestle B into A, using carborundum mixed with turpentine or glycerol. The grinding should be finished with a fine powder to give a reasonably fine tooth to the finished surface.

It is essential that the two grinding surfaces be well fitted and that the clearance established by the neck of the pestle should exceed in

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