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Cross-Reactions of Streptococcal Group N Teichoic Acid in Antipneumococcal Horse Sera of Types VI, XIV, XVI, and XXVII

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The group antigen of *Streptococcus lactis* (group N) is an intracellular teichoic acid. It contains glycerophosphate and galactose phosphate, and the latter appears to form part, at least, of the immunological determinant reactive with group N antiserum (S. D. Elliott, Nature **200:** 1184, 1963). Cross-reactions occur among teichoic acids, and we now record a series of cross-precipitations between the group N substance (designated strep N) and considerable portions of the antibodies to four different pneumococcal (Pn) capsular polysaccharides (designated S, with appropriate numeral). In two instances the reactivity was anticipated, whereas in the other two the cross-reactions permit deductions concerning the possible constitution of the Pn type-specific substances.

The group N streptococcal teichoic acid was prepared from strain C559 ("Orla Jensen") obtained from R. C. Lancefield, as were also anti-N rabbit sera used in this study. Cultures were grown for 18 hr at 37 C in 20-liter amounts of dialysate broth made from Pfanstiehl peptone and containing 1% of dextrose. Part of the teichoic acid was liberated into the culture supernatant fluid from which it could be precipitated by the addition of $(NH_4)_2SO_4$ to 0.8 saturation. The serologically active material thus obtained was partially purified as already described, and contained, in addition to an undetermined amount of glycerophosphate, 7% galactose (mainly as galactose phosphate), 4.2% phosphorus, and about 30% nucleic acid. Some polyglycerophosphate was also present. The strep N was used without further purification, especially as polyglycerophosphate (M. McCarty, J. Exptl. Med., **109:**361, 1959) precipitated only anti-Pn XVI and weakly (Table 1).

Quantitative data are given in Table 1 and are discussed below in terms of the quantitative theory of immune precipitation proposed by M. Heidelberger and F. E. Kendall (J. Exptl. Med. **61:**563, 1935). It is clear from the footnotes to the Table that at least with types VI and XIV, for which highly purified polysaccharides were available, the antibody precipitated by the N substance was cross-reactive type-specific antipneumococcal globulin and not antiteichoic acid. Had it been the latter, the sum of anti-strep N and anti-S would have exceeded the known homologous anti-S content of the sera. This appears to be true for the other two types as well.

Cross-reaction with anti-Pn VI. This was considered a possibility because of the known structure of S VI (R. A. Rebers and M. Heidelberger, J. Am. Chem. Soc. **81:**2415, 1959; **83:**3056, 1961) and that attributed to strep N (S. D. Elliott, Nature **200:**1184, 1963). The observed cross-precipitation could be due either to the presence of multiples of D-galactose phosphate in both S VI and strep N (in S VI, the PO_4 is linked to the 2 position) or to multiples of nonreducing endgroups of D-galactose, as such groupings in polysaccharides were shown to react in anti-Pn VI sera (M. Heidelberger and P. A. Rebers, J. Bacteriol. **80:**145, 1960). Both of the available anti-Pn VI horse sera gave precipitates (in one instance 23% of the antibody), and the extent of reaction of each was in accord with previously noted relative reactivities toward polysaccharides containing terminal residues of D-galactose.

Cross-reaction with anti-Pn XIV. Precipitation in this antiserum has the same chemical basis as in anti-VI, except that the most likely relevant antigenic determinant in S XIV consists of nonreducing endgroups of D-galactose. Since S XIV also contains 1,3-linked D-galactose, such residues in strep N might reinforce the effect of any terminal groups. Pn VI and Pn XIV cross-react in both directions. The cross-precipitation of strep N in antisera to both of these Pn types strengthens evidence given earlier that the galactose in strep N is at least partly the D isomer.

Cross-reactions with anti-Pn XVI and anti-Pn XXVII. According to R. Brown (J. Immunol. **37:** 445, 1939), to whom we are indebted for these

TABLE 1. *Cross-reactions of streptococcal group N teichoic acid in antipneumococcal horse sera*[a]

Substance	Amt used	Antibody nitrogen precipitated at 0 C from anti-Pn type				
		VI 681C[b]	VI 771C[b]	XIV 635C[b]	XVI 594C[b]	XXVII 668C[b]
	μg	μg	μg	μg	μg	μg
Homologous polysaccharide[c]		690	760	910	900	260
Strep N	300					96[i]
	400	147[d]			164	
	600		46[e]			100[i]
	800	164[d]		89	262[h]	
	1,200		48[e]			
	1,600			143[f]	290[h]	
	3,000			171[g]		
Xanthomonas campestris	100					63[j]
	200					70[j]
Rhizobium radicicolum	60					51[k]
	200					62[k]
Physarum polycephalum	50				4	
	100				11	
	200				9	
Streptococcus group A polyglycerophosphate	60				5	
	120				5	

[a] Calculated to 1.0 ml of antiserum.

[b] Absorbed with pneumococcal C substance. Serum 681C from which oxidized *Shigella dysenteriae* polysaccharide (Heidelberger, Rao, and Davies, Pathol. Microbiol. **28**:691, 1965) had precipitated 48 μg of antibody N gave 139 μg of N with strep N.

[c] At maximal precipitation.

[d] Supernatant fluids plus S II at the 40-μg level gave 17 μg of N instead of 21 as in intact serum; plus S VI gave 522 μg of N; total, 677.

[e] Supernatant fluids plus S II at the 100-μg level gave 92 μg of N instead of 155 as in intact serum; plus polysaccharide of *S. dysenteriae* at the 50-μg level gave 108 μg of N instead of 127; plus S VI gave 654 μg of N; total, 701.

[f] Supernatant fluids plus anthrax (Smith) gave 57 μg of N; intact serum gave 219 μg of N.

[g] Supernatant fluids plus S XIV gave 740 μg of N; total, 911.

[h] Supernatant fluids from the 290 μg precipitate plus S XVI gave 452 μg of N; total, 742. Supernatant from the 262 μg precipitate plus *Physarum* gave 13 μg of N; supernatant fluids from this plus S XVI gave 465 μg of N; total, 740.

[i] Supernatant fluids plus polysaccharide of *X. campestris* at the 200-μg level gave 58 μg of N; plus S XXVII gave 168 μg of N; total, 266.

[j] Supernatant fluids plus *Rhizobium radicicolum* at the 100-μg level gave 12 μg of N; after both absorptions, strep N gave 87 μg.

[k] Supernatant fluids plus strep N gave 91 μg of N.

polysaccharides, S XVI and S XXVII contain 2.8 and 3.1% of P, respectively. Using Brown's preparations, Z. A. Shabarova, J. G. Buchanan, and J. Baddiley (Biochim. Biophys. Acta **57**:146, 1962) obtained chromatographic evidence of the following in S XVI: galactose, glucose, rhamnose, glucosamine, galactosamine, and glycerophosphate; in S XXVII, galactose, glucose, rhamnose, glucosamine, and phosphate. One might therefore reasonably ascribe the large cross-reaction of strep N in anti-Pn XVI to similarly spaced residues of D-galactose or D-galactose-PO_4, or both, with possible reinforcement by glycerophosphate; the reaction in anti-Pn XXVII would be due to D-galactose or D-galactose-PO_4, or both. In both instances, one-third of the antibody was involved. The cross-reactions also make it probable that much of the galactose, at least, in both S XVI and S XXVII is the D isomer.

The data in footnote *h* of Table 1 show that excess strep N or an inhibitor partially inhibits precipitation of S XVI and anti-Pn XVI. From

footnotes *j* and *k*, it is evident that the polysaccharides of *Xanthomonas* and *Rhizobium* precipitate a fraction of the antibodies to S XXVII different from that reactive with strep N. The first of these substances contains D-glucose, D-mannose, D-glucuronic acid, pyruvic acid, and *O*-acetyl (J. H. Sloneker, D. G. Orentas, and A. Jeanes, Can. J. Chem. **42**:1261, 1964), whereas the second is made up of D-glucose and D-glucuronic acid (E. Schlüchterer and M. Stacey, J. Chem. Soc., p. 776, 1945). S XXVII does not appear to contain glucuronic acid, so that the cross-reactivities in question are probably due to multiples of similarly linked residues of D-glucose.

S XVI was the only polysaccharide of the above types to show marked cross-reactivity in anti-strep N sera. Rabbit antiserum R2013, which precipitated 840 μg of nitrogen with the current preparation of strep N, gave a maximal value of 21 μg of nitrogen with 30 μg of S XVI.

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