Immunochemistry, 1976, Vol. 13, pp. 67–80. Pergamon Press. Printed in Great Britain.

IMMUNOCHEMICAL RELATIONSHIPS BETWEEN BACTERIA BELONGING TO TWO SEPARATE FAMILIES: PNEUMOCOCCI AND *KLEBSIELLA*

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(*Received* 18 *February* 1975)

Abstract—The cross-reactions of more than 60 capsular (K) type-specific polysaccharides of *Klebsiella* in antipneumococcal sera of 26 specific types and in several anti-*Salmonella* sera are described and discussed, many for the first time. Quantitative data on the extent of cross-reactivity are given in many instances. From these, and from a study of residual fractions of antibody in the supernatants, it has been possible to confirm the presence of non-reducing end-groups of D-glucuronic acid in K2, K8 and K20. Such groups were also correctly predicted for K9 and K59 and will most probably be found upon chemical examination of K15, K23, K27, K30, K33, K45, K51 and K55. Non-reducing end-groups of L-rhamnose were correctly predicted for K47 and K56 and will likewise be found in K17 and K19.

Other correlations of chemical structure and immunological specificity are pointed out, as well as limitations of the methods used.

INTRODUCTION

Knowledge of the molecular basis for the frequent cross-reactions which occur between microorganisms belonging to widely disparate families is not only of practical interest, but also has a broader theoretical significance, for it is capable of revealing strict relations between the chemical constitution of certain microbial antigens and their immunological specificities. Several such aspects of cross-reactivities have already been reviewed [1–4].

The bacterial polysaccharides provide a rich source of antigenic material, and since they are often the principal antigenic determinants of the parent microorganisms, the corresponding antisera are also frequently available. Hence the cross-reaction of a polysaccharide of known chemical structure with antibodies to a polysaccharide of uncertain or unknown structure may yield information as to one or more sugars contained in the unknown and even as to the positions at which the sugars are linked. Conversely, cross-reactivity of a polysaccharide of unknown structure with antibodies to a polysaccharide of known composition and linkage may be equally informative. If the cross-reactions are massive, qualitative tests could suffice, but if only a small proportion of the antibody is involved, quantitative estimations are necessary, as differences in titer might easily be minimal. Quantitative analyses have the further advantage of yielding information as to which fractions of the usual complex mixture of antibodies are cross-reactive, and which fractions remain in the supernatant fluid. The data so obtained must, however, be interpreted with caution, for it has been shown that, in certain instances of cross-reactivity, part of the precipitable antibody behaves as if it were multivalent with respect to the cross-reactive antigen and part as if it were univalent, being coprecipitated only in the presence of the multivalent portion [4*b*]. Precipitation and reactivity are therefore not synonymous. An additional and occasionally observed difficulty is that a second cross-reactive antigen may serve to coprecipitate soluble antigen–antibody complexes remaining from the first precipitation. Instances illustrative of these difficulties will be given.

The present review encompasses the cross-reactivities of some 60 of the 80 or more capsular, type-specific polysaccharides of *Klebsiella* in a series of antipneumococcal sera chosen for their availability. Most of these antisera were raised in horses before it was known that the tendency of this animal to produce macromolecular anticarbohydrate [5] diminished its usefulness for supplying quick and efficient type-specific antibodies for the cure of pneumococcal pneumonia [6]. This very property, however, appears important for the tendency of equine anticarbohydrate to be more cross-reactive than that of the rabbit, which is usually IgG. Accordingly, most of the antisera used in this study were those of equine origin which were not discarded when the purpose for which they were produced became obsolete. Some data are also given for antisera raised in rabbits and these are designated by the letter R. Limited data are also included on cross-reactions in a few anti-*Salmonella* sera.

MATERIALS AND METHODS

Klebsiella K polysaccharides were mainly isolated by Nimmich [11, 12]. Samples of K1 and K3 were also obtained from Prof. S. D. Henriksen and Dr. Jorunn Eriksen; K6

* Aided by grants from the National Science Foundation, U.S.A.

from Prof. R. W. Wheat; K11 from Dr. S. Stirm; and K5, deacetylated K5, K18, 20, 21, 24 and 62 from Prof. G. G. S. Dutton.

Antipneumococcal (anti-Pn) sera were supplied by the Bureaus of Laboratories of the New York City and New York State Departments of Health. Antipneumococcal type-specific rabbit globulins were given by E. R. Squibb & Sons; anti-*Salmonella* sera by Dr. Anne-Marie Staub. Anti-Pn sera containing more than a few μg/ml of antibody to the pneumococcal group-specific C-polysaccharide were precipitated with this substance before use and carry the letter C after the number of the animal.

Qualitative tests are recorded on a scale of − to + + + +, on which + + is roughly equivalent to 8–20 μg of antibody nitrogen per ml (by actual comparison) and + + + + to 100 and up. The tests were usually carried out with 0·5–1 ml of antiserum, depending upon its content of antibody, in tubes immersed in ice-water. Polysaccharide (0·05 mg) was first added, followed by 0·05 mg more if precipitation became evident at the interface. The contents of the tubes were thoroughly mixed, and the rack of tubes, usually 4 rows of 10 tubes in each, was placed in a cold room for 6–8 days. After immersion in ice-water, readings were taken. Quantitative analyses at several levels of antigen were made of the heaviest reactions (Table 4) and an additional 0·1 or 0·15 mg/ml was added to the remaining tubes, since a large excess of antigen was frequently required for maximal cross-precipitation. After 6–8 days second readings were taken, after which the sera were used for a second series of tests. In these, the second polysaccharide was added to the remaining tubes of two rows in order to lessen the possibility of negative tests due to inhibition by the excess of one or the other of the substances first tested. Results of such second (or occasionally, third) tests are recorded in parentheses. Strong cross-reactions usually appeared rapidly, whether or not the sera had been negative or weakly reactive in earlier tests. A reading of + + ± would be slightly greater than + + if read in the same series on the same day, but might be considered + + at another time, a failing common to all qualitative tests.

Quantitative analyses were carried out as in earlier papers [7–9]. Antigen and antibody were mixed at 0°C and the tubes were capped and immersed at least to the level of their contents in a 0°C bath (Forma Scientific, Inc.). After 4–14 days, depending upon the rapidity of flocculation, if any, the tubes were centrifuged at 0°C. Supernatants were saved for eventual analysis and the inverted tubes were allowed to drain in a cold box [8] before the rims were wiped and the precipitates dislodged and washed in the tubes at 0°C with ice-cold saline. As cross-reactions may be sensitive even to small differences in temperature (for example, [10]), the contents of the tubes should be kept as close to 0° as possible. All values are reported as micrograms of antibody nitrogen precipitated at 0°C per ml of antiserum, even though in some instances of massive cross-reactivity as little as 0·05 ml was actually used. Analyses were run in duplicate, with a serum blank, at two or more levels of polysaccharide. Only maximal values are given in the tables.

RESULTS AND DISCUSSION

Several anti-Pn sera which were known to cross-react heavily in other instances (e.g. [1]) failed to give tests stronger than + + with any of the *Klebsiella* K polysaccharides tested and are omitted from Table 4. The sera were: anti-Pn XI613 (− to ±); anti-Pn XII296C [+ +, (+ +) with K10, K58]; anti-Pn RXIII Squibb, (K44+ +); anti-Pn XIV635C [K7, K12+, K41, K50+ +, K57(+)]; anti-Pn XVI594C (K22+, K36+ + = 16 μg N); and anti-Pn RXVII (− to ±). Anti-Pn XXVIII510C, which precipitated 35 μg N with K52, is also omitted as it reacted only + with K2, K26, K54 and + ± with K8.

Klebsiella K type polysaccharides 1–59, 62–64, 66, 72, 75 and 81 were tested. Their composition is given in Table 1, which is adapted from [11, 12]. Structures, insofar as presently known, are shown in Table 2, with corresponding data for pneumococcal capsular

Table 1. Constituents [a] of capsular polysaccharides (K) of *Klebsiella* tested for cross-reactivity

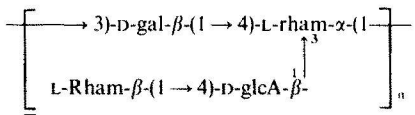
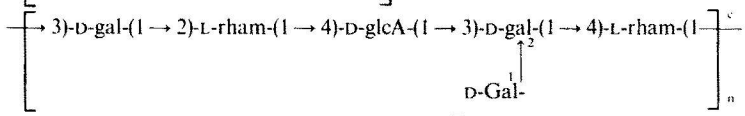
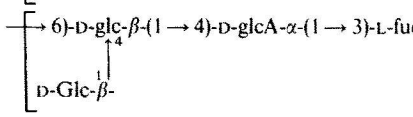
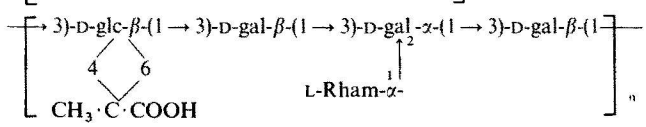
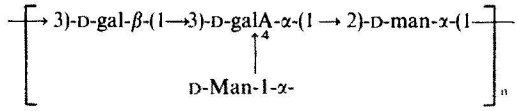
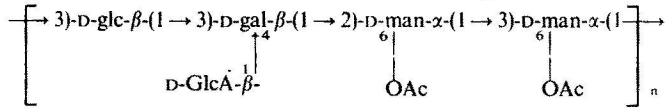
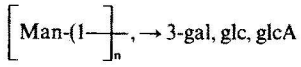
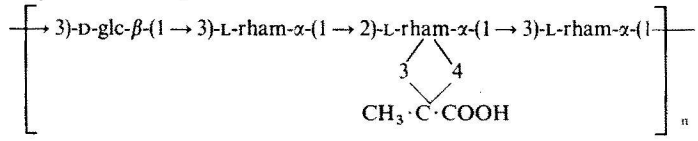
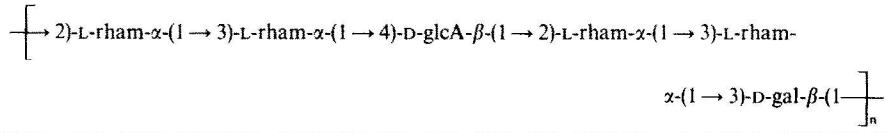
GlcA, gal, glc	K8PyA, 15, 25, 27PyA, 51
GlcA, gal, man	20, 21PyA, 29PyA, 42PyA, 43, 66
GlcA, gal, rham	9, 47, 52, 81
GlcA, glc, man	2, 24
GlcA, glc, rham	17, 44
GlcA, glc, fuc	1, 54PyA
GlcA, gal, glc, man	4, 5PyA, 7PyA, 10, 11PyA, 13PyA, 26PyA, 28, 30PyA, 31PyA, 33PyA, 35PyA, 39, 46PyA, 50, 59, 62
GlcA, gal, glc, rham	12PyA, 18, 19, 23, 36PyA, 41, 45PyA, 55PyA
GlcA, gal, glc, fuc	16, 58PyA
GlcA, gal, man, rham	40, 53
GlcA, glc, man, rham	64PyA
GlcA, glc, man, fuc	6PyA
GlcA, gal, glc, man, rham	14PyA
GalA, gal, man	3PyA, 49, 57PyA, 75
GalA, glc, rham	34, 48
GalA, gal, rham, fuc	63
PyA, gal, rham	32
PyA, gal, glc, rham	56, 72
3-Deoxy-L-*glycero*-pentulosonic acid, gal, glc	38 (13)
Unknown keto acid, gal, glc	22, 37. K37 has 4-O-(1-carboxyethyl)-D-glcA

[a] Abbreviations used in this and subsequent tables: gal, galactose; glc, glucose; man, mannose; rham, rhamnose; fuc, fucose; ara, arabinose; galA, galacturonic acid; glcA, glucuronic acid; PyA, pyruvic acid; DPA, 3-deoxy-L-*glycero*-pentulosonic acid; galN, galNAc, galactosamine and its N-acetyl derivative; glcN, glcNAc, glucosamine and -NHAc; manN, manNAc; fucN, fucNAc. In Tables 2 and 3, sugars with a capital first letter, as Glc, indicate non-reducing end-groups.

Table 2. Known partial and complete structures of *Klebsiella* K polysaccharides tested

K Type		
1	[D-Glc-(1—]$_n$, most sugar linkages 1,3-; D-glcA, L-fuc	(14)
2	—→ 3)-D-glc-β-(1 → 4)-D-man-β-(1 → 4)-D-glc-α-(1— ↑3 1 D-GlcA-α-	(15)
3	D-Gal, D-man; galA → D-man	(16)
5	—→ 4)-D-glcA-β-(1 → 4)-D-glc-β-(1 → 3)-D-man-β-(1— ↑2 ⋱4 6⋰ OAc CH_3CCOOH	(17)
7[a]	—→ 3)-D-glcA-β-(1 → 2)-D-man-(1 → 3)-D-glc-(1— ↑3 ⋱7 1 4 6⋰ D-Gal CH_3CCOOH	(18)
8	—→ 3)-D-gal-β-(1 → 3)-D-gal-α-(1 → 3)-D-glc-β-(1— ↑4 1 D-GlcA-α-	(19)
9	—→ 3)-D-gal-α-(1 → 3)-L-rham-α-(1 → 3)-L-rham-α-(1 → 2)-L-rham-α-(1— ↑4 1 D-GlcA-β-	(20)
11	—→ 3)-D-glc-β-(1 → 3)-D-glcA-β-(1 → 3)-D-gal-α-(1— ↑4 1 D-Gal-α- 4⋱6 PyA	(21)
18	[D-Glc-(1—]$_n$, → 3)-D-gal-(1 —, → 3)-L-rham-(1—, glcA.	(22)
20	—→ 2)-D-man-α-(1 → 3)-D-gal-β-(1— ↑3 --OAc 1 -α-D-gal-(3 ← 1)-β-D-GlcA	(23)
21	—→ 3)-D-glcA-α-(1 → 3)-D-man-α-(1 → 2)-D-man-α-(1 → 3)-D-gal-β-(1— ↑4 4 COOH 1 ⋱ ⋰ -α-D-Gal C ⋱ ⋰ 6 CH_3	(24)
24	—→ 2)-D-glcA-α-(1 → 3)-D-man-α-(1 → 2)-D-man-α-(1 → 3)-D-glc-β-(1— ↑4 1 D-Man-β- ---OAc	(25)
28	—→ 2)-D-gal-α-(1 → 3)-D-man-α-(1 → 2)-D-man-α-(1 → 3)-D-glc-β-(1— ↑2 1 D-Glc-β-(1 → 3)-D-glcA-β-	(26)
37	XA[b] ↓4 —→ 4)-D-glc-(1—, → 6)-D-glc-(1—, → 3)-D-gal—,	(27)
38	DPA ↓2 —→ 6)-D-glc-β-(1 → 3)-D-gal-β-(1 → 4)-D-gal-α-(1— ↑2 1 D-Glc-β-	(13)

(Table 2—*continued*)

K Type		
47		(28)
52		(29)
54(A3S1)		(30)
56		(31)
57		(32)
59		(33)
62		(34)
64	glcA → man, D-glc, D-man, L-rham	(35)
72		(36)
81		(80)

— Partial substitution. Sugars are pyranoses unless otherwise indicated.

[a] Repeating unit is double that given, with lateral substituents only on alternate half-units.

[b] XA = 4-O-(1-carboxyethyl)-D-glucuronic acid.

[c] Not uniquely determined.

polysaccharides, the principal determinants of pneumococcal type specificity, in Table 3. Qualitative and quantitative data are summarized in Table 4 and its footnotes.

*Cross-reactions in anti-*Pn I

Although seven of the K polysaccharides contain galacturonic acid, only two, K49 and K63, cross-react heavily (Table 4). In these, then, the sugar acid belongs to the D-series,* and is linked, at least in part, as lateral non-reducing end-groups and/or attached at the 1 and 3 positions, as in Pn SI [37]. Footnote a (Table 4) shows that both substances precipitate a portion of the same fraction of anti-Pn I; also that ketha gum, in which D-galA was first identified as a result of the gum's massive cross-reactivity in anti-Pn I [57], removes all of the fraction of anti-Pn I precipitable by K49 and K63, K57, which contains D-galA linked 1,3,4-[32], did not precipitate anti-Pn I.

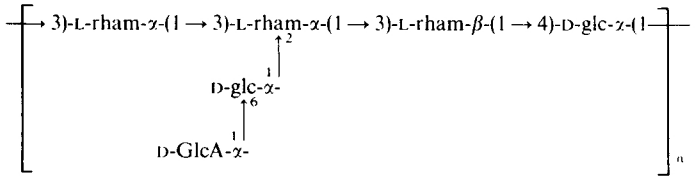
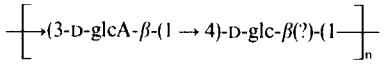
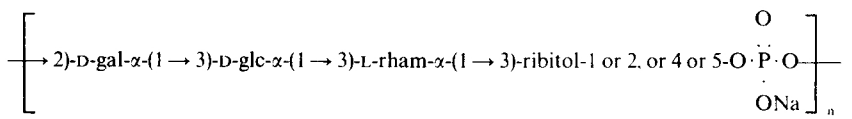
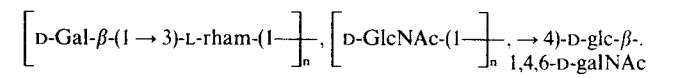
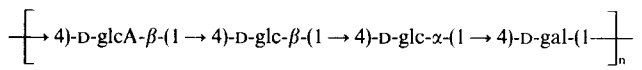
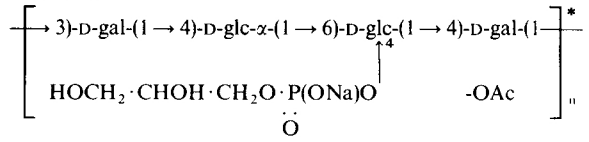
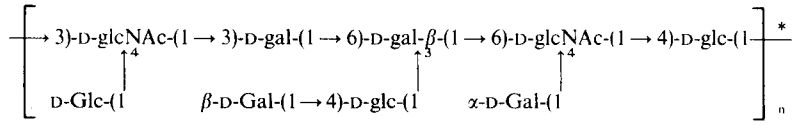
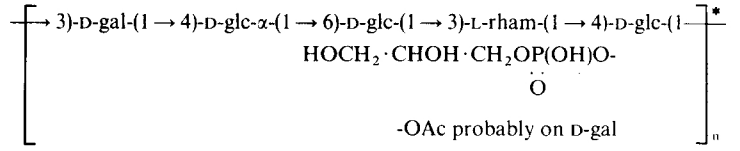
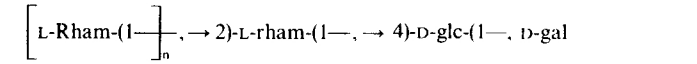
Two strong anti-Pn I rabbit sera were tested with K49 and K63; one gave no precipitate with either; the other, RI937, with about 2000 μg anti-Pn I nitrogen per ml, gave 267 μgN with K49 and ± with K63.

*Cross-reactions in anti-*Pn II

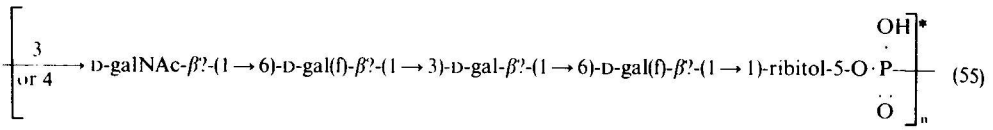
The type-specificity of Pn II is separable into several partial specificities: an immunodominant one

* For an example of differences in the specificity of D- and L-forms of the same sugar cf. [79].

Table 3. Constitution of pneumococcal capsular polysaccharides of types used

Pn type		
I	galA → galN, galA → glcN, galA(1 → 3)-glcN-(1 → 3)-galA. D-GalA, probably partly O-acetylated, glc.	(37)
II		(38)
III		(39)
IV	D-gal, D-galNAc, manNAc, fucNAc, PyA possibly 2,3- on gal	(40)
V	D-gal, D-glc, → 2)-D-glcA-β-(1 → 3)-L-fucN, D-glcA-β-(1 → 3)-L-fucN-(1 → 4)-D-glc, 2-AcNH-2,6-dideoxy-L-talose	(41)
VI		(42)
VII		(43)
VIII		(44)
IX	-D-glcA-α-(1 → 3)-D-glc, glcA-(1 → 3)-glcNAc, manNAc-(1 → 3)-glc-(1 → 3)-manNAc	(45)
X	gal(f), galN, glcN, ribitol, PO_4.	(46)
XIA (XLIII)		(46a)
XIV		(75)
XV	gal, glc, galN, glcN, glycerol, PO_4.	(46)
XVIII		(47)
	or alternative with single glc and isomaltosyl residues exchanged	
XVIIIA	D-gal, D-glc, rham, glcNAc, glycerol, PO_4, 2:3.5:1:1:1:1; no -OAc.	(48)
XIX	glc, rham, D-manNAc, PO_4, 1:2:1:1 (49); gal, glc, rham, galN. glcN, PO_4	(46)
XX	gal, glc, glcN, PO_4	(46)
XXII	D-GlcA-α-(1 → 3)-D-glc, D-gal-β-(1 → 3)-L-ara, D-gal-β-(1 → 5)-L-ara, → 4)-rham D-glc-β-(1 → 4)-D-glc, erythritol, PO_4.	(50)
XXIII		(51, 52)
XXV	gal, galA, 2 aminosugars	(53)
XXVII	gal, glc, rham, glcN, PO_4, PyA	(46, 54).
XXVIII	glc, rham, glycerol, PO_4	(46)

(Table 3—*continued*)

Pn type		
XXIX		(55)

*Not uniquely determined.

Table 4. Qualitative and quantitative Data on *Klebsiella* K Polysaccharides

K polysacch.	Anti-Pn: Homologous:	I1057C 1024	II513C 4040	III792C 600	IV608C 2340	V555 4060	VI681C 724	VII937C 887	VIII1008 1288	IX623C 1655	X627C 864	XV628 770
1		–		+	57[g]	–	±	–	+ +	–	83[n]	±
2		+	1670[b]	–	±	39[j]	–	+	–	+ +	+	121[q]
3		–	–	–	±	±	–	±	±	–	±	–
4		–	560[b]	–	±	24	+ ±	–	–	–	–	–
5		–	+	55[f]	–	–	+ ±	–	24[n]	–	±	±
6		±	9	–	+ + ±	±	+	–	–	–	±	–
7		–	+	–	–	–	+	–	–	–	–	±
8		–	710[b]	–	–	331[j]	–	–	±	±	–	–
9		±	1080[b]	+	–	223[j]	+ + ±	+ + ±	–	–	+ ±	–
10		–	–	+	–	–	16	–	–	+ +	+ ±	–
11		–	–	–	±	–	–	–	–	–	±	–
12		–	–	–	–	–	52[l]	–	–	–	±	–
13		–	–	±	82[g]	–	+	–	+ +	+ +	–	–
14		–	–	–	–	±	–	–	–	–	–	(–)
15		–	1050[b]	+ ±	–	332[j]	–	41[m]	–	+	+ + ±	–
16		–	–	+	–	–	–	–	–	–	–	–
17		–	–	–	–	–	–	–	±	–	–	–
18		–	–	–	–	–	+ +	±	28[n]	+ + ±	+	±
19		(±)	–	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(±)	(–)
20		–	895[c]	–	–	+ +	+	–	–	+	+	–
21		–	–	–	±	±	+	–	–	11[o]	±	–
22		–	+ +	–	–	+	–	–	–	–	+ + ±	145[q]
23		(–)	1300[c]	(–)	(–)	137	(+)	(–)	(±)	(–)	(±)	–
24		–	±	–	–	–	–	+ + ±	–	–	+ + ±	–
25		(–)	785[c]	45[f]	(–)	650[k]	(+)	(–)	(–)	(–)	(±)	(–)
26		–	–	–	±	±	–	–	+	–	±	–
27		–	1450[c]	–	–	510[k]	±	–	–	–	–	(–)
28		–	–	–	–	–	–	–	–	–	–	–
29		–	–	–	92[g]	–	–	–	–	–	–	–
30		(–)	1925[c]	(–)	(–)	±	(–)	(–)	(–)	(–)	±	+ ±
31		(–)	(–)	+ ±	(–)	(–)	(–)	(–)	(–)	(–)	52[n]	(–)
32		±	+ ±	–	990[h]	–	+	+	–	–	+	±
33		(+)	1785	(+)	(+ + ±)	±	(–)	(–)	(–)	(–)	(±)	109[q]
34		±	187[b]	±	–	–	+ + ±	–	–	–	±	–
35		–	360[d]	–	±	+	±	±	–	–	±	–
36		–	535[d]	–	–	208[k]	–	–	–	–	±	(–)
37		–	+ ±	+ + ±	435[i]	–	–	±	–	–	±	(–)
38		(–)	(+)	(±)	(+)	(–)	(–)	(–)	(–)	(–)	(±)	(±)
39		(–)	–	76[f]	(±)	(–)	(+ +)	(±)	177[n]	(–)	51[n]	–
40		(–)	+ ±	(±)	–	–	(+)	(±)	(–)	(–)	(–)	–
41		–	(–)	–	–	–	+ +	–	–	–	–	–
42		–	(–)	±	56[i]	–	+	–	–	+	+ + ±	±
43		–	(–)	–	–	–	+ ±	–	–	–	–	271[q]
44		–	104[d]	–	(–)	(–)	(±)	(±)	42[n]	(+ + ±)	(±)	(–)
45		(±)	1300[d]	(–)	(–)	176[k]	25[l]	(–)	(–)	(–)	(±)	(–)
46		–	–	–	+ +	–	+	–	–	–	–	–
47		–	–	–	–	–	21	+ + ±	–	±	±	–
48		+ +	156[c]	+	–	–	–	–	–	–	+	–
49		255[a]	+ +	–	–	–	±	–	–	–	–	–
50		–	+ ±	–	–	–	102[l]	–	–	50[n]	–	–
51		(–)	2700[c]	–	(–)	+	(±)	(±)	(–)	(±)	(±)	(–)
52		–	–	+	–	–	–	+ + ±	–	–	+	+
53		(–)	(±)	(–)	(–)	(±)	(–)	(±)	(–)	±	(±)	–
54		–	+	–	±	–	–	+	+ +	–	+	–
55		–	1970[c]	(–)	(–)	+	(–)	(–)	(–)	(–)	(–)	±
56		(–)	(–)	(–)	(±)	(–)	(–)	(–)	(–)	–	(±)	(–)
57		–	+	–	–	–	–	–	–	–	–	–
58		–	–	–	–	–	–	±	–	–	–	(–)
59		–	700[e]	+ +	–	160	+ ±	–	–	+	56[n]	–
62		–	+	+ +	±	149[k]	–	–	–	–	±	–
63		148[a]	±	–	219[i]	–	15	–	–	–	+	–
64		–	±	–	±	±	–	±	–	41[o]	+	±
66		–	–	+ + ±	–	–	–	–	–	–	–	–
72		–	256[c]	–	–	–	19	–	–	±	–	–
75		±	+	(±)	(±)	(–)	(–)	(–)	(–)	(–)	(±)	(–)
81		–	+ + +			+	+ +					(–)

due to lateral non-reducing end-groups of D-glucuronic acid [1, 58, 59] linked α-1,6- to D-glucose [60], a minor specificity arising from consecutive residues of L-rhamnose linked 1,3- in the main chain, and another due to 1,6- or 1,4- linked D-glucose. End-groups of D-glucose, such as those in glycogen and certain dextrans, may also fit into sites on antibodies designed for D-glucuronic acid [59, 10].

Of the 52 K-substances tested which contained glcA, 20 reacted extensively with anti-Pn II. One of the earliest pneumococcal cross-reactions noted, that of the K2 polysaccharide in anti-Pn II [61], is now known to be caused by the presence, as lateral substituents in the repeating units (Table 2), of multiple [62] non-reducing end-groups of D-glcA [15]. Such end-groups have also been found in K8 [19], K9 [20] (in which they were predicted on the basis of its heavy cross-reaction), K20 [23] and K59 [33]. These serological reactions have the advantage over chromatographic methods of identifying the glcA as D-glcA, but anti-Pn II does not distinguish between D-glcA and 4-O-methyl-D-glcA. Multiple lateral non-reducing end-groups of D-glcA will probably be found in the remaining heaviest precipitators of anti-Pn II, namely,

Maximal precipitation at 0°C; quantitative Analyses calculated to 1·0 ml

K polysacch.	XVIII495C 2200	XIX631C 2250	XX616C 355	XXII566 878	XXIII912C 418	XXV513C 186	XXVII668C 277	XXVIII510C 735	XXIX642C 389	Ty	Para Ty A 730	Para Ty B 342	S. senftenberg 300
1	±	±	±	+ ±	–	–	7	±	±	–	±	+ –	
2	+	+ +	+ ±	+ +	+ ±	±	+	+	–	±	–	–	
3	–	–	–	–	–	–	–	–	+	±	±	±	
4	±	–	–	±	±	–	–	±	+	(–)	(–)	(–)	
5	±	–	–	(–)	(–)	(–)	45'	(–)	(±)	(–)	(±)	(–)	
6	–	–	–	–	+	–	33'	–	–	+	±	±	
7	±	–	±	±	–	–	57'	–	±	–	+ + +	±	
8	–	–	+ +	+	–	±	+ ±	+ ±	–	±	±	–	
9	–	+ +	–	+ +	±	–	–	–	+ ±	–	+ +	–	
10	–	–	–	(+ +)	18'	(–)	(–)	(–)	(+ +)	(±)	(+ +)	(±)	
11	±	–	–	–	–	–	±	±	–	±	–	–	
12	–	–	–	(±)	(±)	(–)	(+ +)	(–)	(±)	(+ +)	(±)	(–)	
13	–	–	–	(–)	(–)	(–)	±	(–)	(–)	(±)	(±)	(±)	
14	(–)	(–)	(–)	–	66'	–	–	–	–	–	–	(–)	–
15	±	–	–	±	+ ±	(–)	–	(–)	(±)	(±)	+ +	(–)	(+ +)
16	–	–	–	–	–	(–)	(–)	(–)	(–)	(±)	(–)	(–)	(+)
17	–	–	–	33"	232'	(–)	(–)	(±)	(+)	(+ +)	(±)	(–)	(±)
18	+ ±	±	–	+ +	–	–	–	–	–	+ ±	±	±	
19	(–)	(–)	(±)	±	193'	–	–	–	–	±	±	–	
20	±	–	–	+	–	–	–	–	–	–	–	–	
21	±	–	–	+	–	–	–	–	–	–	–	–	
22	–	–	16'	–	–	–	–	–	+ + ±	–	+	(–)	–
23	–	–	+ +	–	–	–	–	–	–	±	±	±	
24	–	–	9	±	–	–	–	–	–	±	+ + ±	±	
25	(±)	(+)	(–)	(–)	(±)	+	(–)	(–)	(–)	(±)	(±)	(–)	(–)
26	+ + ±	±	±	+ +	+ ±	±	+	+	–	+	(+ + +)	(–)	
27	(–)	(–)	(–)	–	–	–	+ +	–	±	+ ±	(±)	(+)	
28	–	±	–	(±)	(±)	(–)	(–)	(–)	(–)	(–)	(+ + ±)	(–)	(+ + ±)
29	–	+	–	(–)	(–)	(–)	(–)	(–)	(–)	(–)	±		(–)
30	–	±	–	(–)	(–)	(+ +)	(–)	(–)	(–)	(–)	(–)	(±)	(–)
31	(–)	(–)	(±)	–	–	–	+ + ±	–	+ +	(–)	+	–	–
32	–	+ ±	±	–	–	–	84'	–	±	+ +	20	–	
33	(–)	(–)	(–)	+ ±	+ +	+ + ±	±	–	+ +	(–)	–	–	–
34	–	85'	–	+ ±	64"	±	–	–	±	–	+ + ±	–	+
35	(–)	(±)	(±)	+	±	–	–	–	+ ±	(±)	+ ±	–	–
36	(–)	(±)	(±)	–	–	–	+ +	–	+ ±	(±)	–	–	–
37	(–)	(±)	(–)	(–)	(–)	(–)	(±)	(–)	(+)	(+ +)	(±)	(–)	(±)
38	(–)	(–)	±	–	±	–	±	–	–	+	–	(–)	+ +
39	29'	–	16'	13	(+ ±)	(–)	(–)	(–)	+	+ + ±	32	(–)	(+ + +)
40	–	–	–	(+ ±)	(–)	(–)	(–)	(–)	+		(±)	(–)	(±)
41	+ ±	–	±	±	–	–	–	–	+ + ±		+ ±	–	
42	–	–	+	–	–	–	–	–	+ ±			±	
43	500'	–	+ +	–	–	–	–	–	–			±	
44	(+ + ±)	(–)	(±)	–	–	–	–	–	+ +		–	±	
45	(–)	(–)	(±)	–	–	–	–	–	±		–	–	
46	±	–	+ ±	–	–	–	+ + ±	–	–		+ ±	–	
47	+ +	–	–	84"	260"	–	–	–	+ +	±	+ +	–	
48	–	240'	–	+ +	+ + ±	–	–	–	±	±		–	–
49	±	–	–	–	43	(266)	±	±	–	±		–	±
50	–	+ ±	+ ±	+ ±	–	+	60'	–	20		+ + ±	+	
51	(–)	(+)	(+ +)	(+ ±)	(–)	(–)	(–)	(–)	(–)		(–)	(+ +)	
52	–	±	±	65"	–	–	–	35	±	+ ±	+	+	
53	±	±	–	53"	+ + ±	–	–	–	–		–	–	
54	–	±	±	+	±	–	+	+	–				
55	±	–	–	–	–	–	–	–	–		–	–	
56	(–)	(–)	(–)	–	96"	–	73'	–	–	–	–	(–)	–
57	813'	(–)	–	(±)	+ +	–	±	±	±	–		±	±
58	(–)	±	–	(–)	(–)	(–)	(–)	(–)	(–)		(–)	(±)	
59	–	–	+	+ +	–	–	–	–	–	±	+	+	
62	–	–	–	–	–	–	–	–	±	–	±	–	
63	–	–	±	+	±	62'	16	–	+	+		±	+ ±
64	–	±	±	+	85"	–	–	–	±				
66	–	–	–	–	–	–	–	–	–				
72	±	+		+	+	+	+ +	+	+ +	–	±	–	
75	(–)	(–)	(–)		(+ +)		(+ + +)		–				
81	(–)	(–)											

Footnotes to Table 4 on pp. 8–9.

Footnotes to Table 4.

Sera with the designation C were absorbed with pneumococcal group-specific C-polysaccharide; sera not marked with C contained little or no anti-C.

[a] K49 supernatants + K63 gave 62 μg N; further data as well as pptn. in anti-Pn I 704, in [56]. K63 supernatants + K49 gave 101 μg N [56]. Serum absorbed with ketha gum [57] failed to ppt. with K49 or with subsequently added K63.

[b] K2 supernatants + K9 pptd. 208 μg N; K9 supernatants gave 1025 μg N with K2 [56]. K15 supernatants + K2 gave 800 μg N; + K23 gave 313 μg N, after which K25 pptd. 70 μg N; K15 supernatants + K25 gave 106 μg N, after which K23 pptd. 280 μg N.

[c] K20 supernatants gave 819 μg N with K2, 46 μg N with K 8; after pptn. with K20 and K8, K23 gave 287 μg N. K23 supernatants + K25 gave 188 μg N. K25 supernatants + K8 pptd. 86 μg N; + K9, 664 μg N; + K15, 523 μg N, after which K2 gave 800 μg N. K27 supernatants + K2 pptd. 511 μg N and reacted weakly with K8. K30 supernatants + K2, K9, K55 gave 17, 123, 600 μg N, resp. Serum absorbed with K30 and K55 gave 933 μg N with K51; the cross-reactive ppts. totaled 3283 μg N. K34 supernatants pptd. 777 μg N with K9 [56].

[d] K35 supernatants gave 275 μg N with K36; K36 supernatants pptd. 56 μg N with K35; the combined K35, K36 and K36. K35 supernatants gave a further 196 μg N with K25. K44 supernatants pptd. 138 μg N with K72, 245 μg N with K35; serum absorbed with K44 and K72 gave 350 μg N with K8. K45 supernatants gave 935 μg N with K2.

[e] K48 supernatants pptd. K35 as did intact serum and gave 610 μg N with K4, possibly owing to copptn. of soluble K48–antibody complexes; K48, K35 supernatants gave 240 μg N with K8. K51 supernatants gave no ppt. with K2 but pptd. 106 μg N with K72. K55 supernatants + K51 gave 840 μg N. K72 supernatants gave 128 μg N with K48, 630 with K4, 418 with K36.

[f] Deacetylated K5 gave 57 μg N; K5, deAcK5 supernatants pptd. 55, 54 μg N with Pn S VIII; intact serum gave 112 μg N. When the K5, S VIII and deAcK5, S VIII supernatants were mixed no further pptn. occurred. Subsequent addition of the polysaccharide of *Fomes annosus*, which also contains β-linked D-glc and D-glcA residues, but not cellobiouronosyl [64], gave 83 μg N; intact serum gave 115. Anti-Pn III absorbed with deAcK5 gave 22 μg N with deacetylated *Arthrobacter* NRRL B-1797 polysaccharide which contains D-gal, D-glc, D-glcA, PyA [65]. Because of this reaction and a much stronger one in anti-Pn VIII it may be considered to have cellobiouronosyl groups; intact anti-Pn III gave 60. K25 supernatants + S VIII gave 96 μg N. K39 supernatants reacted with K25 and gave 21 μg N with K5; supernatants from the second pptn. gave 28 μg N with Pn S VIII. Anti-Pn III 792C absorbed with S VIII gave no ppt. with K39.

[g] K1 supernatants + K13, K63 gave 48, 179 μg N, resp.; K1, K13 supernatants + K29 pptd. 50 μg N. K13 supernatants gave 212 μg N with K63, as in intact serum. K29 supernatants pptd. 42, 17, traces, 203 μg N with K1, K13, K42, K63, resp.

[h] From [54], in which additional data are to be found.

[i] K37 supernatants from which an average of *ca.* 400 μg N had been pptd. gave 552 μg N with K32, 656 out of 928 with depyruvylated Pn S IV, and reacted with K29, K63. K42 supernatants which had pptd. an average of 46 μg N gave 37, 64, 69 μg N with K1, K13, K29, resp. The 42, K13 supernatants pptd. 323 μg N with K37; the K42, K29 supernatants gave 327 μg N. K63 supernatants gave 698 μg N with K32 [56]. + + with K27.

[j] K2 supernatants pptd. 30 μg N with Pn S II; intact serum gave 63 at level used [69]. K8 value from [56]. Serum which had pptd. 450 μg N with *E. coli* K85 [71] gave 53 μg N with K8. K9 supernatants + degraded gum arabic gave 48 μg N [56]; intact serum gave 283 [69]. K15 supernatants + K8, K9 gave 0, 9 μg N, resp.

[k] K25 supernatants gave 3 μg N with K8. K27 supernatants pptd. 127 μg N with K25. K36 supernatants + K8 gave 7 μg N. K45 supernatants + K59, K9 gave 37, 31 μg N resp.; supernatants from K45, K9 + K8 pptd. 8 μg N. K62 supernatants + S II, K8, K27 gave 36, 19, 453 μg N, resp; serum absorbed with K62 and K8 gave 530 μg N with K25.

[l] K12 supernatants + S II gave 5 μg N; intact serum pptd. 21 [7]. K12 supernatants pptd. guar gum much as did intact serum. Anti-Pn VI which had pptd. 65 μg N with guar gave 45 μg N with K12. K45 supernatants pptd. 14 μg N with K47. K50 supernatants + K12 gave 45 μg N, as in intact serum at the level used; + S II, 12 μg N, + K47, + + ±; supernatants from pptn. with K12 gave 10 μg N with K45.

[m] Supernatants gave 11 μg N with S XIV; intact serum pptd. 35 [9].

[n] Deacetylated K5 pptd. 18 μg N. Serum absorbed with K5 or K18 pptd. as much N with K18 or K5 as did intact serum; the doubly pptd. sera gave 208, 48 μg N with S III, oat glucan: intact serum gave 203, 63 μg N at the levels used. K39 supernatants gave 15 μg N with *Lipomyces starkeyi* out of 44 [66, 67]; + K5 gave 10 μg N; supernatants from this pptd. 97, 100 μg N with S III, S XIX, the latter as in intact serum. K44 supernatants pptd. S III, S XIX as did intact serum. Supernatants from the K44, S III absorptions gave no ppt. with K5.

[o] K21 supernatants + K64 gave 31 μg N out of 41 [72]. K50 supernatants + K64 pptd. 24 μg N.

[p] K1 supernatants pptd. 12 μg N with K39, 53 μg N out of 104 [73] with streptococcal group L substance [74]. K31 supernatants + K1 gave 19 μg N; + S XIV, 48 μg N out of 60 [9]; + oxidized–reduced *E. coli* K85, 57 μg N out of 70 [71]. Supernatants from the pptns. with K1 and oxd.–red. *E. coli* K85 gave 50, 45 μg N, resp., with S XIV. K39 supernatants pptd. 30, 14 μg N with K1, K31, resp.; serum absorbed with K39 and K31 gave 23 μg N with S XV; intact serum gave 60 [73]. K59 supernatants pptd. with K1 as did intact serum.

[q] K2 supernatants + depyruvylated *Rhizobium meliloti* B (dp Rh.m.B) gave 26 μg N out of 111 [54]. K22 supernatants pptd. with K2, K33, dp Rh.m.B, gave 44, traces, 19 μg N resp. K33 supernatants + K2 gave 18 μg N; + K22, 48 μg N; supernatants from this second pptn. gave 4 μg N with K43. K43 supernatants gave no ppt. with K2.

[r] K39 supernatants + S VII gave no ppt., + dextran N236 gave 35 μg N, (intact serum pptd. 47 μg [47]); serum absorbed with K39 and dextran gave no ppt. with S VIII. K43 supernatants failed to ppt. with K57, S XIA; the latter [46a] gave 278 μg N with intact serum [73]. K57 supernatants + oxidized–reduced *Sporobolomyces* acetylphosphoglucogalactan gave 688 μg N; intact serum gave 1200 [77]; + *Lipomyces lipoferus* [66], pptd. 72 μg N out of 173 [67].

[s] K34 supernatants + K48 gave 171 μg N [56]. K48 supernatants pptd. 48 μg N with K34; + S VIII, 66 μg N out of 124 [9, 68].

[t] K22 supernatants gave 10 μg N with K24; with S II, 20 μg out of 40 [9]; serum absorbed with K22 and K24 gave 16 μg N with S II. K39 supernatants + K22 pptd. 16 μg N, as in intact serum, but pptd. only 24 μg N with S II.

[u] K17 supernatants pptd. 40, 34 μg N with K47, K52, resp. K39 supernatants gave 50 μg N with K47, + + ± with K17. K47 supernatants + K52 gave 30 μg N; K52 supernatants + K47 pptd. 40 μg N [56]. K53 supernatants + K47, K52 gave 25, 9 μg N, resp.

[v] K10 supernatants gave as much ppt. with K14, K56 as did intact serum; absorption with K10 and K14 left 111 μg N for K17, 47 for K56; pptn. with K10 and K56 left 114 μg N for K17. K14 supernatants pptd. 26 μg N with K49, after which K47 gave 187 μg N. K17 supernatants + streptococcal group B gave 2 μg N; intact serum pptd. 209 [73]. K17 gave + + + in a potent Squibb anti-Pn XXIII rabbit serum.

[w] K34 supernatants + K64 gave 71 μg N; serum which had pptd. 39 μg N with streptococcal group F zl [68] gave 34 μg N with K34. K47 supernatants from an earlier bleeding gave no ppt. with streptococcal group B; intact serum gave 97 μg N at the level of B-substance used [51]; the serum, absorbed with B, gave 82 μg N with K47 [54]. K49 supernatants pptd. K47 as did intact serum and gave 70 μg N with K64; serum absorbed with K49 and K64 yielded 35 μg N with K34. *cf.* also [56]. K56 supernatants gave 111 μg N with K17, 112 with K19, 176 with K47. K64 supernatants pptd. 31 μg N with K34.

[x] Supernatants which had averaged 47 μg N with K63 gave 150 μg N with S XXV.

[y] K5 supernatants + K32 gave 60 μg N. K6 supernatants pptd. 18 μg N with K7; with *Rhizobium trifolii* UNZ29, 18 μg N out of 78 [54]. K7 supernatants failed to ppt. with K6 but gave 62 μg N with K32. K32 supernatants [54]. K50 supernatants + K56 gave 64 μg N; + K5 pptd. 40 μg N. K56 supernatants + K32 pptd. 60 μg N; supernatants from this gave 7 μg N out of 80 with deacetylated *Rhizobium trifolii* TA_1 [54].

[z] Serum from which carob mucilage had pptd. an average of 32 μg N gave 3 μg N with K52.

K4, 15, 23, 25*, 27, 30, 33, 45, 51, and 55, although all contain D-glc as well.

K4, dissolved with the aid of brief exposure to alkali, precipitated much more antibody (1600 μg N) than did the solution listed in Table 4 and [56], possibly owing to removal of a hindering -OAc.

Footnote [b], Table 4, shows that K2 precipitates most of the fraction of antibody reactive with K9; the reverse is not true, possibly because the end-groups of D-glcA in K9 are in the β-anomeric form, while in Pn SII, the antigenic determinant of Pn II, and in K2, they are the α-form. Precipitation of K9 in anti-Pn II is probably reinforced by the tandem 1,3-linked residues of L-rhamnose similar to those which give rise to a minor partial specificity of Pn II [58]. However K8, in which the end-groups are α-, precipitates less antibody than K20, in which they are β- and there is no rhamnose. In K2, K8, K9 and K20, the end-groups are not linked to D-glucose, as in SII, so that spatial and conformational relations must be modifying factors in addition to the chemical nature and linkage of the adjoining sugar.

Footnote [b] also shows that K15 precipitates about one-half of the antibody reactive with K2 as well as a large part of the fraction of antibody precipitable by K23 and K25.

Similar information is given for other anti-Pn II-precipitating K polysaccharides in footnotes [c,d] and [e]. With respect to K44, a rather weak reactor, the data are explicable if K44 has few or no end-groups of D-glcA, or if such end-groups are partially blocked by -OAc. The fraction of anti-II precipitated is one-half of that reactive with K72, which contains no glcA. Its string of L-rhamnose residues (Table 2) is undoubtedly responsible for the cross-reaction and it is therefore likely that two or three such consecutive residues will be found in K44, which contains glcA, glc, and rham. Prior precipitation with K72 scarcely affects the cross-reaction of K48 (footnote [e]). Since K48 contains galA and no glcA, but does have glc and rham, the last is either hindered by a substituent or linked otherwise than 1,3-. Evidently the reaction is mediated by D-glucose.

K51 cross-reacts more massively in anti-Pn II513 than any of the others tested, precipitating 67% of the antibodies. Of the large number of polysaccharides of varying origin which react specifically with this serum, only that of *Brachychiton* exceeds this proportion, precipitating 75% in the bleeding presently in use. K30 and K55 are the next best, and it would be interesting to know whether or not in these two, and in K51, the glcA is linked α-1→6 to D-glc, as in Pn SII. Such linkage would not necessarily be expected, since D-GlcA-α-(1→2)-L-rham has been isolated as a partial hydrolytic product of *Brachychiton* gum [70].

A solution containing 6000 μg anti-Pn II nitrogen per ml, made from Squibb's purified, freeze-dried, rabbit anti-Pn II, gave 36 μg N with K2 and 122 μg N with K30, also the following qualitative tests: with K8 ±, K15 (+ + ±), K23 (+ + + +), K33 (+ + +). A New York State Department of Health Bureau of Laboratories rabbit anti-Pn II serum, bleeding of 11/10/45, with 3100 μg anti-Pn II nitrogen per ml, gave + + + with K2, 23, 33, ± with K8, 25, + + with K9, 15, + + ± with K4, 27, 45, + + + ± with K30, + + + + with K51, 55.

*Cross-reactions in anti-*Pn III *and* -VIII

Multiple residues of cellobiouronic acid in the main chain of the repeating unit are obviously the cause of the cross-reaction of K5 [17, 63] and will undoubtedly prove to be the reason for the heavier precipitation by K39 which includes more than one-half of the antibody precipitated by K5 (footnote [f]). K5 and its deacetylated derivative precipitate the same amount and the same fraction of anti-III, and this is a portion of the antibody reactive with S VIII and with other polysaccharides containing, or probably containing, multiple cellobiouronosyl residues (footnote [f]). The behavior of K25 is atypical, since it does not precipitate anti-Pn VIII or remove much of the antibody

* Niemann and Stirm (personal communication) report that K25 has D-Glc-(1— and →2)-D-glcA-(1—, either of which could account for the cross-reactivity, especially as 1,2-linked sugars may function as somewhat hindered end-groups (see anti-Pn V and VI).

precipitated by S VIII from anti-Pn III. K66 also precipitates anti-Pn III but not anti-Pn VIII. The cellobiouronic acid units of Pn S III are linked through the 3-position of glcA and all linkages in S VIII are $1 \rightarrow 4$.

In anti-Pn VIII, K5 and K18 apparently precipitated different fractions of antibody (footnote [n]), but the amounts were small. K18 did not precipitate anti-Pn III and therefore probably does not contain cellobiouronosyl residues. Possibly the reaction in anti-VIII is caused by →4)-β-D-glcA(1— residues. Tests of the supernatants from precipitates by K39 showed that a corresponding portion of the antibody precipitable by S III had disappeared. A part of the fraction reactive with *Lipomyces starkei* [66, 67] was also involved, but not the fraction which precipitates with S XIX [9]. This is believed to react because of 1,4-linked glucosyl residues [68]. The supernatants from K44 precipitated S III and S XIX as did intact serum: after the reaction with S III, K5 gave no precipitate.

Cross-reactions in anti-Pn IV

K1 reacts partly with the portion of anti-Pn IV reactive with K13 and K63; serum absorbed with K1 and K63 gave 50 μg N with K29 (footnote [g]). However, K13 supernatants precipitated K63 as did intact serum, possibly owing to coprecipitation of soluble K13 anti-Pn IV complexes. K29 supernatants showed little overlapping with K1 and K63 but much with K13 and K42. K13, K29, and K42 all contain galactose and pyruvic acid (Table 1), as does Pn S IV [40], but the structures of the three Ks are unknown. K1 contains no sugars in common with S IV, but if its fucose has the D-configuration and is linked 1,4- it might fit into anti-Pn IV spaces designed for 1,4-linked D-galactose. K63 has no pyruvic acid (unpublished data), but contains galactose and fucose. Its structure is not known.

K32, also of unknown structure, was the heaviest reactor in anti-Pn IV608C (42% of the antibody, footnote [h]) and precipitated every anti-Pn IV tested [54, 56]. Like S IV, it consists partially of gal and PyA. K37 also precipitated anti-Pn IV strongly (footnote [i]). It contains D-galactose, D-glucose, and 4-O-(1-carboxyethyl)-D-glcA [27]. There seems to be no obvious reason for its cross-reactivity, which involves a portion of the fractions of antibody precipitated by K32 and depyruvylated S IV. The antibody reactive with K42 is partly a portion of that precipitated by K1, K13, K29, and K37. E. R. Squibb and Sons' purified rabbit anti-Pn IV, at 4850 μg N with PnS IV, gave 1610 μg N with K32 and 44 μg N with K63.

Cross-reactions in anti-Pn V

As in studies with other polysaccharides [69], many of the Ks which react strongly in anti-Pn II precipitate less heavily in anti-Pn V, a difference ascribed to the →2)-D-glcA-β-(1— linkages of S V [41], which, in certain conformations, might leave positions 3, 4 and 6 of the glucuronic acid available for reactivity with antibody designed for the unsubstituted molecule. However, some of the heaviest precipitators of anti-Pn II, such as K20, K30, K33, K48, K51 and K55 show little or no reactivity in anti-Pn V. K62 precipitates anti-V but not anti-II, and perhaps contains 2-substituted D-glcA, like S V, which also fails to precipitate anti-Pn II513 [69].

Cross-reactions in anti-Pn VI

K50 was the strongest reactor in anti-Pn VI681C, followed by K12, which removed much of the anti-Pn VI precipitable from this serum by S II (footnote [l]). K12 will probably be found to contain L-rham linked 1,3-, as the cross-reaction between Pn II and -VI has been traced to the presence of multiple residues of 1,3-, as the cross-reaction between Pn II and VI has of both types [7]. (See also Table 3). Footnote [l] also shows that D-gal, as non-reducing end-groups, or linked 1,2- as in S VI, is not involved in this cross-reaction. K50 appears not to react with the fraction of antibody which precipitates with multiples of 1,3-linked L-rham, but it is possible that soluble K50 anti-Pn VI complexes might have coprecipitated with K12. K45 precipitates a portion of the antibody reactive with K47, the structure of which is known. However, there are three sugars in common.

Cross-reactions in anti-Pn VII

K15 precipitates a portion of the fraction of antibody cross-reactive with Pn S XIV (footnote [m]), which also contains D-gal and D-glc [75], as does S VII (Table 3). The limited cross-reactivity (+ + ±) of K9 may be due to residues of 1,3-linked L-rham common to S VII and K9. Precipitation by K24 and K47 is difficult to explain, (*cf.* Tables 2 and 3), while that of K52 may be due to its non-reducing end-groups of D-gal. Possibly the two aminosugars of S VII, D-galactosamine and D-glucosamine, give rise to specificities which cross-react with D-gal and D-glc.

Cross-reactions in anti-PnIX

K50 is the strongest reactor in this antiserum, followed by K64. After precipitation with the former, K64 still gave 24 μg N (footnote [o]), as well as yielding 31 μg N from supernatants from which K21 had precipitated 11 μg N. Intact serum gave 41 μg N with K64 [71]. The structure of K50 is not known, but K21 has →3)-D-glcA-α-(1 → 3)-D-man-(1—(25). The internal D-glcA→ man of K64 is probably also linked 1,3 between the sugars [35]. S IX has an internal D-glcA-α-(1 → 3)-D-glc [45b] and this would account for the cross-reactions of K21 and K64 if the 3, 4 and 6 positions of the mannosyl residues could fit into antibody spaces designed for glucose, as has already been postulated for many of the cross-reactions of *Lipomyces* [67]. In K21 the glcA is also branched at C4, while in K24, which does not precipitate anti-Pn IX623C, glcA is linked 1,2,4. K2 has non-reducing end-groups of D-glcA attached α-(1 → 3) to 1,4-linked D-man in the main chain and reacts + + in anti-Pn IX.

Cross reactions in anti-Pn X

K1, K31, K39 and K59 are the best precipitators of the available anti-Pn X. S X is said to contain galactofuranose, galactosamine, glucosamine, ribitol, and phosphate [46], but its structure is not known, nor have those of the first three K substances been elucidated, other than that K1 has non-reducing end-groups of D-glc. Footnote [p] shows that K1, K31, and K39 precipitate much of the same fraction of antibody, and that this is the portion which reacts with Pn S XV and with the group-specific polysaccharide

of streptococcal group L, composed of D-gal, rham, D-galNAc and D-glcNAc [74]. Although no single sugar is shared by all, these five substances and S X will eventually be found to contain at least one immunologically equivalent sugar and linkage. On the other hand, K59, S XIV, and oxidized-reduced *E. coli* K85 precipitate mainly a different fraction. Reactions rated at ++± were given by K15, K22, K24 and K42. Of these, only the structure of K24 is known [25].

Cross-reactions in anti-Pn XV

The best reactors in this antiserum were K2, K22, K33 and K43, the last being the most potent. All four of these must contain a similar immunologically reactive structural feature in their repeating units, as they precipitate much of the same fraction of anti-Pn XV (footnote [q]). This is also the portion reactive with the depyruvylated extracellular polysaccharide of *Rhizobium meliloti B* [54], which, in its intact form, contains end-groups of D-gal substituted at 4 and 6 with PyA, →3)D-gal-β-(1—, and D-glc linked in various ways and partly acetylated [76]. S XV is said to contain gal, glc, galN, glcN, glycerol, and PO_4 [46], but its structure is not known.

Cross-reactions in anti-Pn XVIII

Only K43 and K57 showed noteworthy behavior in this antiserum, both giving massive precipitates of the same fraction of antibody (footnote [r]). The supernatants from the smaller reaction with K43 failed to precipitate with K57 or Pn S XIA, indicating that K43 had reacted with all of the multivalent cross-reactive antibody and that only antibody univalent with respect to these substances remained (*cf.* p. 000). The precipitate with K57 also consisted mainly of antibody cross-reactive with oxidized-reduced *Sporobolomyces* acetylphosphoglucogalactan [77] and partly of the fraction which precipitated *Lipomyces lipoferus* [67]. Possibly an acetylated sugar residue is involved.

Cross-reactions in anti-Pn XIX

K34 precipitated a portion of the same reactive fraction of antibody as did K48 (footnote [s]). The structures of K34, K48 and S XIX are not known, but glucose is the only sugar in common and is probably responsible for the cross-reactions, as the supernatants from K48 gave only one-half of the antibody precipitated from intact serum by S VIII (footnote [s] and ref. [68]).

Cross-reactions in anti-Pn XX

S XX contains galactose, glucose, and glucosamine [46]. All of the K substances which precipitate ++ or more in this antiserum contain glucose and all but K24 contain galactose as well. K22 and K39 react with a portion of the fraction of antibody precipitated by S II (footnote [t]). Since they do not contain L-rham, as does S II, this is possibly an indication that they might have their D-glucose linked 1,6- and/or 1,4- [10, 59].

Cross-reactions in anti-Pn XXII

Not much explicit information can be derived from this series. Footnote [u] shows that K17 precipitates a portion of the same fraction of antibody as K47 and K52, both of which contain 1,4-linked D-glcA as well as L-rham bound 1,3,4- in the former and 1,4- in the latter. 1,4-linked rhamnose and variously linked glcA are believed to occur in S XXII [50]. K53 also contains rhamnose and precipitates a portion of the same antibody.

Cross-reactions in anti-Pn XXIII

The heavy precipitation of K47 in this antiserum has already been noted [54, 56] and prompted the prediction that K47 would be found to have non-reducing end-groups of L-rhamnose, as proved to be true [28]. A similar prediction for K56 was also well-founded [31] and it is equally evident that such end-groups will be found in K17 and K19, possibly also in K14 and K64. The small precipitation by K10 is due to another component. These relationships are detailed in footnotes [v] and [w].

Cross-reactions in anti-Pn XXV

The anomalous, heavy cross-reactions of K49 and other substances in this antiserum have already been commented upon, as well as the reaction of K63, which appears to be entirely due to antibody ([56, 71] and footnote [x]).

An anti-Pn XXV raised in a mule, through the kindness of Dr. Gerald M. Ward, New York City Department of Health Laboratories, Otisville, New York, with 610 μg anti-S XXV N per ml, gave 13 μg N with K34, which did not precipitate antiserum 513, and reacted only + with K63. It failed to precipitate K33 or K49. A New York State Department of Health rabbit anti-Pn XXV was negative with K30, K49 and K63, ± with K33.

Cross-reactions in anti-Pn XXVII

The sugars in S XXVII are gal, glc, rham and $glcNH_2$ [46], but their order and linkages are unknown. A pyruvylated sugar is a principal antigenic determinant [54]. K6 and K7 precipitate mainly the same fraction of antibody (footnote [y]). K32 evidently reacts with a different portion of the antibody than do K5, K7 and K56, while K5, K50, and K56 scarcely overlap. The pyruvylated sugar in K5 is D-man [17], in K7 [18] and K56, D-glc [31], attached as a ketal at 4- and 6-.

Cross-reactions in anti-Pn XXVIII *and anti*-Pn XXIX

Only K52 reacted markedly with anti-Pn XXVIII510C (p. 000). The principal structural features of K52 have been determined (Table 2) but those of S XXVIII, which contains glucose, rhamnose, glycerol, and PO_4 [46], are not known.

An unusual feature of S XXIX is its content of both D-galactofuranosyl and D-galactopyranosyl residues [55]. It also contains galNAc and ribitol-PO_4 ([46] and Table 3). One can only guess at the reasons for the limited cross-reactions recorded in Table 4.

Cross-reactions in anti-Salmonella *sera*

Since no massive precipitations occurred in the limited number of available antisera, the reactions recorded in Table 4 are mainly qualitative. All of the

K substances which precipitate + + ± or + + + contain at least two sugars in common with the corresponding O antigen. K24, like the antigen of group A, contains 1,2- linked D-man, as does K28 [26]. Unfortunately, little is known of the structures of the other reactive Ks, except for K7, which precipitates *anti-paratyphi A* for no obvious reason. K28 and K38 are said to have non-reducing end-groups of β-D-glc, but the former precipitates anti-*paratyphi A* and anti-*S. senftenberg* (antigen 1?) while the latter fails to react with anti-*para A* and precipitates anti-*senftenberg* rather weakly. K1 is also said to contain end-groups of D-glc but reacts + + only in anti-*paratyphi B*, of the three sera tested (Table 4).

CONCLUSION

The present compilation of new and previously obtained data provides insights relating the chemical constitution and structure of many *Klebsiella* K polysaccharides to their immunological specificity. Both the qualitative and quantitative serological tests are relatively simple and rapid. Positive cross-reactions vary from the immediate, even at 0°C, the temperature preferred cf. for example, [10]), to approximately 2 weeks for completion, and, when clear-cut interpretation is possible, yield structural information that could take months to obtain by purely organic chemical means. Examples are:

1. Although the structure of Pn S I is only partially known (Table 3), its content of D-galA permits the prediction that in K49 and K63, which react strongly with anti-Pn I, the galA will be found to be in the D-form and at least partly in linkages similar to those in S I.

2. The strong precipitation of K2, K8, and K20 in anti-Pn II confirms the assignment, based on chemical studies, of non-reducing end-groups of D-glcA to the repeating units of these substances (cf. Tables 2 and 4).

3. The presence of such groups in K9 and K59 was predicted from their heavy precipitation of anti-Pn II and later confirmed chemically [20, 33].

4. It is probable that non-reducing end-groups of D-glc might fit into sites on molecules of anti-Pn II homologous for D-glcA. This explanation of the reactivity of glycogen [10, 78], certain dextrans [59] and synthetic polyglucoses [78] in anti-Pn II somewhat weakens the prediction (p. 75) that end-groups of D-glcA would be found in K15, K23, K27, K30, K33, K45, K51 and K55. However, K1, K18, K28, K38 and K54, which are known to possess end-groups of D-glc, react not at all or only very weakly in the anti-Pn II used. Moreover, glycogen and a large number of dextrans precipitated much smaller amounts of antibody than did the first series of K-substances in the same antiserum.

5. Presence of cellobiouronosyl residues in K5 was suspected during structural studies [17] and confirmed by the cross-reactivity of K5 in anti-Pn III and -VIII (Table 4) and by its precipitation (footnote [f]) of a portion of the same fraction of anti-Pn III as was reactive with S VIII. Although similar reasoning was used to identify a glcA- and glc-containing aldobiouronic acid isolated from the slime elaborated by *Sphaerotilus natans* [81], it now appears that a polysaccharide such as that of the fruit bodies of the mold *Fomes annosus*, which contains the residues -D-glcA-β-(1 → 4)-D-glcA and -D-glcA-β-(1 → 3)-D-glc [64] can also precipitate anti-Pn III and -VIII (unpublished results).

6. The very heavy precipitation of anti-Pn XXIII by K47 [54] showed that it must contain non-reducing end-groups of L-rham. A prediction of similar end-groups in K56 was made as a result of lower, but still strong reactivity in the same antiserum. Both deductions were verified ([28, 31] Table 2).

7. Other instances of reactivity in different anti-pneumococcal sera are discussed in the text, but the interpretation of many promising cross-reactions remains unclear because of lack of knowledge of the structure of the polysaccharide of the pneumococcal type which stimulated the antibody.

8. Comparison of the presently known compositions of the *Klebsiella* K and pneumococcal capsular polysaccharides shows that : (a) many K substances contain D-mannose, which has not yet been found in any pneumococcal type-specific substance; (b) K substances apparently never contain aminosugars, while many Pn S do, some showing as many as three. The extent of participation of aminosugars in the overall specificities of the pneumococcal types containing them is still uncertain and requires investigation.

9. In a current extension of earlier studies on cross-reactivities of *Klebsiella* [16] the present investigation has served as a guide to numerous hitherto unsuspected relationships between the K types.

Acknowledgements—The technical assistance of W. P. Grosvenor and C. T. Fang is gratefully acknowledged.

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