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Precipitating Cross-Reactions Among Pneumococcal Types

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Data accumulated over many years are brought together on cross-reactions of 46 among the more than 80 pneumococcal serological types, with the idea of correlating cross-reactions with the structures of the relevant type-specific capsular polysaccharides, insofar as these have been determined. The precipitin reaction was carried out with the polysaccharides and antibodies raised in horses, rabbits, and a mule. Quantitative values (micrograms of antibody nitrogen per milliliter of antiserum at 0 to 1°C) are given in many instances and discussed, together with arbitrary qualitative data, in terms of the known structures of the polysaccharides. Some precise relationships are uncovered, and an attempt is made to determine why some of the cross-reactions are reciprocal and why others are only unilateral.

Even before the structures of any of the capsular type-specific polysaccharides of *Streptococcus pneumoniae* (pneumococci) were known, unilateral and reciprocal cross-reactions between the many serological types were recorded (46, 58) as a result of attempts to raise type-specific diagnostic antisera in rabbits.

The development and use of purified microbial polysaccharides as vaccines are presently expanding. There is also anticipation that demonstrated cross-reactivities might afford some measure of cross-protection. These considerations and the rapidly increasing knowledge of the structures of the relevant polysaccharides prompted this collection of published and hitherto unpublished data accumulated over the years. Cross-reactions among the pneumococcal (Pn) types, as demonstrated by the precipitin reaction, are shown in the tables. Data, mainly quantitative, from earlier papers have been added to make the tables more complete and because recently identified chemical structures of the Pn capsular polysaccharides have made some of the previously described cross-reactions more explicable. Quantitative values of antibody nitrogen precipitated are given for most of the more marked reactions. Data are given for cross-reactions of 46 serological types of *S. pneumoniae* with anti-Pn sera of 36 types. Cross-reactivity was measured by precipitation of antisera by type-specific capsular polysaccharides. Although the precipitin reaction is relatively insensitive, this was considered an advantage since positive results might be all the more significant in relating chemical structure to immunological specificity, the main objective of the study. Predictions of structural features of Pn polysaccharides of unknown structure could often be made from cross-reactivity in antisera to types for which the structure of the capsular polysaccharides was known. In most instances these predictions were verified by subsequent studies.

The cross-precipitations are assembled in Table 1, and the reciprocal ones are brought together in Table 2 and treated separately. Insofar as possible, the results are discussed in relation to the chemical structures of the type-specific capsular polysaccharides involved as dominant immunogens in the raising of antisera and as antigens in the cross-reactions.

MATERIALS AND METHODS

All tests were carried out at or near 0°C to obtain maximal values. My co-workers and I have repeatedly called attention to the usually larger differential shown by cross-reactions between 0 and 37°C than is observed with homologous precipitation. This is because cross-reacting antibodies usually combine with only a portion of the repeating unit of a polysaccharide, sometimes a single sugar. It therefore is not surprising that cross-precipitation at low temperatures does not always parallel immunogenicity in humans or other animals at 37°C or higher (cf. reference 41). An extreme example of the difference in precipitation between 0 and 23°C is given in reference 19.

Methods used and sources of most of the polysaccharides and antisera have been described in the papers cited. Antisera containing appreciable amounts of antibodies to the group-specific C-polysaccharide were absorbed with this polysaccharide before use; this is denoted by the letter C after the number of the serum. Qualitative tests were allowed to stand in a cold room at 3 to 5°C for 6 days or more and were read on a scale of − to ++++; quantitative determinations were placed in a cold bath at 0 to 1°C for 2 days to 2 weeks or longer, depending on the rapidity of precipitation. Qualitative data are given within parentheses

when carried out in antisera which were negative or weakly positive in other tests. Quantitative figures within parentheses refer to single analyses instead of the usual mean of duplicates. The following abbreviations are used: S, specific capsular polysaccharide; Gal, galactose; Glc, glucose; Rham, rhamnose; Man, mannose; GalA, galacturonic acid; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; dp, depyruvylated. Except as indicated, the antisera used had been raised in horses. All sugars are in the pyranose form unless indicated as furanoses by f, as in Galf.

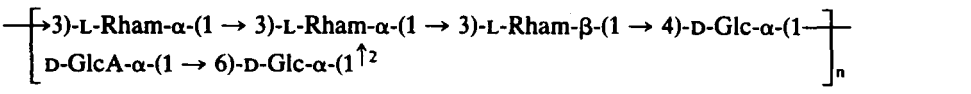
RESULTS AND DISCUSSION

Cross-precipitation of other Pn type-specific polysaccharides in anti-Pn1. S3 gave small precipitates in three of the four equine anti-Pn1 sera tested: 27 μg of antibody nitrogen per ml at 0°C was the largest amount, with 884C. S33 and S10A (34 U.S.) precipitated 10 and 27 μg of N, respectively, from 1057C. Although the significance of these small reactions is not clear, a D-Galf residue of S10A (11, 50) might fit into antibody spaces designed for S1, the structure of which is given (44) as:

$$\text{-}\alpha\text{-}(1 \rightarrow 3)\text{-D-GalA-}\alpha\text{-}(1 \rightarrow 4)\text{-D-GalA-}\alpha\text{-}(1\text{-})_n$$

with the repeating unit beginning at 3)-2-acetamino-4-amino-2,4,6-trideoxy-D-Gal.

S2, S5, S6, S6B, and anti-Pn sera (references 17, 18, and 28). The structure of S2 is (37):



Accordingly, there is no 1,4,6-linked Glc, as previously thought, nor is there GlcA in the main chain, so the discussion of the Pn2-Pn20 cross-reaction in reference 30 requires revision. The immunodominant group of S2 is the isomaltouronic side chain (29), making it possible that the phospho-α-D-glucosyl residue of S20 (52a), with or without the adjacent α-1,6-linked D-Glc, might fit partially into combining sites on anti-S2 molecules designed for insomaltouronic acid. As S18, like S2, has Glc in 1,4- and 1,6-linkages and 1,3-linked L-Rham in addition (16), it is not surprising that S2 precipitates anti-Pn18. The reverse reaction does not occur with the anti-Pn2 sera available.

A tentative structure for S5 retains the 1,2-linked D-glcA (B. Lindberg et al., unpublished data). Although precipitation of anti-Pn5 by S2 is appreciable (18), S5 failed to react in the anti-Pn2 sera at hand.

S6B differs from S6 only in its 1,4-linkage between L-Rham and ribitol (36); in S6 the connection is 1,3- (52). If the specificities are determined almost entirely by the sugars, this would explain why S6 and S6B precipitate practically the same amount of antibody from anti-Pn6. Otherwise S6B cross-reacts very weakly (+) only in anti-Pn28 and therefore is omitted from Table 1. Reciprocal cross-reactions of type 2 in anti-Pn19, anti-Pn20, and anti-Pn23 are given in Table 2 and discussed in a separate section, as are the other reciprocal reactions encountered.

S4 and dp S4. Removal of pyruvic acid from its acetal linkage on positions 2 and 3 of D-Gal (43) in S4 occurs without marked degradation, but dpS4 precipitates far less antibody (21) from anti-Pn4, showing that the 2,3-pyruvyl-D-Gal is immunodominant. However, dpS4 is more cross-reactive than S4 (Table 1), owing to the liberation of two hydroxyl groups of the Gal. Moreover, dpS4 precipitates C-reactive protein and antibodies to Pn C-polysaccharide almost quantitatively (33). *N*-Acetyl-D-galactosamine is the sugar common to PnC and dpS4, but the extent of the reaction is unusual if only a single sugar in the repeating units is actually the cause. The 1,4-linked D-Gal (32) in dpS4 presumably is responsible for the acquisition of cross-precipitation in anti-Pn8: intact S4 does not precipitate. Reactivity of both forms in anti-Pn14 probably occurs because the 1,3-linked *N*-acetyl-D-galactosamine of S4 (34) fits partially into antibody sites designed for 1,3-linked D-Gal.

S7 and S7B. Although the structure of S7 is not yet fully known, nonreducing lateral end groups of D-Gal in the repeating unit have been identified (10), as surmised in reference 30 and predicted in reference 56 because of the cross-reaction of S7 in anti-Pn14. Precipitation in anti-Pn19 is probably due to the 1,4-linked D-Glc, as well as to 1,2-linked L-Rham in S19 (47) and S7. The long-known reaction of S7B in rabbit anti-Pn24 (7) was confirmed. Several other weak cross-reactions were observed (Table 1). The structure is not known.

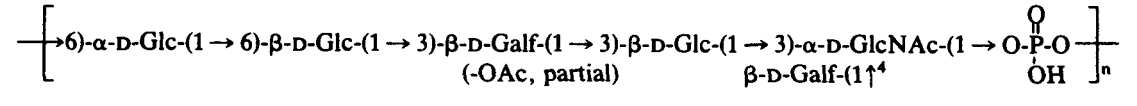


TABLE 1. Cross-precipitation of Pn capsular polysaccharides in anti-Pn sera

Polysaccharide[a]	Cross-precipitation[b] in anti-Pn:										
	1	2	3	4	5	6	7	8	9	10	11
Homologous	1,024	4,000	600	2,390	4,060	724	893	1,288	1,655	864	792
S1		++	+	+±	−	±	−	±	−	±	−
2	(−)		+	−	63[d]	21[e]	±	±	±	3[f]	±
3	++±	+		±	−	+	±	205	±	±	−
4	0	−	−		−	−	±	−	±	±	−
dp4	26	++	20	765	±	10	+	70	++	++	+
5	+	0[d]	+±	+±		−	−	58	−	++	−
6	(−)	0[e]	(−)	(−)	(−)		(±)	(−)	(−)	(±)	(±)
7	(−)	(−)	(−)	(+)	(−)	(−)		62	±	(−)	+
7B (48 U.S.)	±	±	+	+	−	−	+	−	−	−	−
8	(−)	(−)	113	(+)	(−)	(−)	(−)		(−)	(−)	(−)
9	−	−	+	±	−	−	−	9		−	−
9A (33 U.S.)	(−)	(++±)	(−)	(±)	(−)	(−)	(−)	(−)	(++++)	(−)	
10	[	(−) to (±)	]	(++±)	[		(−) to (±)			]	−
10A (34 U.S.)	++±	+	(−)	(±)	(±)	(−)	+	++±	(−)	51	(±)
11	(±)	(−)	(−)	(−)	(−)	++	(±)	(−)	(−)	(−)	
11A (43 U.S.)	++[j]	−	(−)	++±	(−)		(−)	(−)	(−)	(−)	125
12	(±)	(−)	(±)	+	(±)	+	+	(−)	(−)	−	−
12A (83 U.S.)	−	±	±	−	−	++±	−	−	++±	−	−
13	−	−	−	−	−	+±	−	−	−	25	−
14	±	±	−	±	±	17[e]	35[f]	−	−	79[f]	−
15	−	−	−	(78)	−	+	−	±	+	62	(+±)
15A (30 U.S.)	−	−	−	(77)	−	−	−	−	−	28	−
15B (54 U.S.)	−	−	±	++±	−	−	−	−	−	−	(−)
15C (77 U.S.)	+±	±	++	+++	±	±	+±	++	−	++	±
16	(−)	(−)	(−)	(±)	(−)	(−)	++	(−)	(−)	(−)	(±)[k]
18	−	±	−	−	−	10	7[f]	47[f]	−	+	−
18A (44 U.S.)	−	−	−	−	−	−	−	−	±	±	(++)
18C (56 U.S.)	±	−	−	++	−	±	−	−	−	−	−
19	(+±)	20[f]	(+±)	(−)	(−)	(−)	70[f]	105[f]	(−)	(−)	−
19A (57 U.S.)	+	−	++	++	+	+±	−	++	−	+±	
20	±	56[f]	−	+	−	±	−	+	+	163[f]	−
22	+±	++±	−	−	−	−	−	+±	−	++±	−
23	(−)	7	[			(−) to (±)				]	−
24	−	−	±	47[m]	++±	−	−	68	−	+	−
24A (65 U.S.)	−	−	−	−	−	−	−	−	−	−	−
25	±	−	−	++±	+±	−	+	+++	−	43	−
27	−	−	−	140[n]	−	−	−	−	−	11[o]	(−)
28	−	++±	−	−	−	±	−	−	−	+	(−)
29	−	±	−	±	−	++	−	−	++	61	(−)
32	(−)	(−)	(−)	(+±)	(−)	(±)	(±)	(+±)	(±)	(−)	(±)
32A (67 U.S.)	−	±	++	±	−	±	+±	±	+	±	−
37	[				All (−) or (±)					]	±
72	[								All − or ± through 15		

S9 (Danish 9N). A tentative structure, possibly overly complex, has been proposed for S9 (14). Simpler repeating units were shown to occur in other members of the group i.e., in 9A (type 33 U.S.) (6) and 9V (type 68) (49). Cross-reactions within the group are discussed in reference 55: D-GlcA was immunodominant in 9A, 9V, and 9L since reduction of -COOH to -CH_2OH greatly diminished serological activity. In the present study, only small cross-reactions of 9N were found with anti-Pn's 3, 8, 12, 14, 18, 19, 22, and 25. S9 and the capsular polysaccharides of all of these types except possibly S25, the structure of which is not known, contain 1,4-linked D-Glc in their repeating units. This may be the cause of these minor precipitations.

S10 and S10A (34 U.S.) The structure assigned for S10 (M. B. Perry, V. Daoust, and R. Lowe, Abstr. W.H.O. 3rd Int. Conf. on Immunity, Immunization, and Cerebrospinal Meningitis,

TABLE 1—*Continued*

	12	14	15	16	18	19	20	22	23	25	27	28	29
	1,240	1,010	770	872	2,200	2,250	355	878	420	620	277	785	389
1	−	+	±	±	±	−	−		−	3[c]	+	−	+±
2	+	+	−	±	−	8[f]	44[f]	++	+±	++	−	−	+±
3	±	±	−	−	±	−	−	−	−	±	−	−	±
4	5	65	−	−	−	−	−	++±	+	16[c]	18[g]	−	++
4	±	57	−	25	−	++	29	++	++	8[c]	25[g]	±	+±
5	++	+	±	−	−	+±	−	−	±	±	−	−	−
6	(−)	15[e]	(−)	(−)	+±	±	±	±	−	(±)	(−)	(−)	++
7	+±	34[f]	(−)	(−)	91	2[f]	+±	(−)	22	(++±)	(±)	++	(±)
7B	±	+	±	−	++	±	±		−	++	±	−	−
8	−	(−)	−	−	14[h]	127[f]	(−)	(±)	(−)	+	−	(−)	(−)
9	+	11[i]	−	−	2	(−)	−	10	±	++	−	−	
9A													
10	−	15[f]	++	++±	−	±	35[f]	±	−	49[c]	86	±	57
10A	(±)	++±	(−)	(±)	(−)	(±)	(+)	(±)	(−)	12[c]	5	−	20
11	−	(−)	448	+	−	±	−	+	−	±	±	−	−
11A	(−)		(682)	++++	278	(−)	(−)	−	−	++±	−	−	+±
12		< 45	−	−	(−)	(±)	(−)	(−)	(−)	(±)	(−)	(−)	−
12A	++++	±	+	−	±	±	−		−	−	−	−	−
13	−	+±	−	−	−	−	++	−	−	+±	−	−	−
14	(−)		(21)	(−)	−	+	−	+++	−	+±	−	−	++
15	(−)	47		(−)	57	(−)	++	−	−	++	+++	−	++
15A	−	++±	301	−	−	±	−	+	−	+±	95	−	−
15B	(−)	++++	293	(+)	+++	(−)	(+)	−	++	−	+	−	−
15C	++	+±	+++	−	−	±	−		−	++±	++±	−	++
16	(+±)	(−)	(−)		(−)	(±)	(+)		−	−	−	+±	±
18	−	−	++	+++		±	+		++±	+	−	±	−
18A	(±)	(±)	(−)	(++++)	357[f]	(±)	−		(±)	(−)	(±)	(±)	−
18C	−	−	−	150	1,685	−	−	−	++	−	−	−	−
19	±	±	−	±	−		+		−	−	−	−	±
19A													
20	±	±	−	±	+++	±			−	++	+±	−	−
22	±	−	−	−	−	+	±		++	(− or ±)			
23	±	±	−	±	−	+	−	(±)		(++)	(±)	(++)	(−)
24	−	++	−	±	−	−	−	−	±	+±	+	+	++
24A	−	++	−	−	−	±	−	−	109	++	−	189	−
25	±	−	−	±	−	±	−	(±)	(−)		(−)	(±)	(−)
27	(−)	(±)	(−)	(±)	−	±	+		+	±	123[p]	+	±
28	(−)	(±)	(−)	(+)	(−)	(±)	−		−	−	+±		++±
29		++	(−)	(±)	(−)	(−)	+		−	++	±	−	
32	(+)	(−)	(−)	(−)	(−)	28	(+)	(±)	(++±)	(−)	(++±)	(+±)	(±)
32A	−	++	−	+±	++±	−	±	−	134	++±	38	++	++±
37	34	(++)	(+)	(+)	(−)	(±)							
72			]	290	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)

[a] S17 was + only in anti-Pn7 and anti-Pn9; S17A was ++ in anti-Pn11 and + in anti-Pn's 12, 15, and 20. They are omitted from the table.

[b] Data are expressed either qualitatively (see text) or quantitatively (as micrograms of antibody N per milliliter).

[c] From reference 1.

[d] From reference 18.

[e] From reference 28.

[f] From reference 30.

[g] From reference 21.

[h] From reference 16.

[i] From reference 49a.

[j] In anti-Pn1 1057C; − in anti-Pn1 884.

[k] +++ in rabbit anti-Pn11 59 (New York State).

[l] From reference 22.

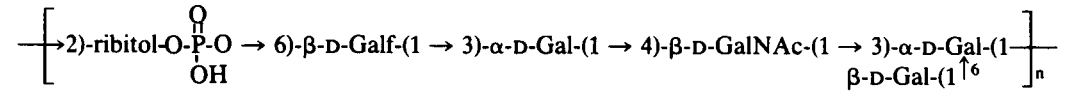
[m] Mean of 43 and 50.

[n] dpS27 gave 176 μg of N per ml.

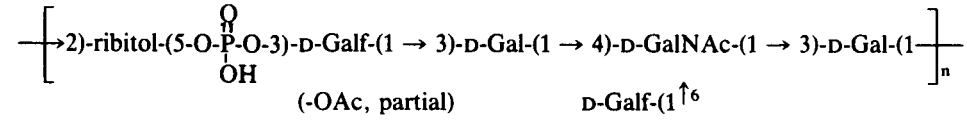
[o] dpS27 precipitated 45.

[p] Precipitation by dpS27.

Marburg, 1980) is:

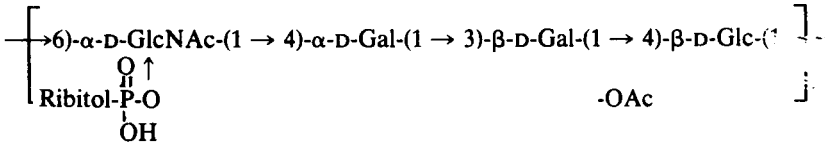


and that for S10A (11, 50) is:

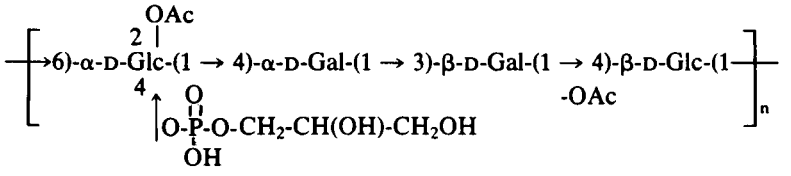


In spite of structural similarities, cross-precipitation of S10 in rabbit anti-Pn10A (R13 N.Y. State) was only about 80 of 1,965 μg of N per ml and 6% of the total in the reverse direction with H627. There were marked differences in the cross-reactivities of S10 and S10A in other antisera (Table 1). Rabbit anti-10A was tested only with S1, S8, S14, S25, S27, and S29, giving ±, +, ±, +±, −, and ++, respectively.

S11 and S11A (43 U.S.). The structure for S11 (M. B. Perry, personal communication) is:



and that for S11A (38) is:



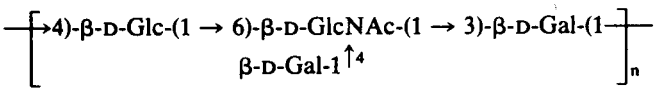
Removal of the acetyl groups from S11A abolished the precipitin reaction in a rabbit anti-Pn11A (38). The smaller-than-expected precipitation of S11A in anti-Pn11 613 (16%) is perhaps due to blocking of antibodies by the as yet unlocated -OAc groups in S11 and S11A. Both polysaccharides react heavily in anti-Pn15 528C, probably because of the -OAc sugar in their repeating units, since O-deacetylation of S15, as with S11A, abolishes its precipitation in homologous rabbit serum. Precipitation of S4, S25, and S29 in a potent rabbit anti-11A was +±, +, and +±, respectively. Precipitation by S11 in anti-Pn16 594C and anti-Pn18 495 was only + and −, respectively, but S11A gave ++++ and 278 μg of N per ml (Table 1). As pointed out in reference 38, S11A and S18 have the probable sequence →3)-D-Gal-(1→4)-α-D-Glc-(1→6)-D-Glc-(1- and also glycerophosphate and O-acetyl groups (16) in common, so that massive cross-reactivity is to be expected. The structure of S16 is not known.

S12, S12A (83 U.S.), and S13. The only marked cross-reaction of S12 was in anti-Pn14 635C and was predictable since both S12 (15, 42) and S14 (4, 45) have nonreducing lateral end groups of D-Gal in their repeating units. S12A precipitated anti-Pn12 very heavily and anti-Pn6 and anti-Pn9 only moderately. S12A differs from S12 only in the substitution of *N*-acetyl-D-glucosamine for *N*-acetyl-D-galactosamine in the main chain. (K. Leontein, B. Lindberg, J. Lönngren, and D. J. Carlo, personal communication).

S13 (57) gave only small cross-precipitations (Table 1). In anti-Pn6, reactivity might be due to a small amount of anti-ribitolphosphate in the antiserum; in anti-Pn14 and anti-Pn20, it might be due to 1,3-linked D-Gal.

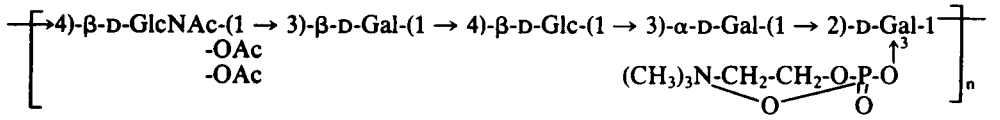
S14. New methods have established a simpler

structure for S14 (45):



Anti-Pn14 635C has been a useful reagent for detecting and predicting the presence of immunodominant lateral D-Gal in many other microbial and plant polysaccharides. Cross-precipitation of S14 in anti-Pn22 is apparently caused by the 1,4-linked D-Glc residues in the repeating units of S14 and S22 (9).

S15, S15A, S15B, and S15C. The structure of S15 (48) is:



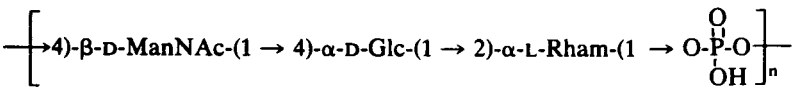
S15 and S15A precipitated anti-Pn4 609C to the same extent. Possibly the 1,3-linked D-Gal residues of S15 fitted partially into combining sites of anti-Pn4 designed for the 1,3-linked D-GalNAc residues of S4. The structures of S15A, S15B, and S15C have not yet been determined, but S15C has the same main chain as S15B but lacks the -O-acetyl group of the latter (P. E. Jansson, unpublished data). In this group, S15 reacted most heavily in anti-Pn10 627C, S15A and S15C precipitated less, and S15B did not react at all. The 1,3-linked residues of D-Gal in S15 and S29 are apparently the mediators of the reaction of S15 in anti-Pn29, whereas the 1,4-linked residues of D-Glc are undoubtedly the reason for precipitation of S15 in anti-Pn20 616. S15A and S15B precipitate anti-Pn15 to the same extent but differ sharply in anti-Pn14, anti-Pn18, and anti-Pn27. Because of the strong reaction of S15B in anti-Pn14, S15B may be expected to have a lateral nonreducing end group of D-Gal in its repeating unit; S15C gave only +. Although S15A precipitated anti-Pn4 609 quite strongly (Table 1), the reciprocal reaction in a potent rabbit anti-Pn15A was only +±. Precipitation of S4 in rabbit anti-Pn15B was only (±). S20, however, gave ++, but the reciprocal reaction was only (+).

S16. Small cross-reactions occur in anti-Pn7, anti-Pn12, and anti-Pn27, but the structure of S16 is not known.

S17 and S17A (78 U.S.). S17 and S17A are omitted from Table 1 because the strongest reaction was of S17A, +± in anti-Pn11 613. However, S17 (for structure, see M. B. Perry, D. R. Bundle, V. Daost, and D. J. Carlo, Can. J. Biochem. Cell Biol., in press) precipitated massively in a rabbit antistreptococcal group F, type 4 serum (31), as did the group F type 4 polysaccharide in rabbit anti-Pn17 (N.Y. State no. 29). S17A has several structural differences from that of S17 (P. E. Jansson et al., in press).

S18, S18A (44 U.S.) (22), and S18C (56 U.S.). Two alternative probable structures have been proposed for S18 (16). 1,4-Linked D-glucosyl residues in S18 and S23 are undoubtedly responsible for the cross-reaction of S18 in anti-Pn23. As the structures of S16, S18A, and S18C are not known, reasons for the heavy cross-precipitation of the S18 group in anti-Pn16 cannot be given.

S19 and S19A (57 U.S.). The structure of S19 (47) is:



In anti-Pn2 and anti-Pn7, S19 gave definite precipitates, but S19A (40, 41) did not (Table 1). Precipitation of anti-Pn8 1008 by S19 was much stronger than that by S19A, probably because of inhibition by the side chains of S19A on the 1,4-linked Glc. S19A reacted weakly in anti-Pn4, anti-Pn5, anti-Pn6, and anti-Pn10, in which S19 was negative. Except for 1,3-linked D-Gal in S10 and S19A, the reasons for the other reactions are obscure.

S20 (p. 1237) and S21. The strong precipitation of S20 in anti-Pn18 appears to be due to the 1,6-linked D-glucosyl and 1,3-linked D-galactosyl residues in the repeating units of both S18 and S20. The latter residues and those of 1,4-linked

D-Glc probably account for the weaker reaction in anti-Pn27. S21, − to ± in all antisera tested, is omitted from Table 1.

S22. As in other instances, the stronger reactions (Table 1) of S22 (9) could be due to the 1,4-linked D-linked D-Glc of the polysaccharides of the types involved.

S23. The weak reaction in anti-Pn2 might be caused by 1,4-linked D-Glc even though (or because) it is substituted by a phosphoryl group on S23 (Perry et al., Abstr. W.H.O. 3rd Int. Conf. in Immunity, Immunization, and Cerebrospinal Meningitis, 1980.) D-GlcPO_4 might even fit loosely into the combining sites of anti-Pn2 meant for D-GlcA.

S24 and S24A (65 U.S.) (composition and structures not known). S24 precipitated in anti-Pn4 and anti-Pn 8 (Table 1), but S24A did not; both reacted equally weakly in anti-Pn14 and nearly equally weakly in mule anti-Pn25. S24A was the only Pn S tested which precipitated strongly in anti-Pn28 510C, giving 189 of μg N per ml. It also gave 109 μg of N in anti-Pn23 912C; reciprocal reactions were negligible in the rabbit anti-Pn24A available.

S25. Precipitation was +± in anti-Pn5 and +++ in anti-Pn8. S25 contains D-GalA (13), but the structure has not been determined. Cross-reactions are shown in Tables 1 and 2.

S27. S27 (5) was the first Pn S in which pyruvic acid was found (21); because of the cross-reactivity of the extracellular polysaccharide of pyruvyl-containing *Klebsiella* type K32 in both anti-Pn27 and anti-Pn4, pyruvic acid was suspected in S4 as well and was identified (21).

S28. The structure of S28 is not known. Cross-reactions are given in Table 1.

S29. S29 has two residues of 1,6-linked D-Galf in its repeating unit (51), and these might function as somewhat hindered nonreducing end groups. This would explain the reactivity of S29 in anti-Pn6 and anti-Pn14. Both S29 and S14 have 1,3-linked D-galactopyranose in their repeating units. This could reinforce the precipitation in anti-Pn14 and account for that in anti-Pn9 as well, since S9 contains 1,3-linked D-GalNAc which would be partly equivalent immunologically to similarly bound D-Gal.

S32 and S32A (67 U.S.) (structures unknown). S32 contains Gal, Glc, Rham, and uronic acid (1) and does not precipitate as strongly in anti-Pn23 (a long-known cross-reaction [46, 58]) as does S32A (Table 1). The latter, at least, may therefore be assumed to have nonreducing lateral end groups of L-Rham in its repeating units (P. Allen, B. Prescott, and M. Heidelberger, unpublished data); phosphorylcholine is also a constituent. S32A reacts in anti-Pn's4, 14, 16, 18, and 25, whereas S32 does not, but gives 28 μg of N in anti-Pn19 631C. Both cross-reacted roughly equally in anti-Pn27.

S37. The only noteworthy cross-reaction of the unusual glucan S37 (39) (not tested in all antisera) was in anti-Pn12 296C, giving 34 μg of N per ml. S37 is said to have the nonreducing lateral end groups D-Glc-β-(1→2)-D-Glc-β-(1-, in contrast to the corresponding α-linked kojibiosyl of S12 (15).

S72. All tests of S72 were − to ±, except for heavy precipitation in anti-Pn16 594C (290 μg of N per ml). As structural formulas have not been proposed for S16 or S72 (12) and there are several sugars in common, an explanation is presently lacking.

RECIPROCAL CROSS-REACTIONS

Reciprocal, as well as unilateral, cross-reactions were often encountered in early efforts to obtain a reliable supply of strictly type-specific, diagnostic rabbit anti-Pn sera (46, 58). In this part of the present paper, an attempt is made to find out why some cross-precipitations between serological types are unilateral and others are reciprocal. One factor that might be involved is that different animals of a single species produce antisera mainly reactive toward different portions of the repeating units of bacterial polysaccharides (see, for example, reference 28). Therefore, if one could have a sufficiently large collection of potent antisera, possibly all cross-precipitations would be reciprocal. It is also likely that a target sugar in the main chain of the more highly branched or more highly O-acetylated of two polysaccharides would be relatively inaccessible to cross-reactive antibodies. This would tend to make a reaction unilateral. These considerations apply also to bacterial agglutination, especially as this is a precipitation reaction at the cellular surface (23).

Data on reciprocal cross-precipitations among the Pn types are summarized in Table 2. Except for certain intergroup reactions, those are omitted which are recorded as less than ++ in either direction. Types 2,5 and 2,6 are included, even though S5 and S6 failed to precipitate several anti-Pn2 sera, since types 5 and 6, originally 2A and 2B, were "weakly and incompletely agglutinated" (3) in anti-Pn2.

Types 2,5; 2,6; 2,19; 2,20; and 2,22. Reciprocal cross-precipitation of types 2,5 is undoubtedly due to the lateral nonreducing end groups of D-GlcA in the repeating unit of S2 and 1,2-linked D-GlcA in S5. Like 1,2-linked D-Gal in S6 (52), 1,2-linked D-GlcA in S5 may react serologically as a somewhat hindered lateral end group (18). The cross-reaction may also be reinforced by the 1,4-linked D-Glc in the main chains of both S2 and S5, a feature presumably also involved in the two-way 2,19 reaction. The rather small precipitation in the 2,6 pair is referable to the 1,3-linked L-Rham in the repeating units of both. It is difficult to account for the 2,20 pair unless

TABLE 2. Reciprocal cross-precipitation of pneumococcal types[a]

Polysaccharide	Antiserum	Polysaccharide	Antiserum
2[b]	V555 63, V606 79, VI681 21 VI771 155, XIX631 8, XX616 44, XXII566 ++	5, 6, 19, 20, 22	II464 +, II513 0, 20, 56, ++±
3	VIII1008 205	8	III792 113
4	RXXIV ++, MXXV 16, XXVII668 18[c]	24, 25, 27	IV609 50, ++±, 140[d]
5	RXXIV ++±	24	V555 ++
6	XIV635 15, XVIII495 +±, XXIX641 ++	14, 18, 29	VI 681 17, 10, ++
7	XIV 34, XVIII 91	14, 18	VII937 35, 7
8	XVIII 14, XIX 127, RXXIV ++±	18, 19, 24	VIII 47, 105, 68
10	RXA < 79, XIV 15, XV628 ++, RXVA, ++± XX 35, MXXV 49, XXVII 86, XXIX 57	10A, 14, 15, 15A, 20, 25, 27, 29	X627 51, 79, 62, 28 163, 43, 11[e], 61
10A	XXIX 20	29	RXA ++
11	RXIA +++	11A	XI613 125
11A	XV (682)[f], XVI594 +++±, XVIII 278	15, 16, 18	RXIA ++, +++±, ++
14	XV 21, RXVA +++	15, 15A	XIV 47, ++±
15	RXVA ++++, RXVB ++++, XVIII 57	15A, 15B, 18	XV 301, 293, ++
15A	RXVB ++++, XXVII 95	15B, 27	RXVA ?[g], +++
18	RXVIIIA 321[h], RXXXIIA +++[i]	18A, 32A[i]	XVIII 357[h], ++±

[a] Qualitative data are expressed on a scale of − to ++++; quantitative values are expressed as micrograms of antibody nitrogen per milliliter of antiserum. M, Mule; R, rabbit (antisera without these letters were raised in horses). Sera containing appreciable antibody to Pn group-specific C-polysaccharide were absorbed with C before use. In this table serum types are given in Roman numerals to avoid confusion with serum numbers and amounts of precipitated nitrogen.

[b] See text for reason why group pairs 2,6 and 6,2 are included.

[c] dpS4 gave 25 μg of N.

[d] dpS27 gave 176 μg of N.

[e] dpS27 gave 45 μg of N.

[f] Single determination only.

[g] Test was not made.

[h] From reference 22.

[i] P. Z. Allen, B. Prescott, and M. Heidelberger, unpublished data.

the glucose PO_4 of S20 could fit loosely into combining sites of anti-Pn2 designed for GlcA. As for types 2 and 22, the polysaccharides of both contain sugars bound in the same ways, yet the cross-precipitations are small.

Types 3,8. Since one-half of S8 consists of the repeating unit of S3 (35), heavy mutual cross-precipitation is to be expected. This long-known reciprocal cross-reaction has been extensively discussed (24, 25), as have also the inhibitory properties of oligosaccharides formed on partial hydrolysis of each S (8). The strong reciprocal cross-precipitation between types 8 and 19 may now be accounted for by the presence of α- and β-1,4-linked D-Glc (35) in S8 and by α-1,4-linked D-Glc and β-1,4-linked D-ManNAc (partially equivalent immunologically to D-Glc) in S19.

Types 4,24; 4,25; and 4,27. S24 and S25, of unknown structure, appear not to have been tested for pyruvic acid. This acid, however, has little to do with the reciprocal cross-reactivity of 4,27, as precipitation is greater with the dp polysaccharides (Table 1). A possible reason for the reaction is the partial serological equivalence of 1,4-D-ManNAc in S4 and 1,4-linked D-Glc in S27.

Types 5,24. There is no explanation for the reciprocal reactions of 5,24 at present.

Types 6,14; 6,18; and 6,29. The rather weak reciprocal cross-reactions of 6,14 are accounted for by the evidence (28) that the 1,2-linked D-Gal of S6 shows the serological behavior of a partially hindered lateral end group of D-Gal such as exists unobstructed in S14 (see above). The still weaker 6,18 reactions reflect the presence of 1,3-linked L-Rham in both polysaccharides. In the 6,29 pair, the two 1,6-linked D-Galf residues of S29 might fit loosely into antibody sites designed for the D-Gal of S6.

Types 7,14 and 7,18. Reciprocal cross-reactiv-

ity of types 7 and 14 is due to the nonreducing lateral end groups of D-Gal in the repeating units of S7 and S14, with probable reinforcement by the β-1,4-linked D-glucosyl residues present in both. The 7,18 pair also has 1,4-linked D-Glc and, in addition, 1,3-linked L-Rham.

Types 8,18; 8,19; 8,24. Each repeating unit of S8 and S18 has two 1,4-linked D-Glc residues; these appear to suffice for a limited degree of reciprocal cross-reactivity. In the much heavier cross-reactions of the 8,19 pair, it is possible that the phosphoryl-(1→4)-β-D-ManNAc residues of S19 might fit to some extent into anti-Pn8 combining sites intended for the cellobiouronic acid groups of S8, aided and abetted by the 1,4-D-glucosyl residues of S19. For the opposite direction, the cellobiouronic acid residues of S8 might fit into the anti-Pn19 combining sites for PO_4-ManNAc residues. The composition and structure of S24 are not known.

9A, 9L, 9N (9 U.S.), and 9V. Cross-precipitation among the members of this group has been studied quantitatively (55). Reciprocal crossing between 9A and 9N is massive, as would be expected from their similar main chains. Mutual cross-reactivity of the 9A,9L pair is almost complete, but the structure of 9L is not known. 9V has O-acetyl groups on GlcA and Glc; these probably give it an individual specificity since 9N does not precipitate the rabbit anti-9V used (55).

Types 10,10A; 10,14; 10,15; 10,15A; 10,20; 10,27; 10A,25; and 10A,29. S10, S10A, and S15 (see above for structures) have two 1,3-linked D-galactosyl residues, and S14 and S27 have one in their repeating units; these sugars appear to be principally responsible for reciprocal reactions 10,10A; 10,14; 10,15; and 10,27. In the 10,20 pair the nonreducing lateral end groups of β-D-Galf are probably the main cause, whereas S10A and S29 have the same furanose in the chain of sugars and 1,3-linked D-Gal in addition. The structures of S15A and S25 are not yet available.

Types 11,11A and 11A,15. The structures of S11, S11A, and S15 are given above. As all three polysaccharides contain the sequence 1,3-β-D-Gal-1,4-D-Glc in their repeating units, this accounts for the reciprocal precipitation in antisera to both pairs. Why the reaction of S11 and S11A in anti-Pn15 is so massive is not clear.

Types 14,15 and 14,15A. The reason for these reciprocal reactions appears to be the same Gal-Glc sequence as described above, since this also occurs in S14.

Types 15,15A; 15,15B; 15A,15B; 15,18; and 15A,27. S18 also has the 1,3-D-Gal-1,4-D-Glc sequence, but the anomeric forms of the sugars were not determined (16). A rabbit anti-Pn15A gave +++ with S27; S15A precipitated anti-Pn27 668C similarly (95 μg of N per ml).

Types 18,18A and 18,32A. S18A contains all components of S18 except the O-acetyl group and has GlcNAc in addition (22), but its structure is not known, nor is that of S32A.

Types 19,19A. This reciprocal cross-precipitation was demonstrated in reference 40. S19A differs from S19 in that it has two side chains which are lacking in S19 (40).

In summary, in most of the reciprocal cross-reactions between Pn types of which the structures of the capsular polysaccharides are known, at least two sugars or their immunologically partially equivalent amino analogs are present in common. This is not, however, a rigid requirement. In four of the pairs listed, a single immunodominant sugar, or a presumably equivalent combination, suffices to make the cross-reactions reciprocal. This seems reasonable. In only two instances, 2,6 and 6,8, probably due to unusually favorable steric factors, does reciprocal cross-reactivity appear to be due to a single non-immunodominant sugar in common. Accordingly, reciprocal cross-reactivity, like the nonreciprocal reactions, has an orderly structural basis. Where the chemical structures of the capsular polysaccharides are known, it should now be possible to predict the likelihood, but not the certainty, of reciprocal cross-reactivity.

The use of more sensitive methods than those in the current exploratory studies might make such prediction more certain. For example, as early as 1939 use of capsular swelling and agglutination (46) in rabbit anti-Pn sera revealed the following reciprocally cross-reactive pairs: 2,20; 3,8; 8,19; 10,20; 10,29; 13,17; 13,29; 18,28; 20,31; 22,31; and 23,32. In reference 58, the pairs 8,19; 10,20; 10,29; 16,28; 20,31; and 23,32 are recorded. No information on the complete structure of any Pn polysaccharide was available at that time.

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