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# The Specific Carbohydrate from Pneumococcus Type VIII.

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Following previous work on the specific carbohydrates of the pneumococcus, a study of preparations from a type-VIII strain has been undertaken in order to determine differences in activity and chemical composition. This culture was isolated in 1930 from pneumonic sputum and has since been maintained at a maximum virulence by frequent mouse passage. It is agglutinated by type-III antiserum in low dilution and by type-VIII in high dilution.

Specific carbohydrate was prepared from the cellular sediment and also from the supernatant fluid of from 16- to 20-hour cultures grown in an infusion-free peptone broth[1] which contained 0.2% cane sugar. The cellular extracts were purified by methods similar to those reported for type-I pneumococcus.[2] The soluble specific substance was best removed from the broth concentrates by precipitation as the barium or calcium salt and by repeated alcoholic precipitations. Purification through the calcium salt resulted in the purest product yet obtained, but the yield was low.

The reactions which distinguished the cellular carbohydrate from the soluble specific substance of the type-I pneumococcus failed to indicate any difference in the type-VIII fractions prepared as described. Although both fractions induced purpura, neither was observed to have immunizing activity in mice; furthermore, precipitation tests with adsorbed sera showed no difference between them.

The type-VIII soluble specific substance was readily soluble in water and gave an acid reaction. It passed through collodion membranes rapidly but through cellophane slowly and only after prolonged dialysis. Analysis of one preparation gave 0.19% nitrogen, 0.06% phosphorus, 0.70% ash, and 3.90% moisture. The specific rotation was about +126°. Before hydrolysis there was no reduction of Fehling's solution, but, when boiled for 4 hours with 10% sulfuric acid, the polysaccharide yielded 69.5% of reducing sugars calculated as dextrose. The Molisch test was positive in a 1:2500 dilution and the naphthoresorcinol in a 1:100. In the latter

[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1931, **21**, 245.

[2] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

concentration, the ninhydrin, biuret, and xanthoproteic-acid tests were negative. The soluble specific substance was readily precipitated by basic and neutral lead acetates, calcium chloride, barium hydroxide, silver nitrate, mercuric and mercurous nitrates but not by copper sulfate, uranium nitrate, ammonium sulfate, tannic acid, picric acid, phosphotungstic acid, nor trichloracetic acid.

The highest dilution of type-VIII soluble specific substance which gave precipitation with type-VIII antipneumococcus serum was 1:4,000,000 and with type-III antiserum 1:2,000,000. The homologous reaction, however, was the stronger. This precipitate tended to be more voluminous and floccular, except in high concentration, while the heterologous precipitate was compact and transparent. When a type-VIII antiserum was adsorbed with type-III soluble specific substance, it still precipitated with the type-VIII carbohydrate in the same dilution as before adsorption; but, when adsorbed with the latter, it failed to precipitate with either. Also, type-III soluble specific substance removed from type-III antipneumococcus serum the precipitins for both types III and VIII soluble specific substance, but type-VIII carbohydrate removed only the homologous precipitins. When tested qualitatively for complement-fixing activity, type-VIII soluble specific substance, in 1:100,000 dilution, reacted with type-VIII antiserum from the rabbit but not with type I, II, or III. The purpura-inducing activity in mice of a preparation of type-VIII cellular carbohydrate was partially neutralized by types III and VIII antipneumococcus sera from the rabbit and was intensified by these two antisera from the horse. Treatment of this carbohydrate solution with pepsin or trypsin did not affect the purpuric activity. The type-VIII specific carbohydrate induced fatal anaphylactic shock in guinea pigs which had been passively sensitized with the homologous antipneumococcus serum from the rabbit but not from the horse.