# Cross-Reactions of Various Microbial, Fungal, Mold, and Yeast Polysaccharides in Antipneumococcal and Other Antisera

**Michael Heidelberger**†

*Contribution from the Department of Pathology, New York University Medical Center, 550 First Avenue, New York, New York 10016*

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**Abstract:** An immunochemical study of the cross-reactions of microorganisms has brought to light many relationships between chemical structure and immunological specificity. (A few instances are given in refs. 1–4.) Use of the relatively insensitive precipitin reaction under controlled conditions, the availability of antipneumococcal and anti-*Klebsiella* horse and rabbit sera, and the limitation of the studies to the relatively open polysaccharides as antigens greatly facilitated the accumulation of significant data. It was clearly shown that the order, linkage, and spatial arrangement of constituent sugars and other components of the polysaccharides were the dominant factors in the establishment of their specificities. In the present article, cross-reactions of numerous microbial polysaccharides are brought together because too few of these species were tested over some 30 years to warrant separate publication.

## Materials and Methods

Polysaccharides are listed in alphabetical order of the microbial species from which they were derived. Antisera had been raised in horses, unless otherwise indicated by R for rabbit on M for mule, and were generously given by the New York City and New York State Departments of Health Laboratories; anti-*Salmonella* sera were given by Anne-Marie Staub, then at the Pasteur Institute, Paris.

Qualitative and quantitative analytical methods have been described in earlier articles.[1-7] Qualitative data are given on a scale of – to ++++; when in parentheses, the antisera had been used previously with negative or weakly positive results: –, ±, +, or ++.

## Results and Discussion

*Achromobacterium mucosum* nov sp. (Names and numbers refer to polysaccharides of the bacterial species from which they were derived, not the bacteria.) A crude preparation was sent by Hideo Suzuki (Chiba City, Japan). It contained D-gal, D-glc, D-man, and D-glcA.[8,69] The strongest reactions were ++, ++, and ++±, in anti-Pn22, anti-Pn23, and anti-*Salmonella paratyphi* B, respectively. In the absence of structural data regarding *A. mucosum*, precipitation in anti-Pn22 and –23 is possibly due to the presence of 1,4-linked D-glc, as in PnS22[9,10] and PnS23,[11] while that in anti-*S. paratyphi* B may occur because of D-man in 1,4- or 1,6-linkage.[12]

*Acinetobacter* 23B3, differing from *Acinetobacter calcoaceticus* BD4,[13] was sent by E. Juni (Ann Arbor, MI). It consists of gal and glc. Its only cross-reaction greater than + was 49 μg of antibody nitrogen per milliliter of anti-Pn1 1057C which was +++ on the original qualitative test and difficult to account for unless the gal fits partly into antibody sites designed for 1,3- and/or 1,4-linked D-galA and/or the amino sugar of PnS1.[14]

*Arthrobacter viscosus* NRRL B1797 and 1973 and their deacetylated derivatives were from Allene Jeanes (Peoria, IL). 1973 was tested in all antisera listed in Table I except those of *Salmonella*. It gave no cross-precipitation stronger than +, nor did 1797 in the few tests made. Deacetylation, however, resulted in heavy precipitation of both in anti-Pn3 and anti-Pn8 (Table I), and small cross-reactions of dAc 1797 occurred in anti-Pn4 and –16. 1797 contains D-gal, D-glc, D-glcA (pyA), and Ac (Jeanes, A.; Dick, W. E., Jr. Personal communication); gal and glc are present linked 1,3 and 1,4, and one-half of the sugar

† Dr. Michael Heidelberger died on June 25, 1991, at the age of 103 years. He worked at the laboratory bench until a few weeks before his death. For several years, he had been preparing this manuscript for publication summarizing the work of many years, and the data will prove very helpful to carbohydrate chemists and immunologists. I have made a few clarifying footnotes and have checked a number of references and statements in the text. Dr. G. Jeanette Thorbecke arranged for the typing with the competent assistance of Natalie Little and also checked the manuscript. When this article is published, Dr. Heidelberger will have published in every decade of this century. His first two papers appeared in 1908 in the *Journal of the American Chemical Society*, and he wished his last paper to be published here as well. Any errors or misinterpretations of Dr. Heidelberger's ideas are my fault. Please address any correspondence to Elvin A. Kabat at Columbia University.

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hydroxyls are acetylated.[15,16] PnS3 is a polycellobiouronic acid with the units linked β-1,3;[17] one-half of the repeating unit of PnS8 is also cellobiouronic acid and the remainder is β-D-glc and α-D-gal.[18] As few of the type-specific antibody-producing Pn polysaccharides contain OAc, it is not surprising that the highly acetylated 1797 and 1973 show little cross-precipitation in the anti-Pn sera until their OAc is removed. Since dAc 1797 precipitates one-half of the anti-S8 in serum 1008, the anti-Pn8 used, one might predict that it will be found to contain the sequence →4)-D-GlcA-β-(1→4)-D-Glc-β-(1→4)-D-Glc-(1→, which makes up three-fourths of the repeating unit of S8. The reaction in anti-Pn3 is much smaller, perhaps owing to the 1,3-linkage between the cellobiouronic units of S3. dAc 1973 also reacts heavily in anti-Pn3 and -8: it not only contains β-1,4-linked D-glc but also a D-man analog of cellobiouronic acid, thus providing an additional instance of the partial immunological equivalence of D-man and D-glc (cf. ref 19), even extending to their corresponding uronic acids.

*Azotobacter chroococcum* was obtained from M. Stacey (Birmingham, England). The polysaccharide from a local strain consisted mainly of glc with about 4% glcA.[20] The former was mostly β-1,6-linked, with some α-1,4 attached to the glcA.[21] The weak reaction in anti-Pn2 indicates that this is not an unobstructed lateral end group and is probably due to 1,4-linked D-glc. Without further information, it is difficult to account for the cross-reactions listed in Table I.

*Bacillus polymyxa*, B.1828, from Allene Jeanes was tested in anti-Pn1–14,16,19,20 but is omitted from Table I as it gave + only in antiPn7 and ++ in anti-Pn9. The latter might be due to the sequence →3)-D-glcA(1→3)-D-man in the main chain of the repeating unit (cf. refs 19 and 22).

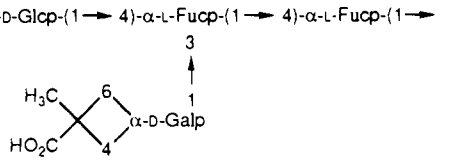
*Bacteroides asaccharolyticus*, a lipolysaccharide from B. Mansheim (Channing Laboratory, Boston, MA)[2] containing gal, glc, glcNH$_2$, and unidentified capsular sugars,[23] reacted ++± in anti-Pn2, -14, -27, and pTA, ++ in anti-Pn6, -10, -20, -23, -25, and K11, and +± in anti-Pn8, -19, and -29. Any discussion must await separation of the components.

*Bifidobacterium bifidum*, formerly *Lactobacillus bifidum*, was from R. Gyorgy (Philadelphia). The extracellular polysaccharide contains gal, glc, galA, and 6-deoxytalose.[24] The weak reaction in anti-Pn1 indicates that the galA might be L-galA or D-galA not linked 1,4- as in PnS1.[14] Precipitation in anti-Pn6 might be due to 1,2-linked D-gal as in PnS6.[25] This would also account for the +± in anti-Pn14[25] since it has been shown that 1,2-linked D-gal behaves in the precipitin reaction like a somewhat hindered nonreducing lateral end group.[26] Reactivity in anti-Pn8 might ensue from a content of 1,4-linked D-gal and/or D-glc. If →4)-α-D-glc(1→4)-β-D-glcNac(1→ is present in *B. bifidum* as in PnS9, the strongest precipitation of 119 μg of antibody N/mL in anti-Pn9 623C would be accounted for.[1,27]

*Brevibacterium oleocaptus nov. sp.* was sent by S. Yamatodani (Osaka, Japan) in 1971. He reported [α]D = +143.5° and a content of gal, glc, man, OAc, pyA and less lactic acid.[28] No structure was given. One can only speculate that the strong precipitation in anti-Pn9 might involve pyA or lactic acid linked 1,3 to glc as D-glcA is in PnS9.[29,30] Precipitation in anti-*Klebsiella* Kl 1 was +++, possibly caused by pyA bound at the 2,3-positions of one of the sugars, as in Kl 1.[31] As there were no other reactions, *B. oleocaptus* is omitted from Table I.

*Clostridium perfringens* Hobbs, RB.5, from R. Cherniak (Atlanta) with gal, glc, man, glcA, and galNH$_2$[32] gave ++± in anti-*S. typhi*, ++ in anti-Pn9 and -14, +± in anti-Pn12 and -22 and anti-PTyA, and + in anti-Pn10. Because of the many alternatives, even guesses would seem far-fetched.

*Corynebacterium insidiosum*, from B. Lindberg (Stockholm), has the following structure:




It is omitted from Table I because it gave only – to ± in the anti-Pn sera. However, it reacted +++ in anti-*Klebsiella* Kl 1. The capsular antigen of Kl 1 also has the sequence →4)-L-fuc(1→3)-D-glc in its main chain plus D-glcA substituted at the 2 and 3 positions by pyA.[31] Kl 58[35] has both of these groupings and is precipitated by anti-Kl 1. Since the capsular polysaccharides of *Klebsiella* Kl 11[36] and Kl 21[37] have the same immunodominant nonreducing lateral end group (3, p402) as *C. insidiosum*, shown above, one would expect *C. insidiosum* to precipitate heavily in R anti-Kl 11 and R anti-Kl 21. It does!

*Epidermorphyton floccosum* precipitated ++± and +± with anti-Pn sera types 25 and 29 and was essentially negative with all others.

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**Table I.** Cross Precipitation of Microbial, Fungal, Mold, and Yeast Polysaccharides in Antipneumococcal and Other Antisera

| Antiserum to: | PnS 1 | ➡ 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 15 | 16 | 18 | 19 | 20 | 22 | 23 | 25[d] | 27 | 28 | 29 | S. typhi | S. pTyA | S. pTyB | Bov Mycop. | Klebs Kl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Homologous polysaccharide | 1024 | 4000 | 600 | 2390 | 4060 | 724 | 893 | 1288 | 1655 | 864 | 792 | 1240 | 1010 | 770 | 872 | 2200 | 2250 | 355 | 878 | 420 | 620 | 277 | 785 | 389 | | 730 | 342 | | 600 |
| Achromobacterium mucosum | (-) | (±) | (-) | (+) | (±) | (+) | (±) | (±) | (±) | (-) | (±) | - | (+) | (±) | (±) | (-) | (-) | (±) | (++) | (++) | (+) | (±) | (±) | (+) | (+) | | (++±) | | + |
| Arthrobac. visc. NRRL B 1797, deA | (-) | (-) | 60[a] | (++) | (±) | (±) | (-) | 662[a] | (-) | (±) | (-) | (-) | (-) | (±) | (+±) | (-) | (-) | (-) | - | - | - | - | - | - | | | | | ± |
| Azotobacter chroococcum | - | ± | ++ | - | ± | - | | 13[c] | 3 | ++± | + | ± | 43 | ++ | ± | - | +± | +± | ++++ | - | M ++± | +++ | - | - | +± | | ± | +++ | |
| Bacteroides asaccharolyticus | - | ++± | + | - | ± | ++ | + | +± | + | ++ | - | - | ++± | - | - | - | +± | ++ | | ++ | ++ | ++± | ± | +± | | ++± | | | ++ |
| Bifidobacterium bifidum | + | - | - | - | ± | ++± | - | ++± | 119 | + | (-) | (±) | +± | (-) | (-) | (-) | (±) | (-) | (±) | (±) | 513 (-) | (-) | (-) | | (+±) | | (++) | | |
| Brevibacterium oleocaptus[b] | - | - | ± | ± | ± | + | - | - | 119 | - | - | - | - | - | - | - | - | - | - | ± | 513 - | + | - | - | - | - | - | ± | ++± |
| Clostridium perfringens | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (++) | (+) | ± | (+±) | (++) | | | (-) | (+) | | (+±) | (±) | 513 (-) | (-) | (±) | (-) | (++±) | (+±) | (±) | (-) | |
| Epidermophyton floccosum | | | | | | | | | | | (-) | (-) | (±) | (-) | (-) | (±) | (-) | (±) | (-) | (-) | 513 (++±) | (-) | (-) | (+±) | (-) | (-) | (-) | | |
| Erwinia amylovora | + | + | + | ± | - | + | 23 | - | ± | ± | - | (-) | + | ± | +± | - | - | ± | (-) | (-) | 513 (-) | (-) | (-) | (-) | (-) | (-) | (-) | | |
| Formes annosus | (-) | (-) | 117 | (-) | (-) | (±) | (-) | 45 | + | (±) | (+) | (-) | (+) | (-) | (+) | (±) | (-) | (+) | (-) | (-) | 513 (-) | (-) | (±) | (-) | | | | | (-) |
| Fusarium moniliforme | - | ++± | - | - | - | +± | + | + | 21 | +± | ± | ++± | ++ | - | ± | - | ± | ++± | ++± | +± | 513 ± | ± | ++± | ++± | ++± | +++ | +± | +++ | |
| Listeria monocytogenes I | | | | | | | | | | | (-) | (-) | (-) | (-) | (-) | (+) | 60 | (-) | (+) | +++ | (-) | (++) | (+±) | (±) | (-) | | (-) | | |
| Listeria monocytogenes II | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - | ++ | - | - | + | | | | (++±) | (+) | | | | | |
| Listeria monocytogenes IVa | (-) | (-) | (±) | (+++) | (-) | (++) | ± | (-) | (-) | (-) | | | + | | | | | | | | | | | | | | | | |
| Listeria monocytogenes IVb | (-) | | (-) | (++) | (-) | (+) | (-) | (-) | (-) | (+) | (±) | | | (±) | | (±) | | | | | | | | | | | | | |
| Mycoplasma mycoides V5 galactan | ± | ± | ± | ± | ± | ± | ± | ± | ± | +++± | ± | ± | ± | ± | ± | ± | ± | ± | + | +± | 513 ± | - | | ± | - | ± | ± | +++± | |
| Physarum polycephalum | (-) | (-) | (±) | 90 | (-) | ++± | ++ | (+±) | (-) | ++ | ++ | + | ++± | ± | 11 | - | +± | +± | | +± | M - | - | | ± | | | | | ++ |
| Pleurotus ostreatus $A_3$ | ± | +++ | + | + | - | +± | - | ± | ++ | ± | - | + | - | - | - | ± | - | ++ | ± | - | 510 - | + | - | - | + | (++) | - | | |
| Pleurotus ostreatus $A_5$ | (±) | 1235 | 306 | (±) | (+) | (+) | (-) | (++) | ++ | (±) | (±) | (-) | (-) | (-) | (-) | (±) | (-) | (±) | (±) | (+) | 510 (-) | (-) | - | (++) | (+) | (±) | (-) | | |
| Pseudomonas aeruginosa I | ± | ± | + | - | - | ++ | ++± | ± | ++ | - | - | ± | ++ | - | + | - | + | ++ | +± | +++ | M ++± | - | - | ± | | | | | - |

| Antiserum to: | PnS 1 | ➡ 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 15 | 16 | 18 | 19 | 20 | 22 | 23 | 25[d] | 27 | 28 | 29 | S. typhi | S. pTyA | S. pTyB | Bov Mycop. | Klebs KI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pseudomonas aeruginosa II | - | + | ++ | +++ | - | ++ | + | ± | ++ | +± | - | | ++ | ± | ++± | - | ± | ± | ++ | ++ | ++ | ± | - | + | | | | | - |
| Pseudomonas aeruginosa III | (-) | (-) | (±) | (-) | (-) | (+±) | (-) | (-) | (-) | (±) | (±) | | (±) | (±) | (-) | (-) | (±) | (-) | (-) | (-) | (±) | (±) | (±) | (-) | | | | | (±) |
| Pseudomonas aeruginosa IV | - | - | +± | - | - | ± | + | - | +± | + | ± | ± | ++± | - | - | ± | - | - | - | - | M 93 | - | +± | ± | | | | | |
| Pseudomonas aeruginesa V | - | - | ± | - | - | - | - | - | +± | +± | - | + | ++± | - | - | ± | - | - | ± | ++± | ++ | - | ± | - | | | | | |
| Pseudomonas aerugonesa VI | - | - | +± | - | - | - | - | - | +± | +± | - | + | ++ | - | - | ± | ± | - | + | +± | ± | - | - | - | | | | | |
| Pseudomonas atlantica, $FcB_1$ | 12 | (-) | (+) | (±) | - | ++± | (-) | | ++± | (-) | (+) | (-) | ++ | | (-) | (-) | | (-) | | (++) | 513 (-) | (++±) | | (±) | (+) | | (±) | (-) | (++) |
| Pseudomonas fluorescence, fc12 | ± | +± | ± | + | + | ± | +++ | - | ++ | ++ | ++ | - | +++± | - | | - | ++± | ++ | +++ | | | + | + | | | | | | |
| Rhizobium japonicum 1809[+] | - | (-) | (-) | +± | (-) | ++± | (-) | (-) | (-) | (±) | - | ± | ++ | - | ± | - | - | - | ++ | +++ | M 25 | ++± | - | - | | | | | ++± |
| Rhizobium japonicum NR7[*] | - | - | ++ | ± | - | +± | - | - | ++± | +± | ± | - | ++± | +++ | - | ++ | ± | + | | ++± | M ++± | +± | + | ++ | | | | | ++± |
| Rhizobium japonicum meliloti B[+] | ± | - | ± | ± | ± | +++ | + | - | + | + | - | - | - | ± | + | ± | - | ± | ± | - | 513 ± | ++++ | - | | - | ± | - | ± | +++ |
| Rhizobium japonicum phaseoli | (-) | (-) | (±) | (±) | (-) | (++±) | (++±) | (++++) | (+) | (+±) | (-) | (-) | ++ | (±) | (±) | (-) | (±) | (±) | | (+±) | M ++ | (++) | | (+++) | | | | | (+++) |
| Salmonella haarlem | (±) | (++±) | (-) | | (±) | (+±) | (-) | (-) | (-) | (+) | (+) | (-) | (+) | (-) | (-) | (-) | (+) | (++) | (+) | (-) | 513 (-) | (-) | (-) | | (++++) | (++±) | (++±) | (+±) | (++) |
| Salmonella typhimurium MZ | | | | 14 | | | | - | | (±) | (±) | (±) | - | (-) | (-) | - | | (-) | | | 513 ++± | 23 | | | | | | | |
| Shigella dysenteriae II | (-) | (-) | (-) | (-) | (-) | (±) | (±) | (++) | (-) | (-) | (+) | (±) | - | (-) | (±) | (-) | (-) | (±) | - | (±) | M (-) | (++±) | (+) | (±) | | | | | |
| Sporothricum schenkii | (±) | (±) | (±) | (-) | (-) | (±) | (±) | (-) | (-) | (-) | (+) | | (+) | (+) | (-) | (-) | | (±) | ++± | +++± | (++±) | - | (++) | (+±) | | | | | |
| Torulopsis | + | +++± | ++ | - | +++ | - | + | ± | ± | ± | + | ± | ++ | + | ± | +++ | ± | ++ | ++ | ++ | 513 ± | ± | ± | | ++± | | ++± | +++ | |

(a) SIII Supernatants + SVIII pptd. 44μgN, intact serum gave 112; VIII supernatants + SIII gave no ppt., intact serum 203μgN; VIII Supernatants + oat glucan (68) gave 26 out of 125μgN. (b) Insid. rosum from B. Lindberg (c) analysis by P.A. Rebers
(d) M (mule), 510 and 513 (above the line of cross reactions) refer to the different anti-Pn 25 used, while the numbers on the line of cross reactions always refer to the actual quantitative precipitin values for the cross reactions. W/e cannot exclude the possibility that more than one antiserum was used also in the analyses with the other anti-Pn, but found no clear indication that this was the case. (G.J.T. and E.A.K.)

[+]Meliloti B probably partially deacetylated when made alkaline during preparation of soln. R. meliloti F gave almost identical pattern.

[*]Nitrate reductase mutant from K. Dillinger.

*Erwinia amylovora*, a galactan[35] toxic to certain plants, was sent by J. S. Huang (Columbia, MO). Except for +± in anti-Pn16 and 23 μg of antibody N (+++) in anti-Pn7, the weak reactions indicate that it has only very widely spaced or no unhindered lateral nonreducing end groups of D-gal or L-gal or that reactive D-gal is linked 1,2.

*Escherichia coli*: Cross-precipitations of the ubiquitous species were recorded by us on polysaccharides of K30, -42, and -85[38,39] and also those of K2, -4, -5, -7-9, -12, -14, -25-29, -31, -51, -52, -54, -57, -87, -100, -08, -09, -013, and -0111.[39]

*Formes annosus* precipitated 117 and 45 μg of N with anti-Pn3 and -8, respectively, and *Fusarium moniliforme* precipitated with a large number of antisera, giving 21 μg with anti-Pn9.

*Hemophilus influenzae* type f[40] from B. Lindberg (Stockholm) gave no positive results in the anti-Pn sera or in anti-Kl 1 and is omitted from Table I.

*Listeria monocytogenes* types 1, 2, 4a, and 4b were furnished by W. V. Ullmann (Connecticut Department of Health, Hartford, CT). In types 1 (ca. 14%) and 2 (ca. 12%), Rha is immunodominant. $GlcNH_2$ (ca. 14%) and glc (1.8%) are present in type 1, while type 2 has gal in addition. In types 4a and 4b, Gal and Glc are immunodominant, respectively[42] (cf. however, ref 42 for type 4b). The strong reactions of types 1 and 2 in anti-Pn23 show that Rha is the L isomer, present as nonreducing lateral end groups or linked 1,2. The fact that type 1 precipitated 60 μg of N/mL from anti-Pn19 631C could indicate that D-glcNAc in *L. monocytogenes* 1 is linked 1,4 since PnS19 has the partially immunologically equivalent 1,4-D-manNAc.[41,42] *L. monocytogenes* type 2 gave ++ in anti-Pn4 and differed only slightly from type 1 in anti-Pn19 and -23, but precipitated anti-Pn28 more strongly. Types 4a and 4b, tested in anti-Pn1-10, reacted +++ and ++, respectively, in anti-Pn4, ++ and + in anti-Pn6, and - and + in anti-Pn10, results not readily explainable with available information. A 4b strain yielded a lipopolysaccharide[42] which, when tested in the higher anti-Pn sera and anti-Kl 1, precipitated +++ in anti-Pn23, indicating the possibility of a nonreducing lateral end group of L-Rha.

*Micrococcus agilis* was from M. Salton (NYU School of Medicine, New York) and is not included in Table I. This succinylated galactorhamnan was tested only in anti-Pn2, -14, -19, and -23, giving a heavy precipitate in anti-Pn23 as did its desuccinylated derivative also. The presence of nonreducing lateral end groups of L-rRha in its repeating unit was therefore predicted and subsequently found.[44]

*Mycoplasma mycoides*, a galactan and a glucan, was sent by A. Rodwell (CS1RO, Parkville, Australia), who also provided anti-*M. mycoides*. The glucan precipitated only in homologous antiserum and is omitted from the table. The galactan is at least partly made up of 1,6-linked D-galf[45] which it shares with PnS10[46] and -29[47] so that it is not surprising that it precipitates antisera to these two Pn types +++± and +++, respectively. Cross-reactions of anti-*M. m.* sera are listed in the table in ref 1 and 48. A porcine antiserum, No. 2130, to *Mycoplasma granularum* from W. Switzer (Iowa State University Ames, IA) gave +± with mangle gum, ++ with fractions of *Mycoplasma pneumoniae* (from R. M. Chanock, NIAID, Bethesda, MD), Albizzia and ghatti gums, an doat glucan, and ++± with *Azotobacter* (from M. Stacey), the only substances tested.

*Physarium polycephalus* precipitated 90 μg of N, pleurotus $A_3$ precipitated +++ with anti-Pn2, and $A_5$ precipitated 1235 and 306 μg of AbN from anti-Pn2 and -3, respectively.

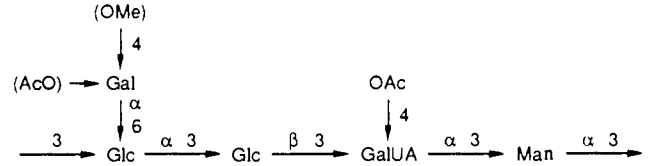
*Proteus mirabilis* F25, F27, P513, and XK (08) were furnished by H. Mayer (Freiburg, West Germany) but are not listed in Table I. F25 was tested in the anti-Pn sera and in anti-Kl 1, giving + only in anti-Pn23; F27 was run in anti-Pn1-10 and was - in all 10. P513, tested in anti-Pn1-10 and -22-29, anti-*S. paratyphi* A, and anti-Kl 1, showed + only in anti-Kl 1.

*Pseudomonas aeruginosa* was Fisher types I-VII (see ref 49).

*Pseudomonas atlantica* fractions B1 and B2 were from W. A. Corpe, (Barnard College, New York, NY). The hydrolyzed, chromatographically homogeneous polysaccharides showed spots corresponding to gal, glc, man, and galA; glc was D, and pyA was found along with rha, glcA, and other sugars, suggesting complexes in which the composition varied with the time of incubation and possibly other factors.[50,51] Fractions B1 and B2 gave ++ and ± in anti-Pn1, indicative of differences in galA, and ± and ++ in anti-Pn28, otherwise showing similar cross-reactivities (only B1 is represented in Table I). Relatively heavy precipitation in anti-Pn27 might indicate 4,6-linked pyA on D-glc or D-man or the sequence →3)-D-glc(1→3)-D-gal(1→ if present. Equally vague possibilities exist for the other reactions in the absence of structural information. For PnS27 see refs 52 and 53; the structure of S28 is not known.

*Pseudomas fluoresence* fc 12 reacted ++ or more with a variety of anti-Pn sera; no new structural insight was gained.

*Rhizobium japonicum* 1809 was sent by W. F. Dudman (Commonwealth Scientific and Industrial Organization, Canberra, Australia). It contains gal, 4-OMeGal (proportions depending upon age of culture), galA, glc, man, OAc, and pyA (Dudman, W. F. Personal communication),[54] and its structure is probably the same as that of other strains in its group, such as 311b and V38. It has a repeat pentasaccharide as follows:




The weak +± precipitation in anti-Pn4 is probably caused either by the 1,3-D-man of 1809 and the 1,3-linked D-manNAc of PnS4[56] or by a loose fit of the partly substituted gal of 1809

(38) Heidelberger, M.; Jann, K.; Jann, B.; Ørskov, F.; Ørskov, I.; Westphal, O. Relations between structures of three K polysaccharides of *Escherichia coli* and cross-reactivity in antipneumococcal sera. *J. Bacteriol.* **1968**, *95*, 2415.

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(49) Heidelberger, M.; Horton, D.; Haskell, T. H. Cross-reactions of lipopolysaccharides of *pseudomonas aeruginosa* in anti-pneumococcal and other antisera. *Infection Immunity* **1986**, *54*, 928-930.

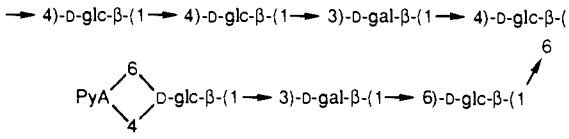
(50) Corpe, W. A. Acid polysaccharide produced by a primary film-forming marine bacterium. *Dev. Ind. Microbiol.* **1970**, *11*, 402.

(51) Uhlinger, D. J.; White, D. C. Relationship between physiological status and formation of extracellular polysaccharide glycocalyx in *Pseudomonas atlantica*. *Appl. Environ. Microbiol.* **1983**, *45*, 64.

(52) Bennett, L. G.; Bishop, C. T. Structure of the type XXVII *Streptococcus pneumoniae* (pneumococcal) capsular polysaccharide. *Can. J. Chem.* **1977**, *55*, 8-16.

into antibody sites designed for the pyA gal of S4. Partly unsubstituted D-gal would also account for ++ in anti-Pn14.[26] Mutual 1,3-linked D-glc is undoubtedly the cause of the stronger ++± in anti-Pn6, possibly reinforced by the 1,3-D-man. Valid explanations of ++, +++, and +++ (28 μg of N/mL) in anti-Pn22, -23, and -M25 are lacking: the first possibly is due to 1,3-linked D-galf in PnS22,[11] while the third might indicate that the GalA of S25[51] is linked 1,3. S27,[52] like 1809, has 1,3-D-gal in its repeating unit. A mutant, *Rh. japonicum* NR7 from K. Dillinger (Cook College, New Brunswick, NJ), contained glc, man, and 12% galA and gave the cross-reactions listed in Table T. In the absence of structural information, one can only say that D-glc and/or D-man might be present in 1,3- and/or 1,4-linkages and that the ++± in anti-Pn25 could indicate that D-galA might be linked as in PnS25.

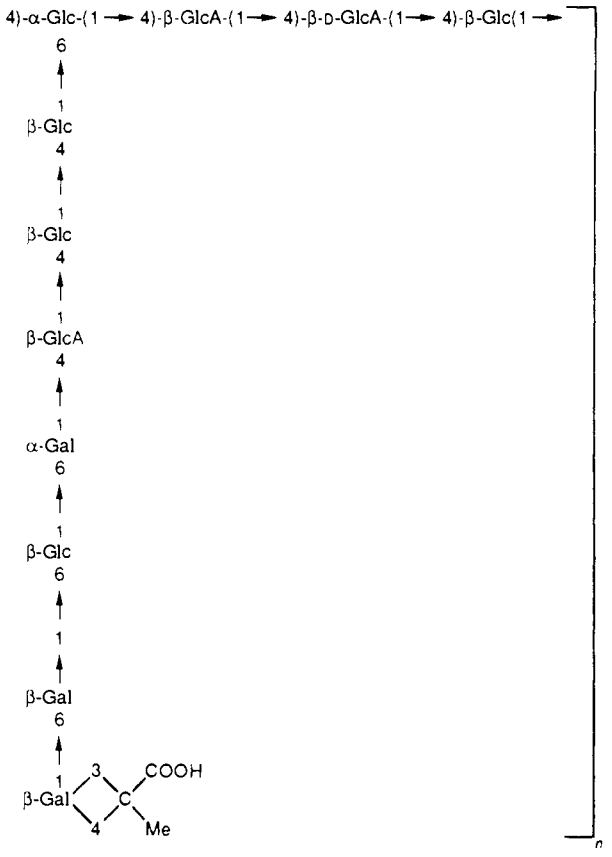
*Rhizobium meliloti* B and F polysaccharides, which gave three lines on gel diffusion against homologous antisera, were brought in by W. F. Dudman and found to contain pyA. Their cross-reactions in anti-Pn were described in ref 58, but as their structure is now considered identical to that of other strains, several new inferences may be drawn. The ++ in anti-Pn4[56] after partial




removal of pyA (and probably OAc and succinyl), may be due to exposure of a 1,4-linked D-glc reacting with an antibody space meant for D-manNAc; +++ in anti-Pn6 may be due to unblocking of two 1,3-linked D-glc residues. Strangely there was no precipitation in anti-Pn8, but in anti-Pn9 nearly 4 times as much antibody came down as with undegraded B (Table 3 in ref 58). In anti-Pn10 the +± appears to be due to the 1,3-linked D-gal present in the main chains of both *Rh. meliloti* and PnS10. (For the latter, cf. ref 46, p 198.) Depyruvylated B also gave ++ in anti-Pn11, explainable since PnS11 also has 1,3- and 1,4-linked D-glc;[61] ++± in anti-Pn 12, possibly owing to the D-glcNAc and kojibiosyl residues of PnS12 (62,63), the terminal and 1,2-linked D-glc of which might have stimulated antibodies capable of loosely accommodating the 1,6-D-glc of B. DepyA B's massive reaction with anti-Pn14 is accounted for by the presence of five sugar residues in its repeating unit capable, by the above reasoning, of reacting with the antibody. PnS15[64] contains four sugar units in common with B, the weaker reactions of which in anti-Pn17 and -19 are similarly explained: structures of PnS17 and 19 are given in refs 65 and 43, respectively. In anti-Pn27 the ++++ of B is due to 4,6-linked PyA, 1,3-D-gal, and 1,4-D-glc in both B and S27.

*Rhizobium meliloti*: Some OAc and succinyl groups were probably removed. Precipitation was +++ in anti-Pn6, doubtless owing to the 1,3-linked D-glc in common with PnS6[25] and perhaps reinforced by a loose fit of the pyAglc into antibody sites in anti-Pn6 designed for the ribitol $PO_4$ of PnS6.

The *Rhizobium phaseoli*, strain 127K87, sent by W. F. Dudman, has a repeating unit of 11 sugars. Its structure[66] is




Since β-D-glcA(1→4)β-D-glc, β-D-glc(1→4)β-D-glc, and 1,4-D-gal are structural elements of PnS8,[18] it is not surprising that the heaviest cross-reaction occurred in anti-Pn8. There was also ++± in anti-Pn6, possibly because the pyAgal end group might have fit loosely into antibody sites designed for the ribitol $PO_4$ of PnS6. The ++± in anti-Pn7 and ++ in anti-Pn14 might also be ascribed to a weak combination with the terminal PyAgal residues. Perhaps an alternative explanation of the reactions in anti-Pn6, -7, and -14 is that the two 1,6-linked D-galactosyls of *Rh. phaseoli* might combine weakly with sites in anti-Pn6 intended for 1,2-D-gal or in anti-Pn7 and -14 sites for lateral nonreducing end groups of D-gal. PnS7 and -S14 both contain 1,4-linked D-glc as well; ++ in anti-Pn10 is probably due to a loose fit of β-1,6-linked D-gal of *Rh. phaseoli* into antibody sites designed for the β-1,6-bound

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(64) Perry, M. B.; Bundle, D. R.; Dasust, V.; Carlo, D. J. Specific capsular polysaccharide of *Streptococcus pneumoniae* type 15F. *Mol. Immunol.* **1982**, *19*, 235.
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D-galf of PnS10.[46] 1,4-Linked D-glc in both substances probably accounts for ++ in anti-Pn23 and +++ in anti-Pn29.[47] The structure of S25 is not known. PnS27s 1,3-D-gal and 1,4-D-glc linkages may also account for the ++ tandem β-1,4-D-glcA of *Rh. phaseoli* fitting loosely into antibody spaces induced for the 3,4-pyA-substituted D-glcA of Kl 11.[31]

*Salmonella adelaide*, from Professor Otto Westphal (Freiburg Germany), precipitated anti-Pn8 1008 appreciably only after the removal of varying proportions of colitose and was tested in other antisera (not in table). In this respect it resembled the *O*-polysaccharide of *E. coli* 111 although much more degraded *S. adelaide* than O111 was required for maximal precipitation. Strains of the two bacteria are reciprocally 100% agglutinable.[58] Like PnS8,[18] in which one-half of the repeating unit is →4)-α-D-glc(1→4)-α-D-gal(1→, *E. coli* 111 has this same sequence (with the glc blocked by two colitoses, linked α1→3 and α1→6[67], and *S. adelaide* is believed to have it as well.

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*Salmonella haarlem* reacted very strongly (++++) with anti-*S. typhi* and with anti-*S. paratyphi* A and 13 (++±). In addition, it reacted with anti-*Klebsiella* K1 and anti-Pn2 and -20 (++).

*Shigella dysenteriae* II reacted ++ with anti-Pn8 and -27.

*Sporothricum schenkii* reacted strongly (++ to +++) with anti-Pn22, -23, -25, and -28 and somewhat less strongly with anti-Pn29 (+±).

*Torulopsis* showed strong reactivity with antipneumococcal sera (PnS2, -3, -5, -14, -18, -20, -22, -23), anti-*Salmonella* sera (*S. typhi*, *S. paratyphi* B), and anti-bovine mycoplasma (++±).

It may be expected that the data in the text and in Table I will prove highly informative to further understanding of the structural bases for cross-reactivity of polysaccharides (G.J.T. and E.A.K.).

(69) Abbreviations: Kl, *Klebsiella*; Pn, *Pneumococcus* or pneumococcal; S, capsular type-specific Pn polysaccharide; C, group-specific Pn polysaccharide; sugars without suffix f for furanose are pyranoses and are capitalized only when end groups or immunodominant; gal, galactose; glc, glucose; rha, rhamnose; man, mannose; galA, galacturonic acid; glcA, glucuronic acid; manA, mannuronic acid; galNAc, *N*-acetylgalactosamine; glcNAc, *N*-acetylglucosamine, etc.; pyA, pyruvic acid; dAc, deacetylated; dpya, depyruvylated.