8516 P

Soluble Specific Substance of Pneumococcus Type III Possessing Properties Distinct from SSS III.

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It has been recently demonstrated that the soluble specific substance of Pneumococcus type I, as originally prepared by Heidelberger, Goebel and Avery, represented a form of the polysaccharide lacking the acetyl group which, when present, conferred upon the substance distinct physico-chemical and immunological properties.[1, 2] In the case of the type specific polysaccharide from Pneumococcus type III, purified according to the procedure described by the same authors, certain observations such as those of Ward[3] have led us to believe that an analogous alteration in chemical structure may have occurred during the chemical manipulations required for its purification. We have, therefore, attempted to prepare this material by a method which avoids insofar as possible the use of strong acids.

Six- to 8-day cultures of Pneumococcus type III in dextrose phosphate broth were concentrated over a boiling water bath to one-tenth of the original volume. The concentrate was precipitated several times with about 1.2 volumes of alcohol. Proteins were precipitated by careful addition of 1N acetic acid, until maximal precipitation was attained. Neutralization and reprecipitation with the acid was twice repeated.

After the last acid precipitation, the supernatant was made alkaline and precipitated with alcohol. The precipitate was dissolved in H_2O and 2 volumes of saturated ammonium sulfate added, the scanty precipitate discarded and the sulfate removed by dialysis against distilled water. The solution of polysaccharide in the dialyzing sack was precipitated with 1.2 volumes of alcohol. After resolution in H_2O and precipitation with acetone, it was washed with alcohol and ether, and dried *in vacuo*.

A pure white product was thus obtained. The substance, while exhibiting a strong reaction with the Molisch reagent, gave negative

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1 Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

2 Enders, J. F., and Wu, C. J., *J. Exp. Med.*, 1934, **60**, 127.

3 Ward, H. K., *J. Exp. Med.*, 1932, **55**, 519.

TABLE I.

Carbohydrate used for absorption	Material used for testing supernatant of absorbed serum	Antipneumococcus type III horse serum absorbed with dilutions of carbohydrates					
		1/500	1/2,000	1/8,000	1/20,000	1/40,000	1/100,000
Carbohydrate H *	Carbohydrate H	—	—	—	—	±	++
SSS III	" "	++	+±	++	+++	+++	+++
Carbohydrate H	SSS III	—	—	—	—	±	++
SSS III	" "	—	—	—	±	++	+++
Carbohydrate H	Unabsorbed type III antiserum	+++	+++	++±	++	±	—
SSS III	Unabsorbed type III antiserum	++++	+++±	+++±	++	+±	+

*The preparation of specific substance described in this paper is thus designated for purposes of convenience in the tabulation of results.

biuret and Millon tests. The material precipitated only with antipneumococcus type III serum.

Like SSS III prepared according to the procedure of Heidelberger and his associates[4] the substance is precipitated from solution by $CuSO_4$, $BaCl_2$ and acetic acid. With all these reagents, SSS III gives a finely divided precipitate with persistent turbidity of the supernatant, while our substance forms large, heavy floccules, leaving at once a water clear supernatant. In a series of buffer solutions of varying pH, SSS III flocculates at pH 1.02 to pH 1.42, whereas at these hydrogen ion concentrations the new product reveals only a slight opalescence. After 3 days in these buffers it, too, exhibited a slight flocculation. This may well indicate a chemical change brought about by the high hydrogen ion concentration, leading to the formation of a substance similar, if not identical, with SSS III.

Our product contains 0.30% nitrogen. In this respect it resembles the material described recently by Heidelberger, Kendall and Scherp,[5] while the present work was in progress, but it apparently differs in its serological behavior, although this may not indicate a fundamental difference. Heidelberger's recent preparations, although precipitating more protein from antipneumococcus type III rabbit serum, nevertheless throws down about the same quantity as SSS III from antipneumococcus type III horse serum. The product which we have obtained, however, behaves differently from these preparations and from SSS III in its capacity to unite with homologous antibody in antipneumococcus horse serum. Thus, if such a serum be absorbed with a quantity of SSS III sufficient to remove all homologous antibody and appear in excess in the supernatant after removal of the precipitate, the supernatant, when brought in contact with the new product, will again yield a precipitate. The results of an experiment demonstrating this fact are recorded in Table I. The procedure was as follows: to each of one series of tubes containing 0.2 cc. of antipneumococcus type III horse serum was added 0.1 cc. of falling dilutions of SSS III and to another series, 0.1 cc. of dilutions of the new material. After thorough mixture, an overnight period at about 20°C. and centrifugation, portions of the supernatant from each were tested (ring test) with 1:1,000 dilution of both carbohydrates and with unabsorbed anti-serum to reveal any excess of the carbohydrates in the supernatant absorbed serum.

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[4] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

[5] Heidelberger, M., Kendall, F. E., and Scherp, H. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 188.

Certain other immunological properties of the material are now being studied.

8517 C

Grouping of Hemolytic Streptococci Isolated in Puerto Rico.*

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While studying the biological properties of hemolytic streptococci isolated from different sources in this Island, we grouped 46 strains using the precipitin reaction described by Lancefield.[1]

All extracts were first tested against group A serum. Those that gave a positive test were not tried against the other sera because cross reactions have never been observed with particular A antiserum.[2] Those that gave a doubtful or a negative result were tested in the same B, C, D, E, F, G and H antisera.

Out of 11 strains from diseased tonsils, 7 belonged to group A, 2 to group C and 2 remained unclassified. All the strains isolated from chronic discharging lesions of the face, from abscesses, from otitis media, from osteomyelitis and from the throat of patients with a diagnosis of scarlet fever were group A. Strains M_6 and M_7, isolated from the throat of cases of agranulocytic angina and rheumatoid arthritis respectively, remained unclassified, but they were not group A.

Of 15 strains from cases of recurrent lymphangitis, 14 were group A and one, group G. Strain D_1, from eczematoid dermatitis, belonged to Group C. Of 2 strains from septicemia, one was group A and the other, G.

Of the 46 strains tested, 37 were group A, 3, group C, 2, group G, and 4 remained unclassified.

* Group sera (A, B, D, E, F, G, H) were kindly sent to us by Dr. R. C. Lancefield.

1 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

2 Lancefield, R. C., personal communication.