IMMUNOCHEMISTRY OF THE PNEUMOCOCCAL TYPES II, V, AND VI

I. The Relation of Type VI to Type II and Other Correlations between Chemical Constitution and Precipitation in Antisera to Tyye VI[1]

MICHAEL HEIDELBERGER and PAUL A. REBERS

Institute of Microbiology, Rutgers, the State University, New Brunswick, New Jersey

Received for publication November 23, 1959

This paper deals with immunochemical aspects of the relation between the pneumococcal Types II and IIB, first noted by Avery (1915) as a weak and incomplete cross-agglutination and studied in greater detail by Stillman (1919). Atypical, IIB strains were later designated Type VI (Cooper, Edwards, and Rosenstein, 1929). Studies with Avery's IIA, now known as Type V, are also under way, for it is believed that if the immunological findings in these three instances of cross-relationship could be correlated with the fine structures of the polysaccharides which constitute the determinants of the type-specificities, a definite contribution would be made to an understanding of the chemical basis of immunological specificity. As will be seen, the work has been facilitated by inclusion of the cross-reactivities, in Types II and VI antipneumococcal horse sera, of several other polysaccharides of known or partially known constitution. The chemical basis for the cross-relation of these two pneumococcal types would now seem to be established.

MATERIALS AND METHODS

The antisera used were supplied by the Division of Laboratories and Research, Department of Health, State of New York, kindness of Jessie L. Hendry, and by the Bureau of Laboratories, New York City Department of Health, through the courtesy of Annabel W. Walter. Antibody content was determined according to Heidelberger and Kendall (1935*a*, *b*, *c*) and cross-precipitations were quantitatively estimated by the use of suitable amounts of antiserum and polysaccharide. For the cross-reactivities the tubes were allowed to stand in a bath at 0 C for 10 to 15 days, with twirling during the first few days (Heidelberger, 1955; Heidelberger *et al.* 1957), and, after centrifugation, were drained in a cold box (Heidelberger and Rebers, 1958). The precipitates, after washing twice at 0 C were analyzed according to Markham (1942).

The specific polysaccharides of Types II, VI, and XIV pneumococci (hereinafter designated SII, SVI, SXIV) were prepared by E. R. Squibb and Sons, and obtained through the courtesy of T. D. Gerlough. The first was further purified by E. A. Kabat, (Beiser, Kabat, and Schor, 1952), the last two in this laboratory (Rebers and Heidelberger, 1959) (Barker *et al.* 1958). The hemolytic streptococcal group A and V substances (McCarty, 1956; McCarty and Lancefield, 1955) were kindly supplied by M. McCarty. The oyster glycogen fraction A_{1b} was prepared by B. Björklund (Heidelberger *et al.* 1957), dextrans N236 and 1355-S-4 were furnished by E. A. Kabat, and the oat and barley glucans by Ian Preece. Carob mucilage and Khaya gums were contributed by E. L. Hirst, guar gum, as well as flax straw hemicellulose, by Fred Smith, and okra mucilage by R. L. Whistler. A sample of partially degraded polysaccharide of *B. polymyxa* was kindly supplied by A. Misaki.

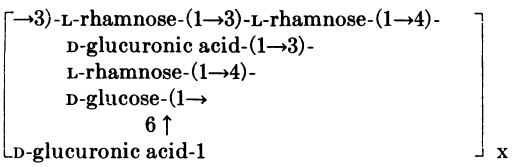
The results of the analyses are given in the tables. All results, including quantities of polysaccharides, are calculated to 1.0 ml of serum, although volumes of sera ranging from 0.20 to 3.0 ml were used, depending upon the intensity of reaction.

RESULTS AND DISCUSSION

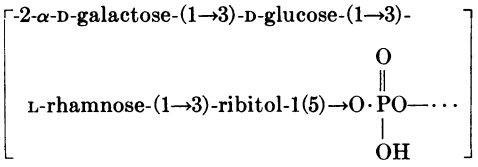
Although several details of the fine structures of SII and SVI remain to be determined, enough is now known to permit designation of the common structural unit, multiples of which permit cross-precipitation. A possible, though not unique

[1] Carried out in part under a grant from the National Science Foundation.

formula for SII is:



Whether the linkages are α, β, or both, has not been determined (Butler and Stacey, 1955). This is partly true of SVI, although the remaining features of the structure appear to be uniquely established (Rebers and Heidelberger, 1959; Rebers and Heidelberger, 1960, *unpublished data*):



The repeating unit of SVI is thus a secondary phosphate of a D-galacto-D-gluco-L-rhamno-ribitol. The phosphate-free portion of the unit, which crystallizes readily, is built up into a linear polymer by means of phosphate bridges. It does not appear to be branched, as is SII, and contains galactose, ribitol, and phosphate as structural units which are absent in SII. On the other hand, SII contains glucuronic acid, which does not occur in SVI, and though the remainder of its molecule is built up of D-glucose and L-rhamnose residues, only the latter are linked in the same way, 1→3, in both substances. If, as would appear likely from the quantitative precipitin theory of Heidelberger and Kendall (1935*b*), cross-precipitation is due to the mutual occurrence in SII and SVI of multiples of a grouping in common, in this instance 1,3-linked L-rhamnose, one would not expect the reaction to be extensive, since end-groups are not involved (for example, Kabat, 1954, 1956), the 1,3-linked rhamnose residues occurring within the main chain of sugars in both polysaccharides. This is also in accord with the weak and delayed agglutination described by Avery (1915) and with the very limited cross-protection observed by Cooper *et al.* (1932). Let us now examine the evidence furnished by the quantitative data in the tables.

It is evident from table 1 that the three available type II antipneumococcal horse sera failed to precipitate SVI. This was unexpected, since type VI (IIB) strains were known to show agglutination, although weak and delayed, in type II antisera. The absence of cross-precipitation in this direction may doubtless be ascribed to the greater sensitivity of the test for bacterial

TABLE 1

Precipitation of types II and VI antipneumococcal horse sera by specific polysaccharides of types II and VI pneumococci

(μg antibody nitrogen from 1.0 ml at 0 C)

Polysaccharide and Amount Used		Type II Antiserum			Type VI Antiserum		
		513	1054C[a]	930C[a]	614	681C[a]	771C[a]
	mg	*μg*	*μg*	*μg*	*μg*	*μg*	*μg*
SII	0.02					18	
	0.03					21	
	0.04						129[b]
	0.06			710		19	
	0.08						152[b]
	0.2						155[e]
	0.4		1010[c]				
	0.5						137
	0.6				4[d]		
	1.25	3600			3		
SVI	0.005	0	0	0			
	0.015		1	0			
	0.13	0	0				760[f]
	0.14					724	
	0.18						751
	0.4	0			1400		
	1.5	0	0				

[a] Adsorbed with pneumococcal C-polysaccharide. Sera 513 and 614 gave practically no precipitate, the others 98, 17, 12, and 171 μg N, respectively.

[b] Supernatants + streptococcal group A V substance gave 3 μg N (Table 2); subsequent addition of 0.07 mg okra gave 38 μg N (Table 4). SII supernatants + 0.3 mg flax straw hemicellulose gave 10 μg N (Table 2).

[c] Analysis on unadsorbed serum.

[d] Smaller amounts of SII gave only traces of precipitate.

[e] At 37 C 86 μg N were precipitated. On cooling, the supernatants immediately became turbid, and on standing at 0 C, deposited an additional 40 μg N.

[f] At 37 C, with 0.125 mg SVI, 604 μg N were precipitated and the supernatants remained clear at 0 C. In another run with 0.14 mg SVI, 605 μg N were precipitated at 37 C and the supernatants deposited an additional 28 μg at 0 C.

agglutination. In the reverse direction, precipitation was negligible in the strongest of the antipneumococcal VI sera, weak in another, and involved 20 per cent of the antibody in the third. Two antipneumococcal VI rabbit pools failed to precipitate with SII. Type VI antisera thus show great variation in cross-reactivity, and only in two of the five tested were appreciable amounts of antibody capable of engaging closely enough with the 1,3-linked rhamnose residues in SII to form precipitates. That the relatively large precipitate yielded by C-absorbed antipneumococcal VI serum 771 with SII was indeed a cross-reaction differing in its behavior from that of the homologous reaction which might have resulted if the horse had received pneumococcal type II as well as pneumococcal type VI, was shown by carrying out the precipitation at 37 C as well as at 0 C (table 1, footnotes *e* and *f*). In accord with other studies on cross-reactions at the two temperatures (Heidelberger, Kabat, and Mayer, 1942; Heidelberger, Aisenberg, and Hassid, 1954) far less type VI antibody N was precipitated by SII at 37 C than at 0 C: 86 μg as against 155, but the values for the homologous SVI anti-VI reaction were 605 and 760 μg. The supernatants from the cross-reaction deposited an additional 40 μg N on cooling to 0 C, at which point reduced thermal agitation enabled more SII anti-VI aggregates to form. Supernatants from the homologous reaction remained clear when the minimal amount of SVI necessary for maximum precipitation was used, apparently containing only "univalent" antibody (Heidelberger and Kendall, 1935*c*), but deposited 28 μg N with slightly more SVI.

Confirmation of the purely chemical evidence that the pneumococcal II–VI relationship was due to multiple 1,3-linked L-rhamnose residues in the specific polysaccharides of both types was obtained by further quantitative immunochemical studies. Recently (Heidelberger and Adams, 1956) the cross-reactivity of the C-carbohydrate of Group A hemolytic streptococci in Type II antipneumococcal sera was ascribed to its rhamnose content. After the discovery of the V-variant (McCarty and Lancefield, 1955; McCarty 1956) the prediction could be made that the V-carbohydrate would precipitate anti-pneumococcal II better than the A-substance because much of the *N*-acetylglucosamine of the latter had been stripped off, leaving the rhamnose residues in greater proportion and in more accessible, if not terminal positions in the molecule of V-carbohydrate. This prediction was verified, although the difference in one of the two sera was small (Heidelberger and McCarty, 1959). If the basis for pneumococcal II–VI cross-reactivity were actually the common possession of multiple 1,3-linked rhamnose residues in the antigenic determinants of both types, one might expect antipneumococcal VI to behave toward the streptococcal A and V carbohydrates much as did antipneumococcal II. Perusal of table 2 will show this expectation to have been fulfilled, again in the case of the more potent of the two cross-reactive sera. It will also be noted that serum 614, which gave only minute amounts of precipitate with SII, also failed to react with A and V. Thus the reactivity of the streptococcal carbohydrates in anti-pneumococcal VI not only supplies confirmatory evidence that the pneumococcal II–VI relationship is due to multiple 1,3-linked rhamnose residues in both, but also makes it more certain (Heidelberger and McCarty, 1959) that when the chemical structure of the streptococcal substances is elucidated, part of their rhamnose residues, at least, will be found to be linked 1,3-.

Two other polysaccharides known to contain 1,3-bound L-rhamnose were also tested in anti-pneumococcal VI. Flax straw hemicellulose, which is made up, in addition, of D-xylose and 4-*O*-methyl-D-glucuronic acid, reacted more strongly in sera 771C and 681C (table 2) than might have been anticipated, since less than 2 per cent of 2,4-di-*O*-methyl rhamnose was isolated from the hydrolytic products of the methylated hemicellulose (Geerdes and Smith, 1955). Possible reasons for this will be investigated. The other polysaccharide with known 1,3-L-rhamnose residues was a partially degraded rhamnogalactan from *Bacillus polymyxa* (Misaki and Teramoto, 1958). This was tested qualitatively in serum 771C, and reacted readily to give a disc.

Since SVI contains, at intervals, single residues of 1,3-linked D-glucose, it could be predicted that other carbohydrates containing bound 1,3-glucose would also precipitate antipneumococcal VI. Table 3 shows the data obtained in this category. First it will be noted that the β-amylase limit dextrin of oyster glycogen A_{1b} (Heidelberger, Björklund, and Larner, 1957)

TABLE 2

Precipitation of types II and VI antipneumococcal horse sera by hemolytic streptococcal group A and variant (V) carbohydrates and flax hemicellulose

(μg antibody N from 1.0 ml at 0 C)

Polysaccharide and Amount Used		Type II Antiserum 513[a]	Type VI Antiserum 614	Type VI Antiserum 681C	Type VI Antiserum 771C
	mg	*μg*	*μg*	*μg*	*μg*
Group A substance	0.02	14			
	0.03	16			
	0.05	3	0		
	0.08				8
	0.1			12[b]	
	0.15		0		
	0.2			11	13
	0.5		1		21
	1.0				22, 26
Group A V substance	0.03	66			
	0.05	63	0		
	0.08				32
	0.1			15[b]	
	0.16	43	0		68[c]
	0.2			12	
	0.3				63[c]
	0.5		1		
	0.6				38
Flax hemicellulose	0.07			22	
	0.2			40	
	0.3				71
	0.4			56	
	0.6				104[d]
	0.8			59	
	1.0				107

[a] Data from Heidelberger and McCarty, 1959.

[b] Combined supernatants + 0.025 mg SII gave 14 μg N (table 1).

[c] Supernatants + 0.08 mg SII gave 54 μg N.

[d] Supernatants + 0.06 mg SII gave 34 μg N.

and dextran N236 (Kabat and Berg, 1953) fail to yield more than traces of precipitate with antipneumococcal VI sera 614 and 771C, although both polyglucoses react heavily with antipneumococcal II serum 513 by virtue of their α-1,6-bound glucose. Dextran N236 contains 4 per cent of 1,3-like linkages. On the other hand, dextran 1355-S-4, which contains 34 per cent of α-1,3-like linkages (Rankin and Jeanes, 1954; Jeanes *et al.*, 1954), precipitated 2.5 per cent of the antibodies in serum 614 and 6 per cent of those in serum 681C. Oat and barley glucans (Preece and Mackenzie, 1952; Aspinall and Telfer, 1954; Acker, Diemair, and Samhammer, 1955), with roughly equal numbers of β-1,3- and β-1-4-linkages, gave appreciable but smaller precipitates with the same two sera. It will be recalled that these glucans reacted with antipneumococcal VIII by virtue of multiples of adjacent β-1,4-bound glucose (cellobiose) residues (Heidelberger and Rebers, 1958). As indicated in the footnotes to table 3, prior precipitation of the sera with either α-1,3- or β-1,3-containing glucans failed to alter greatly the amounts of antibody precipitable by the other. The meaning of this in terms of the chemistry of SVI is not yet clear. Notable, also, is that serum 771C, the most reactive toward carbohydrates containing 1,3-linked rhamnose, gave negligible precipitates with those in which glucose was similarly bound.

TABLE 3

Precipitation of types II and VI antipneumococcal horse sera by polyglucoses

(μg antibody N from 1.0 ml at 0 C)

Polyglucose and Amount Used		Type II Antiserum 513	Type VI Antiserum 614	Type VI Antiserum 681C	Type VI Antiserum 771C
	mg	*μg*	*μg*	*μg*	*μg*
Oyster glycogen A_{1b}	6.0	188[a]			
β-Amylase limit dextrin A_{1b}	0.2		0	11[c]	
	0.6	400[a]	0	17[c]	1
	2.0			20	
Dextran 1355-S-4	0.05				2
	0.15		35[d]	34	1
	0.2		39[e]		
	0.3		36[d]	42[f]	
	0.6		29[d]	43[f]	
	0.7	575[b]			
Dextran N236	0.1	500[b]			
	0.15		0		
Barley glucan	0.03	2			
	0.05				1
	0.06	3			
	0.1			11	
	0.15				1
	0.2	0	25		
	0.3		26[g]	14	
	0.6	1	10[h]		
Oat glucan	0.05				1[i]
	0.15		16		3[i]
	0.3		22		0
	0.5		19		

[a] From Heidelberger *et al.*, 1957.
[b] From Goodman and Kabat, 1960.
[c] Supernatants + 0.3 mg barley, dextran 1355-S-4, gave 10, 27 μg N, respectively.
[d] Combined supernatants + 0.15 mg oat, 0.3 mg barley gave 16, 31 μg N, respectively.
[e] Supernatants + 0.15 mg oat, barley, 0.013 mg SXIV (table 4) gave 16, 20, 23 μg N, respectively.
[f] Supernatants + 0.075 mg barley gave 9 μg N.
[g] Supernatants + 0.3 mg dextran 1355-S-4 gave 31 μg N.
[h] Single determination.
[i] Supernatants + 0.05, 0.15 mg dextran gave 0, 0 N.

An apparently discordant result is the appreciable precipitation of antipneumococcal VI 681C by β-amylase limit dextrin. About 1 per cent of periodate-resistant glucose has been found in glycogen (Abdel-Akher *et al.*, 1952) and may accordingly be linked 1,3-. If such glucose residues were responsible one would have expected precipitation in serum 614 as well, for it is the best reactor of the three sera with oat and barley glucans. It is more likely that the reaction in 681C is another instance of the precipitation of glycogen (and the β-amylase limit dextrin derived from it) in occasional so-called "normal" horse sera (Staub and Grabar, 1943; Heidelberger, Aisenberg, and Hassid, 1954). If this were true, the greater reactivity of dextran 1355-S-4 in serum 681C than in 614 would be accounted for by addition of the 1,6-reactive portion of

TABLE 4

Precipitation of type VI antipneumococcal horse sera by polysaccharides with nonreducing end groups of galactose

(μg antibody N from 1.0 ml at 0 C)

Polysaccharide and Amount Used		Serum 614	Serum 681C	Serum 771C
	mg	*μg*	*μg*	*μg*
SXIV	0.02	23[a]		
	0.06	25[a]	17	
	0.12		15	
Carob	0.1	41	78[d]	1
	0.2	54, 54[b]	89[d]	3
	0.4	56[b]		
Guar	0.1	89[c]		
	0.2		106	
	0.3	91[c]		
	0.4		105	
Khaya grandifolia	0.25	58		
	0.5	71, 76		
	1.25	65		
Khaya senegalensis[g]	0.08	1		
	0.2	4		
	0.5	0		
Okra	0.1	237[e]		41
	0.2		218	47[f]
	0.3	298[e]		
	0.4		228, 210	42
	1.0	276[e]		

[a] Supernatants + carob gave 26 μg N. With antipneumococcal XIV #635C, SVI gave a maximum of 15 μg N/ml; SVI treated with 0.02 N NaOH at 25 C for 1½ hr. (5 per cent sec. PO_4 split) gave 9 μg N.

[b] Supernatants + 0.4 mg okra gave 213 μg N, with 0.3 mg barley, 33 μg N. Supernatants from the last + 0.4 mg *Khaya grandifolia* gave 51 μg N, supernatants from this + okra gave 143 μg. Total cross reactions: 281, minus barley, 248 μg N.

[c] Analyses by Mrs. Esther Hurwitz.

[d] Combined supernatants + 0.2 mg okra gave 116 μg N.

[e] Supernatants + 0.2 mg carob gave 9 μg N; the supernatants from this, with 0.3 mg *Khaya grandifolia* gave no precipitate.

[f] Supernatants + 0.14 mg SII gave 153 μg N.

[g] Crude gum. After standing in alkaline solution, as for *K. grandifolia*, 0.2, 0.5 mg precipitated 36, 35 μg N from serum 614.

the antibodies to the smaller amount precipitated by barley glucan.

In table 4 are given data on a series of cross-reactions in antipneumococcal VI sera which appear to depend upon multiple, nonreducing terminal groups of D-galactose in the precipitating polysaccharides. From the formula on p. 146 it is evident that such groupings do not seem to occur in SVI. However, in this, D-galactose is linked 1,2-, with the acidic —OH of the secondary phosphate close by, and with positions 3,4, and 6 free. It is probable, therefore, that galactose bound in this manner in the complex antigen of pneumococcus type VI, of which the polysaccharide portion is the principal determinant of specificity, can stimulate the production of much the same kind of antibodies as those engendered in animals by pneumococcus type XIV, for example, in which end-groups of galactose actually occur (Barker *et al.*, 1958) and were predicted before their chemical identification on the basis of the cross-reactivity of antipneumococcal XIV with substances such as carob and guar (Heidelberger, 1955; Heidelberger, Barker, and Björklund, 1958). The consequent mutual cross-precipitation of SVI and SXIV therefore comes as no surprise, although the cross-relation of these two pneumococcal types appears to have been known for a long time. Dr. Alfred J. Weil has pointed out the frequent preparation, years ago, of combined antisera for pneumococcal types VI, XIV, by injection of both types of pneumococci into horses. The extent to which carob gum (Hirst and Jones, 1948; Smith, 1948), and guar mucilage (Rafique and Smith, 1950) precipitate antipneumococcal VI sera appears to be in the order of their content of D-galactose end groups, 16 to 20 and 33 per cent, respectively.

Khaya grandifolia and *Khaya senegalensis* gums are said to have much the same structure (Aspinall, Hirst, and Matheson, 1956), being highly branched, acetylated, and composed of galacturonic and 4-*O*-methylglucuronic acids, galactose, 1,4- or 1,2,4-linked L-rhamnose and traces of arabinose. Since the L-rhamnose probably has little or nothing to do with their cross-reactivity in this instance, the far greater precipitation by grandifolia is perhaps due to the occurrence of many more nonreducing end-groups of galactose than in senegalensis. However, the grandifolia gum had been treated with alkali,

resulting in the removal of any acetyl groups present, whereas the senegalensis sample consisted of the crude gum.[2]

The best reactor in this group of gums is okra, composed of D-galacturonic acid, D-galactose, and L-rhamnose (Whistler and Conrad, 1954). Part of the galactose is linked 1,4- and at least part of the rhamnose 1,2-, but since the remaining features of the structure of the mucilage are unknown one can only speculate as to the causes of the extensive cross-reaction.

As in an earlier study on antipneumococcal II sera (Heidelberger and Adams, 1956), the sum of the three partial specificities does not equal the total antibody in any of the three sera studied. In only one of the sera, 771C, does the cross-reactivity of SII approach that characteristic of the pneumococcus III-VIII cross-reaction (Heidelberger, Kabat, and Shrivastava, 1937; Heidelberger, Kabat, and Mayer, 1942) which is due to the presence of multiples of cellobiuronic acid in both SIII and SVIII; that is, a two-sugar portion in common of each unit of the linear molecular chain. Cross-reactions of 10 to 12 per cent of the antibody in type VIII antisera by oat and barley glucans, which contain multiple residues of two glucose units as cellobiose, have been discussed by Heidelberger and Rebers (1958).

The type II–VI relationship has, since its beginning, been recognized as relatively weak and incomplete, and the quantitative data given in the present paper amply confirm this conclusion. The studies on the fine-structure of the type II polysaccharide have thus far afforded no evidence of end-groups other than those of D-glucuronic acid (Butler and Stacey, 1955). As the type VI product appears to be a wholly linear substance (unpublished experiments show a 96 per cent yield of crystalline, phosphate-free repeating unit on cleavage of alkali-degraded SVI with alkaline phosphatase), it is concluded, on the present evidence, that the cross-reactivity between the two types is due to the 1,3-linked L-rhamnose residues which occur at frequent intervals in the main chain of SII and constitute every fifth member (phosphate included) of the main chain of SVI (p. 146). If, then, the cross-reaction is due to multiple interior residues of the same sugar in the same linkage, one would not expect the reaction to be as extensive as if terminal groupings were concerned (for example, Kabat, 1954, 1956; Heidelberger, Björklund, and Larner, 1957; Goodman and Kabat, 1960; Heidelberger, 1960).

The unusual 1,2-linkage indicated for galactose in SVI, and its position adjacent to a phosphate group, apparently enable this sugar residue to function like an end group in the animal stimulated to produce anti-SVI. This would account for the cross-reactions with other polysaccharides containing nonreducing end groups of galactose, notably the galactomannans, which precipitate up to 15 per cent of anti-SVI. The lower reactivity of SXIV in antipneumococcal VI is perhaps due to the greater complexity of this substance and the wider scatter of terminal galactose residues in it.

[2] After treatment with alkali, senagalensis precipitated one-half as much N as did alkaline-treated grandifolia.

ACKNOWLEDGMENT

The technical assistance of Check M. Soo Hoo in part of the work is gratefully acknowledged.

SUMMARY

Recent structural chemical studies on the specific capsular polysaccharides of the cross-reacting types II and VI (formerly IIB) pneumococci are correlated with quantitative microanalytical data on specific precipitation in antisera to the two types. Wide variations are shown in the cross-precipitation of SII in different type VI antisera, as well as in the precipitation of these sera by numerous gums of known constitution. The type-specificity of pneumococcus VI is resolved into partial specificities due to multiple residues in the long, phosphate-linked polysaccharide chain, of 1,3-linked L-rhamnose, 1,3-linked D-glucose, and 1,2-bound D-galactose. These partial cross-reactivities are discussed in detail and the rather limited type II–VI relationship is shown to be due to the presence of multiple 1,3-linked L-rhamnose residues in the specific polysaccharides of both types of pneumococci.

REFERENCES

ABDEL-AKHER, M., HAMILTON, J. K., MONTGOMERY, R., AND SMITH, F. 1952 A new procedure for the determination of the fine structure of polysaccharides. J. Am. Chem. Soc., **74**, 4970–4971.

ACKER, L., DIEMAIR, W., AND SAMHAMMER, E. 1955 Über das Lichenin des Hafers. II. Molekulargewichtsbestimmungen und weitere Untersuchungen zur Konstitution. Z. Lebensm.-Untersuch, u. Forsch., **102,** 225–231.

ASPINALL, G. O. AND TELFER, R. G. J. 1954 Cereal gums. I. The methylation of barley glucosans. J. Chem. Soc., **1954,** 3519–3522.

ASPINALL, G. O., HIRST, E. L., AND MATHESON, N. K. 1956 Plant gums of the genus *Khaya*. The structure of *Khaya grandifolia* gum. J. Chem. Soc., **1956,** 989–997.

AVERY, O. T. 1915 A further study of the biologic classification of pneumococci. J. Exptl. Med., **22,** 804–819.

BARKER, S. A., HEIDELBERGER, M., STACEY, M., AND TIPPER, D. J. 1958 Immunopolysaccharides. X. The structure of the immunologically specific polysaccharide of pneumococcus type XIV. J. Chem. Soc., **1958,** 3468–3474.

BEISER, S. M., KABAT, E. A., AND SCHOR, J. M. 1952 Immunochemical studies on the specific polysaccharide of type II pneumococcus. J. Immunol., **69,** 297–309.

BUTLER, K. AND STACEY, M. 1955 Immunopolysaccharides. IV. Structural studies on the type II pneumococcus specific polysaccharide. J. Chem. Soc., **1955,** 1537–1541.

COOPER, G., EDWARDS, M., AND ROSENSTEIN, C. 1929 The separation of types among the pneumococci hitherto called group IV, and the development of therapeutic antiserums for these types. J. Exptl. Med., **49,** 461–474.

COOPER, G., ROSENSTEIN, C., WALTER, A., AND PEIZER, L. 1932 The further separation of types among the pneumococci hitherto included in Group IV and the development of therapeutic antisera for these types. J. Exptl. Med., **55,** 531–534.

GEERDES, J. D. AND SMITH, F. 1955 The constitution of the hemicellulose of the straw of flax (*Linum usitatissimum* sp.). I. Identification of 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose. II. Hydrolysis of the methylated hemicellulose. J. Am. Chem. Soc., **77,** 3569–3572; 3572–3576.

GOODMAN, J. W. AND KABAT, E. A. 1960 Immunochemical studies on cross-reactions of antipneumococcal sera. I. Cross-reactions of type II and XX antipneumonococcal sera with dextran and of type II antipneumococcal sera with glycogen and Friedländer type B polysaccharide. J. Immunol., **84,** 333–346.

HEIDELBERGER, M. 1955 Immunological specificities involving multiple units of galactose. II. J. Am. Chem. Soc., **77,** 4308–4311.

HEIDELBERGER, M. 1960 The immunological specificity of type II pneumococcus and its separation into partial specificities. II. J. Exptl. Med., **111,** 33–43.

HEIDELBERGER, M. AND KENDALL, F. E. 1935*a* The precipitin reaction between Type III pneumococcus polysaccharide and homologous antibody. II. Conditions for quantitative precipitation of antibody in horse sera. J. Exptl. Med., **61,** 559–562.

HEIDELBERGER, M. AND KENDALL, F. E. 1935*b* III. A quantitative study and a theory of the reaction mechanism. J. Exptl. Med., **61,** 563–591.

HEIDELBERGER, M. AND KENDALL, F. E. 1935*c* A quantitative theory of the precipitin reaction. III. The reaction between crystalline egg albumin and its homologous antibody. J. Exptl. Med., **62,** 697–720.

HEIDELBERGER, M. AND ADAMS, J. 1956 The immunological specificity of type II pneumococcus and its separation into partial specificities. J. Exptl. Med., **103,** 189–197.

HEIDELBERGER, M. AND REBERS, P. A. 1958 Cross reactions of polyglucoses in antipneumococcal sera. VI. Precipitation of type VIII and type III antisera by β-glucans. J. Am. Chem. Soc., **80,** 116–118.

HEIDELBERGER, M. AND MCCARTY, M. 1959 Cross-reactions of streptococcal A and V carbohydrates in type II antipneumococcal horse sera. Proc. Natl. Acad. Sci. U. S., **45,** 235–238.

HEIDELBERGER, M., KABAT, E. A., AND SHRIVASTAVA, D. L. 1937 A quantitative study of the cross reaction of types III and VIII pneumococci in horse and rabbit antisera. J. Exptl. Med., **65,** 487–496.

HEIDELBERGER, M., KABAT, E. A., AND MAYER, M. 1942 A further study of the cross reaction between the specific polysaccharides of types III and VIII pneumococci in horse antisera. J. Exptl. Med., **75,** 35–47.

HEIDELBERGER, M., AISENBERG, A. C., AND HASSID, W. Z. 1954 Glycogen, an immunologically specific polysaccharide. J. Exptl. Med., **99,** 343–353.

HEIDELBERGER, M., BJÖRKLUND, B., AND LARNER, J. 1957 Cross-reactions of polyglucoses in antipneumococcal sera. V. Precipitation by glycogens and limit dextrins. J. Immunol., **78,** 431–434.

HEIDELBERGER, M., JAHRMÄRKER, H., BJÖRKLUND, B., AND ADAMS, J. 1957 Cross-reactions of polyglucoses in anti-pneumococcal sera. III. Reactions in horse sera. J. Immunol., **78,** 419–426.

HEIDELBERGER, M., BARKER, S. A., AND BJÖRKLUND, B. 1958 Immunological specificities

involving multiple units of galactose. III. J. Am. Chem. Soc., **80,** 113–116.

HIRST, E. L. AND JONES, J. K. N. 1948 The galactomannan of carob-seed gum (gum gatto). J. Chem. Soc., **1948,** 1278–1282.

JEANES, A., HAYNES, W. C., WILHAM, C. A., RANKIN, J. C., MELVIN, E. H., AUSTIN, M. G., CLUSKEY, J. E., FISHER, B. E., TSUCHIYA, H. M., AND RIST, C. E. 1954 Characterization and classification of dextrans from ninety-six strains of bacteria. J. Am. Chem. Soc., **76,** 5041–5052.

KABAT, E. A. 1954 Some configurational requirements and dimensions of the combining site on an antibody to a naturally occurring antigen. J. Am. Chem. Soc., **76,** 3709–3713.

KABAT, E. A. 1956 Heterogeneity in extent of the combining regions of human antidextran. J. Immunol., **77,** 377–385.

KABAT, E. A. AND BERG, D. 1953 Dextran, an antigen in man. J. Immunol., **70,** 514–532.

MARKHAM, R. 1942 A steam distillation apparatus suitable for micro-Kjeldahl analysis. Biochem. J., **36,** 790–791.

McCARTY, M. 1956 Variation in the group-specific carbohydrate of group A streptococci. II. Studies on the chemical basis for serological specificity of the carbohydrates. J. Exptl. Med., **104,** 629–643.

McCARTY, M. AND LANCEFIELD, R. C. 1955 Variation in the group-specific carbohydrate of group A streptococci. I. Immunochemical studies on the carbohydrates of variant strains. J. Exptl. Med., **102,** 11–28.

MISAKI, A. AND TERAMOTO, S. 1958 Studies on the bacterial polysaccharide, rhamnogalactan, produced by *B. polymyxa* var. XI. Methyl sugar components of the methylated rhamnogalactan. J. Fermentation Technol., **36,** 266–271.

PREECE, I. A. AND MACKENZIE, K. G. 1952 Non-starchy polysaccharides of cereal grains. II. Distribution of water-soluble gum-like materials in cereals. J. Inst. Brewing, **58,** 457–464.

RAFIQUE, C. M. AND SMITH, F. 1950 The constitution of guar gum. J. Am. Chem. Soc., **72,** 4634–4637.

RANKIN, J. C. AND JEANES, A. 1954 Evaluation of the periodate method for the structural analysis of dextrans. J. Am. Chem. Soc., **76,** 4435–4441.

REBERS, P. A. AND HEIDELBERGER, M. 1959 The specific polysaccharide of type VI pneumococcus. I. Preparation, properties and reactions. J. Am. Chem. Soc., **81,** 2415–2419.

SMITH, F. 1948 The constitution of carob gum. J. Am. Chem. Soc., **70,** 3249–3253.

STAUB, A. M. AND GRABAR, P. 1943 Etude quantitative de la précipitation de sérums anticharbonneux et normaux par différents solutions de gelose. Ann. inst. Pasteur, **69,** 268–274.

STILLMAN, E. G. 1919 A study of atypical type II pneumococci. J. Exptl. Med., **29,** 251–258.

WHISTLER, R. L. AND CONRAD, H. E. 1954 A crystalline galactobiose from acid hydrolysis of okra mucilage; 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose from okra mucilage. J. Am. Chem. Soc., **76,** 1673–1674; 3544–3546.