CROSS-REACTIONS OF ANTITYPHOID AND ANTIPARATYPHOID B HORSE SERA WITH VARIOUS POLYSACCHARIDES*

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While it has been known for many years that the cross-reactivity of polyglucoses such as the dextrans extends beyond precipitation in certain antipneumococcus sera to antisera of the *Salmonella* group (1), the wide scope of the cross-reactivity of the latter sera has not been realized. In the present paper both qualitative and quantitative studies are recorded of the reactivity in an antityphoid and an antiparatyphoid B serum of a synthetic polyglucose, of glycogen, and of a number of bacterial polysaccharides and plant gums. The findings are interpreted in the light of the quantitative theory of the precipitin reaction which postulates the interaction of multiple reactive groupings on antigen and antibody (2). This affords clues as to the linkages likely to be encountered between certain of the sugars, galactose, glucose, mannose, and rhamnose, which occur in the O-antigenic, acetic acid–degraded, specific polysaccharides of both typhoid and paratyphoid B bacilli.

EXPERIMENTAL

Materials and Methods.—The strains of *S. typhi* and *S. paratyphi* B used for immunization of the horses at the Institut Pasteur, Paris, were O 901 and B 50-19, with the antigenic composition IX, XII and I, IV, V, XII, respectively. The antisera, antityphoid 834, bleedings of April 11, 1950, and March 15, 1951, and anti-paratyphoid 1137, bleeding of November 4, 1952, were from the Institut Pasteur at Paris, and were kindly made available by Dr. Anne-Marie Staub of that institute. The writers are also grateful to Dr. Staub for samples of the appropriate polysaccharides and for a copy of the dissertation of Mlle. G. Pon on the chemistry and immunology of these substances (3).

Qualitative tests were usually set up at 0 to 2°C. with 1 ml. of antiserum and 0.05 mg. of polysaccharide. In the event of an immediate reaction several times more polysaccharide were again added. In most instances readings were taken after 5 to 10 days, with the tubes immersed in ice-water until read. Larger amounts of polysaccharide were then added and the procedure was repeated, as in some instances relatively much antigen is required for

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a positive cross-reaction. Readings range from − to ++++ according to the size of the precipitate.

Quantitative analyses of the amounts of nitrogen precipitated were carried out according to reference 4 except that in most instances smaller amounts of reactants were used and the nitrogen was estimated by the Markham method (5). Details of technique are to be found in reference 6.

The results of the qualitative tests are given in Table I; the quantitative data are summarized in Table II.

TABLE I

Qualitative Data on Cross-Reactions in Anti-Salmonella Horse Sera at 0 to 2°C.

Polysaccharide used	Antityphoid serum 834	Antiparatyphoid B serum 1137
S II*	++±; supernatant with yeast mannan, ++	+
S III	±(±)	±(±)
S VIII	+±	±
S IX	+±	±
S XII	+	±
S XIV	+	++
S XVIII	±(+±)	+(+±)
Synthetic polyglucose, fraction A_1	++	+±
Tamarind seed	++±	+
Arabogalactan, Jeffrey pine	++	++
Arabogalactan, larch	−(+±); supernatant with S II, +++	+
Degraded gum arabic	+±	+±
Gum ghatti	++	++
Yeast mannan	++	+
Carob mucilage	+	++++
Okra mucilage	−	+++
Agar, soluble fraction	+	+
Tubercle bacillus, fraction C I		+

Values in parentheses obtained after centrifugation in the cold.

* S, specific polysaccharide of pneumococcus, with type as indicated.

RESULTS AND DISCUSSION

Specific Polysaccharides of Pneumococcus.[1]—

Seven type-specific substances were used. Each was known to contain glucose, one of the four sugars identified (3) in the typhoid and paratyphoid B degraded polysaccharides. Several also contained rhamnose or galactose. The cross-reactions with S II and S XIV appeared especially significant and are discussed more fully than the others.

[1] Designated S, with the appropriate type number.

a. S II.—This polysaccharide is made up of D-glucose (7), L-rhamnose (8, 9), and D-glucuronic acid (10), of which the first two sugars appear in the two *Salmonella* polysaccharides. All of the glucose in S II is said to be in the form of 1,4,6-branch points, while the rhamnose is linked 1,3- (10). Qualitative and quantitative tests (Tables I and II) showed the reaction of S II to be far stronger in the antityphoid serum than in the antiparatyphoid serum. This may indicate that the glucose in the typhoid polysaccharide is similarly linked to that in S II, or that the rhamnose is bound 1,3-, or both. There is some evidence that multiple groupings of 1,6-linked glucose in certain carbohydrates may react in Type II antipneumococcus serum, as well as, or instead of 1,4,6-bound groupings (11, 12), a circumstance also to be considered in this instance. The weak cross-reaction of S II in the antiparatyphoid B serum permits no conclusions to be drawn, as the serum used might have been deficient in the particular cross-reactive antibody involved.

b. S III.—This is a polycellobiuronic acid (13) in which D-glucuronic acid is attached in β-linkage to position 4 of the glucose unit. This is, in turn, linked to position 3 of the next glucuronic acid. The cross-reactions were very slight.

c. S VIII.—The substance contains cellobiuronic acid units, additional glucose (14), and galactose.[2] Since its cross-reactivity is not very extensive, little can be said regarding its basis.

d. S IX.—This polysaccharide contains glucose, amino sugars, and a uronic acid (15) and also cross-reacts only weakly.

e. S XII.—Since S XII contains glucose, galactose, and amino sugars in unknown linkage (15), the slight cross-reaction is uninterpretable.

f. S XIV.—The cross-reaction of this substance, which contains glucose, galactose, and *N*-acetylglucosamine (15, 16), will be discussed below.

g. S XVIII.—This was tested because it is made up of D-glucose, L-rhamnose, and secondarily bound phosphate (17). Its slight cross-reactivity might be related to any of these components.

Polyglucoses, Plant Gums, and Mannans.—

a. Synthetic Polyglucose, fraction A_1.—This material, which cross-reacts heavily in a number of antipneumococcus sera (18), appears to contain all possible linkages of glucose, with 1,4-, 1,6- and 1,4,6- predominating (19). It precipitated about twice as much nitrogen from the antityphoid serum as did S II; the serum, absorbed with polyglucose, precipitated only 4 μg. of N with S II. This affords evidence that the rhamnose in S II plays little part in its cross-reactivity with the antityphoid serum used. The polyglucose reacted less strongly in antiparatyphoid serum, but precipitated many times more N from it than did S II.

b. Glycogen and Tamarind Seed Polysaccharide (jellose).—Jellose, like glycogen, contains 1,4,6-branch points of glucose; two-thirds of its glucose is in this form, while a third is linked 1,4-. The remainder of the substance consists of xylose and galactose, of which all of the latter is in the form of non-reducing end groups (20). Jellose and glycogen precipitate roughly the same amount of nitrogen from the antityphoid serum as does S II, but the reactive antibody is not entirely the same in all three

[2] Personal communication from Prof. J. K. N. Jones, Kingston, Ontario.

TABLE II

Quantitative Data on Cross-Reactions in Anti-Salmonella Horse Sera at 0°C.
Micrograms antibody N precipitated per milliliter

Polysaccharide used	Antityphoid serum 834		Antiparatyphoid B serum 1137‡
	Bleeding 1*	Bleeding 2†	
mg.	*μg.*	*μg.*	*μg.*
S II, 0.01			1
0.025			0
0.03	29	32	
0.05	30	34§	
0.10		31§	
Synthetic polyglucose			
0.3	50‖		19
0.4	53‖		19
0.6	64¶	57	
0.8	66¶		
1.		56	
Glycogen			
0.25	21		
0.5	31		
1.	30**		
Jellose, 0.03	23		6
0.1	28‡‡		6
0.4	23§§		
Yeast mannan			
0.02			1
0.05		39‖‖	0
0.1		41‖‖	

* With typhoid polysaccharide: 512 μg. antibody N per ml. (3).

† With typhoid polysaccharide (3): 430, 442 μg. antibody N per ml.

‡ With paratyphoid B polysaccharide (3): 342 μg. antibody N per ml.

§ Supernatants gave the same amount of N with yeast mannan as did unabsorbed serum.

‖ Supernatants with pine arabogalactan gave 8 μg. N calculated to 1.0 ml.; the supernatants from this gave 11 μg. N with the preparation of yeast mannan that gave 12 μg. N with unabsorbed 834, second bleeding.

¶ Supernatants with S II gave 4 μg. N.

** Supernatant with S II gave 10 μg. N.

‡‡ Supernatant with S II gave 18 μg. N.

§§ Supernatant with glycogen gave 25 μg. N.

‖‖ A second preparation of yeast mannan gave only 12 μg. N. Supernatants from precipitation with preparation 1 gave 32 μg. N with S II.

¶¶ Supernatant with paratyphoid polysaccharide (3) gave 309 μg. N.

*** Supernatant with okra gave 5 μg. N.

‡‡‡ Supernatants with carob gave 47 μg. N.

TABLE II—*Concluded*

Polysaccharide used	Antityphoid aerum 834		Antiparatyphoid B serum 1137‡
	Bleeding 1*	Bleeding 2†	
mg.	*μg.*	*μg.*	*μg.*
Carob, 0.02	4	3	71
0.06		6	
0.2			80¶¶
0.4			64, 73***
Guar, 0.1			66
0.2			72
Kentucky coffee bean			
0.1			61
0.2			60
Okra, 0.05			34‡‡‡
0.1			40‡‡‡
0.2			45‡‡‡, 34
0.4			27

cases. The supernatant from the glycogen precipitate of 30 μg. N (Table II) gave 10 μg. N with S II, while that from the jellose precipitates of 28 and 23 μg. N gave 18 μg. additional N with S II and 25 μg. N with glycogen, almost the total amount possible in the latter instance. Perhaps the jellose, known to contain terminal non-reducing galactose residues, reacts by virtue of a galactoglucose terminal grouping, which is, of course, absent in S II and glycogen. Its presence is suggested by the extent of branching of part of the glucose, although no such disaccharide has actually been isolated (20). If this assumption were correct one would expect to find a terminal galactoglucose grouping in both jellose and the typhoid polysaccharide. Other evidence that terminal, non-reducing galactose residues, *per se*, are not involved in typhoid specificity is given in the next sections.

c. Arabogalactans.—Gum ghatti (21), degraded gum arabic (22), and the arabogalactans of larch (23) and Jeffrey pine (24) show rather limited and not very different cross-reactivity in both antisera (Table I). These gums all contain galactose mainly linked 1,6-, 1,3- and/or 1,3,6-, and probably in the β-configuration. The arabogalactan of larch shows a much higher percentage of non-reducing galactose end groups than that of Jeffrey pine. The latter, however, reacts more heavily in both antisera, indicating that non-reducing end groups of galactose, unless possibly linked to glucose, as discussed above, have little to do with either typhoid or paratyphoid B specificity. The cross-reacting groupings are therefore probably β-1,3-, 1,6- and/or 1,3,6-linkages of galactose. Reasons have been given (25) for the probable occurrence of one or more of these groupings of galactose in S XIV, and such groupings would account for the cross-reactivity of S XIV in both antisera.

d. Galactomannans.—This group of carbohydrates, comprising carob mucilage (26), guar gum (27) and the mucilage of the Kentucky coffee bean (28), reacted more

strongly in the antiparatyphoid serum, a property shared only by okra mucilage (see below), of those tested. The galactomannans are characterized by non-reducing end groups of D-galactose (14 to >20 per cent), with mannose linkages, probably β-, of the main chain at positions 1,4-. There are also some 1,4,6-linked mannose residues, possibly at the points of attachment of the galactose end groups. Carob mucilage, the only one tested in antityphoid serum, reacts in this about as weakly as the larch arabogalactan, which also has many galactose end groups. This would appear to be further evidence against the importance of galactose end groups alone in typhoid specificity. The weak reaction of jellose and larch galactan in the antiparatyphoid B serum would also justify this conclusion for paratyphoid B specificity. However, the cross-reactions of the galactomannans are particularly strong, so that it may be concluded that an appreciable portion of the antibody in the paratyphoid B serum is directed either toward galacto-oligomannose end chains linked 1,4- or 1,4,6- and 1,4-, or toward the corresponding mannose linkages alone. The importance of oligosaccharide end groups in carbohydrate specificity has recently been emphasized (29).

It will be noted (Table II, footnotes‡ and ¶¶) that the supernatant from the precipitation of 80 μg. N by carob mucilage from antiparatyphoid B serum gave a further 309 μg. N with paratyphoid B polysaccharide, whereas the unabsorbed serum yielded only 342 μg. N with the latter. While a number of explanations for the larger total of precipitate with both substances are possible, a probable one is based upon the known state of degradation of the O polysaccharides as isolated after treatment with hot acetic acid. It is unlikely that the para-B polysaccharide precipitates all of the anticarbohydrate in the antiserum. Since the precipitation with carob mucilage is a cross-reaction, characterized as are most of such reactions by a poorer fit between antibody and reactant than is the case with homologous antigen, it is probable that soluble complexes of carob and antibody remain unprecipitated. When para-B polysaccharide is added to such a solution it could not only precipitate the antibodies with which it would normally react, but also a portion at least, of those already complexed or aggregated by carob mucilage.

e. Yeast Mannan.—Unlike the galactomannans, this substance precipitated antityphoid serum more strongly than the antiparatyphoid B serum. The yeast mannan is characterized by non-reducing mannopyranose end groups attached presumably to the 6-position of mannose units in a chain otherwise linked 1,2- and 1,3- (30). This might be taken to indicate that the typhoid polysaccharide will be found to contain multiple units of one or more mannose residues bound in this fashion rather than by the 1,4- or 1,4,6-linkages which one would expect in the paratyphoid B substance. The two available preparations of yeast mannan precipitated different amounts of N from the antityphoid serum, but both reacted only weakly in the paratyphoid B antiserum. Absorption of the former serum with the more strongly precipitating yeast mannan failed to remove appreciable cross-reacting antibody to S II, as might have been anticipated.

f. Okra Mucilage.—This material is made up of D-galactose, L-rhamnose, and D-galacturonic acid. On partial hydrolysis it yields a complex mixture containing three galactobioses, one of which, at least, is linked 1,4-, and acidic products including 2-O-(D-galactopyranosyl-uronic acid)-L-rhamnose (31). The mucilage gives

a negative qualitative test in the antityphoid serum but reacts heavily in the paratyphoid B antiserum. At present the basis for this sharp differentiation is not clear.

The deductions as to the constitution of the typhoid and paratyphoid B polysaccharides permitted by the study of the above cross-reactions are admittedly not very extensive or very precise, since in almost every instance several alternatives are possible. It is hoped, however, that these data will prove useful in providing hints that may shorten the arduous task of the organic chemists whose studies, must in the end, furnish the only secure knowledge of the fine structure of these substances. Should these apparent leads turn out to have been valid, immunochemical methods will once again have shown their power.[3]

SUMMARY

A study was made of cross-reactions of synthetic polyglucose and of numerous plant and bacterial gums in an antityphoid and an antiparatyphoid B horse serum. The observed differences permit conclusions to be drawn regarding certain of the linkages likely to be found in the fine structures of each of the corresponding *Salmonella* polysaccharides:—

1. Cross-reactions of the antityphoid serum with the specific polysaccharide of Type II pneumococcus and with tamarind seed polysaccharide, glycogen and synthetic polyglucose indicate that the acetic acid–degraded O-polysaccharide of *S. typhi*, strain O 901, may contain part, at least, of its glucose as 1,4,6-branch points or in 1,6-linkage, perhaps adjacent to a terminal, non-reducing, galactopyranose unit.

2. Cross-reactions of both antisera with arabogalactans point to the existence of (probably β-) 1,3-, 1,6-, and/or 1,3,6-linkages of galactose in both the typhoid and paratyphoid B polysaccharides.

3. The differential reactivities of the galactomannans and yeast mannan suggest that the mannose in the typhoid polysaccharide is linked 1,2- or 1,3- with possible non-reducing mannopyranose end groups attached 1,6-. In the paratyphoid B polysaccharide the linkages are probably galactooligomannose 1,4-, or 1,4,6-, or the corresponding linkages of mannose alone.

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[3] This manuscript was sent to Dr. A. M. Staub before publication. She has written that preliminary data obtained by methylation and by periodate oxidation of the typhoid polysaccharide, the only one studied, are in accord with the work herein recorded.

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Note Added to Page Proof.—Dr. Paul A. Rebers, in this laboratory, has found that S VI contains galactose, glucose, rhamnose, and phosphorus. S VI gives a $++\pm$ reaction in the antityphoid serum and $\pm$ in the antiparatyphoid B serum.