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The Immunochemistry of *Salmonella* Chemotype VI O-Antigens

THE STRUCTURE OF OLIGOSACCHARIDES FROM *SALMONELLA* GROUP U (O 43) LIPOPOLYSACCHARIDES

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1. A series of oligosaccharides was isolated from *Salmonella milwaukee* lipopolysaccharide by partial acid hydrolysis. 2. Structural studies on these oligosaccharides indicated that the O-specific side chain of this lipopolysaccharide has a repeating pentasaccharide unit that is probably α-D-galactosyl-(1→3)-β-D-galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetyl-D-glucosaminyl-(1→4)-L-fucose. 3. Another oligosaccharide, which is not structurally related to the repeating pentasaccharide unit, has also been isolated and it is indistinguishable from an oligosaccharide obtained from *Salmonella* 'rough' (R) lipopolysaccharides. The isolation of this and similar core oligosaccharides from all chemotype VI lipopolysaccharides supports the view that *Salmonella* S-lipopolysaccharides have a common core that is probably identical with R II lipopolysaccharide.

The present investigations were undertaken to determine the molecular structure of a number of 'smooth' *Salmonella* lipopolysaccharides of chemotype VI, which contain fucose and *N*-acetylgalactosamine in addition to the 'basal sugars' of chemotype I (Kauffmann, Lüderitz, Stierlin & Westphal, 1960). The results of a structural analysis of *Salmonella* group G and N lipopolysaccharides (Simmons, Lüderitz & Westphal, 1965*a*,*b*) have been reported. The present paper is concerned with the structure of *Salmonella milwaukee* (group U) lipopolysaccharide.

MATERIALS AND METHODS

Lipopolysaccharide. The lipopolysaccharide was extracted from an acetone-dried culture of *S. milwaukee* by the phenol–water method and purified in the ultracentrifuge as described by Kauffmann *et al.* (1960).

Isolation of S. milwaukee *oligosaccharides.* The oligosaccharides were prepared from 300mg. of *S. milwaukee* lipopolysaccharide by using the hydrolytic, chromatographic and electrophoretic methods described by Simmons *et al.* (1965*a*). On preparative high-voltage electrophoresis the hydrolysate yielded seven fractions designated E1–E7. Fraction E1 migrated to the anode and proved to be indistinguishable from 3-deoxy-2-oxo-octonate. Fraction E2 was electrophoretically neutral and fractions E3–E6 were shown to be oligosaccharides containing basic amino sugars. Fraction E7 contained free galactosamine and glucosamine. Fraction E2 contained eight neutral oligosaccharides that were separated by preparative chromatography in solvent I and designated C1, C2a, C2b, C2c, C3, C4, C5 and C6 in order of increasing chromatographic mobility. After isolation the homogeneity of these oligosaccharides was investigated chromatographically in solvents I and II. Fractions C2b and C3, which contained small amounts of C2a and C2c respectively, were further purified by high-voltage electrophoresis in borate buffer B, as was fraction C6, which was heavily contaminated with free galactose and glucose.

Structural studies of S. milwaukee *oligosaccharides.* The methods used for the microanalysis, borohydride reduction, periodate oxidation, *N*-acetylation and Morgan–Elson studies of these oligosaccharides were as described by Simmons *et al.* (1965*a*). The complement-fixation inhibition tests were carried out according to the method of Wasserman & Levine (1961) by using an antiserum given by Professor F. Kauffmann, Copenhagen.

β-Galactosidase studies. The method used to test the effect of β-galactosidase on oligosaccharides C1a, C2b and C4 was as described by Simmons *et al.* (1965*a*).

α-Galactosidase studies. The α-galactosidase from coffee beans was a gift from Dr M. J. Osborn. Its action on oligosaccharide C2c was tested as follows: 35μl. of 0·01M-sodium acetate and 2μl. of the enzyme solution were added to 102mμmoles of the oligosaccharide in 10μl. of water. After incubation at 37° for 2hr., 50μl. of water was added to the mixture and the amount of free galactose present determined by the galactose-dehydrogenase method (Wallenfels & Kurz, 1962). Under the above experimental conditions, the enzyme split 100% of the galactose from a positive control containing 100mμmoles of raffinose and only 2·7% of galactose from a negative control containing 100mμmoles of lactose.

RESULTS

Analysis of sugars in S. milwaukee *lipopolysaccharide.* On quantitative microanalysis the sugar composition of *S. milwaukee* lipopolysaccharide (in %, w/w) was as follows: glucose, 6·9; galactose, 24·2; *N*-acetylglucosamine, 12·4; *N*-acetylgalactosamine, 12·5; fucose, 6·9; heptose, 3·1; 3-deoxy-2-

Table 1. *Properties of oligosaccharides obtained from* S. milwaukee *(group U) lipopolysaccharide by partial acid hydrolysis*

N.T., Not tested; * chromatographic evidence only. $GalNH_2$, galactosamine; $GlcNH_2$, glucosamine; Gal, galactose; Glc, glucose; Fuc, fucose. The small amounts of sugar shown in parentheses probably arise from impurities.

Oligosaccharide	Approx. yield (mg.) from 300 mg. of lipopolysaccharide	% of total oligosaccharide yield	R_{Glc} in solvent I	$10^6 \times$ Electrophoretic mobility in buffer A ($cm.^2 v^{-1} sec.^{-1}$)	$10^{-2} \times$ Monosaccharide content (μmoles/10 μl. of eluate)				Nature of oligosaccharide	Sugar components of oligosaccharides	Sugar reduced by borohydride	Relationship of C and E oligosaccharide
					$GalNH_2$	$GlcNH_2$	Gal	Glc	Fuc			
C1	1·0	18	0·24	0·0	3·4	3·4	6·5	(1·0)	3·9	Pentasaccharide	$GalNH_2$, $GlcNH_2$, Gal, Fuc	Fucose	E3 acetylated
C2a	0·5	10	0·38	0·0	2·6	2·9	8·8	(1·0)	(0·3)	Tetrasaccharide	$GalNH_2$, $GlcNH_2$, Gal	Glucosamine*	—
C2b	0·4	7	0·44	0·0	5·9	4·7	4·5	(0·7)	2·8	Tetrasaccharide	$GalNH_2$, $GlcNH_2$, Gal, Fuc	Fucose	E4 acetylated
C2c	0·9	15	0·53	0·0	(0·1)	(0·2)	3·4	(0·4)	(0·6)	Disaccharide	Gal	N.T.	—
C3	0·9	14	0·59	0·0	(0·8)	(0·8)	8·3	7·5	(0·9)	Disaccharide	Gal, Glc	Galactose*	—
C4	0·9	15	0·70	0·0	4·5	3·3	4·5	(0·9)	(0·3)	Trisaccharide	$GalNH_2$, $GlcNH_2$, Gal	Glucosamine	E6 acetylated
C5	0·3	5	0·78	0·0	1·0	0·0	(0·2)	1·0		Disaccharide	$GlcNH_2$, Fuc	Fucose*	—
C6	0·2	4	0·92	0·0	6·1	6·0	(2·5)	(0·2)	(0·8)	Disaccharide	$GalNH_2$, $GlcNH_2$	Glucosamine	E5 acetylated
E3	0·2	4	0·08	3·3	3·6	3·4	6·7	(0·2)	1·6	Pentasaccharide	$GalNH_2$, $GlcNH_2$, Gal, Fuc	N.T.	C1 deacetylated
E4	0·2	4	0·28	4·3	3·9	2·6	2·7	0·0	1·6	Tetrasaccharide	$GalNH_2$, $GlcNH_2$, Gal, Fuc	Fucose*	C2b deacetylated
E5	0·1	3	0·54	5·1	1·4	2·2	(0·4)	0·0	0·0	Disaccharide	$GalNH_2$, $GlcNH_2$	Glucosamine	C6 deacetylated
E6	0·1	1	0·25	6·0	1·6	1·1	1·6	0·0	0·0	Trisaccharide	$GalNH_2$, $GlcNH_2$, Gal	Glucosamine	C4 deacetylated

oxo-octonate, 5·0. The carbohydrate content of this lipopolysaccharide is therefore about 71% (w/w), to which must be added an undetermined amount of lipid A.

Homogeneity of oligosaccharides. All the isolated oligosaccharides appear homogeneous by the criterion of chromatography in solvents I and II and electrophoresis in buffers A and B (Simmons *et al.* 1965*a*). The R_{Glc} values for these substances and their electrophoretic mobilities in buffer A are given in Table 1. The amount of each oligosaccharide isolated and its percentage of the total oligosaccharide yield are also given in Table 1.

Oligosaccharides C6 and E5. On microanalysis, oligosaccharides C6 and E5 (Table 1) contained galactosamine and glucosamine in the ratio 1:1, which indicated that they are probably disaccharides. The chromatographic mobility of fraction C6 and the electrophoretic mobility of fraction E5 support this view. *N*-Acetylation of fraction E5 gave a product that was chromatographically indistinguishable from fraction C6. On borohydride reduction of a sample of fraction C6 the glucosamine content fell from 34 to 1mμmole, whereas the galactosamine content remained unaltered. These findings confirm that fraction C6 is a disaccharide and show that the glucosamine residue occupies the reducing end group of the molecule. The amount of colour produced in the Morgan–Elson reaction by oligosaccharides containing amino sugars depends on a number of factors that have been reviewed by Foster & Horton (1959) and summarized briefly in relation to the present studies (Simmons *et al.* 1965*a*). The colour yield of unhydrolysed oligosaccharide C6 in the Morgan–Elson reaction was 0·9 times that of the hydrolysed oligosaccharide, which favours a (1→3)-linkage between the two residues. From the above evidence the structure of oligosaccharide C6 is considered to be *N*-acetylgalactosaminyl-(1→3)-*N*-acetyl-D-glucosamine.

Oligosaccharides C4 and E6. The *N*-acetylation of fraction E6 gave a product that was chromatographically indistinguishable from fraction C4, and on microanalysis (Table 1) both these saccharides contained galactose, galactosamine, and glucosamine in the proportions 1:1:1. The chromatographic mobility of fraction C4 and the electrophoretic mobility of fraction E6 indicate that they are trisaccharides. On borohydride reduction the glucosamine content of samples of fractions C4 and E6 fell from 33 to 2mμmoles and from 76 to 5mμmoles respectively, and the formation of glucosaminol was demonstrated both by chromatography and high-voltage electrophoresis in borate buffer. These results confirm that fractions C4 and E6 are trisaccharides and that the glucosamine is at the reducing end of the molecule in each instance.

In the Morgan–Elson reaction the colour yield of fraction C4 was 1·8 times that of the hydrolysed substances, which suggests that both hexosamines are substituted in the 3-position and, consequently, that the galactose is terminal at the non-reducing end of the molecule. This finding was confirmed by the observation that β-galactosidase hydrolysed 91% of the galactose in a sample of fraction C4. From the available evidence oligosaccharide C4 is considered to be β-D-galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetylglucosamine.

Oligosaccharides C2b and E4. The acetylation of fraction E4 gave a product that was chromatographically indistinguishable from fraction C2b, and on microanalysis both contained galactose, galactosamine, glucosamine and fucose in the proportions 1:1:1:1. The chromatographic mobility of fraction C2b in solvent I and the electrophoretic mobility of fraction E6 indicate that these substances are tetrasaccharides. On borohydride reduction fucose was reduced in both instances and, with fraction E6, fucitol was demonstrated in the reaction products by paper chromatography. Thus the reducing end group of these oligosaccharides is fucose. In the Morgan–Elson reaction the colour yield with unhydrolysed fraction C2b was 1·2 times that of the hydrolysed substance. After *N*-acetylation fraction E4 also gave a strongly positive reaction, without prior hydrolysis. These results again favour linkages to the 3-position of both the hexosamine residues.

On periodate oxidation 1 mol. of oligosaccharide C2b reduced 3·8 mol. of periodate and produced 2·7 mol. of formic acid, a trace of formaldehyde and no acetaldehyde. After 24 hr. a gradual linear increase in the periodate oxidation products was noted and attributed to overoxidation. The values given were obtained by extrapolation to zero time. From these results it appears that the terminal galactose reduces 2 mol. of periodate/mol., yielding 1 mol. of formic acid/mol., and that the fucose reduces 2 mol. of periodate/mol. with the production of 2 mol. of formic acid/mol. The small amount of formaldehyde is probably due to overoxidation. If this explanation is correct, then the hexosamine residues are protected and therefore most probably not substituted in the 6-position. This appears to be the case, as both galactosamine and glucosamine were demonstrated chromatographically in the hydrolysis products of the periodate-oxidized oligosaccharide. When the monosaccharide composition of *S. milwaukee* lipopolysaccharide was studied quantitatively before and after periodate oxidation, the percentage (w/w) of each hexosamine fell from 11 to about 9, and that of fucose fell from 7 to 0. These results confirm that the *N*-acetylhexosamine residues are largely protected, which is in agreement with the view that they are 3-*O*-substituted. By contrast, the oxidation of fucose in the intact lipopolysaccharide indicates it is not 3-*O*-substituted. The quantitative periodate oxidation studies on fraction C2b, and especially the absence of acetaldehyde formation, suggest that the fucose is 4-*O*-substituted. The behaviour of fraction C2b in the Morgan–Elson reaction excludes 2-*O*-substitution.

On the basis of the above evidence the structure of oligosaccharide C2b is considered to be β-D-galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetylglucosaminyl-(1→4)-L-fucose.

Oligosaccharide C2c. Oligosaccharide C2c behaved like a disaccharide in solvents I and III, and on quantitative microanalysis contained galactose only. When a 52 mμmole sample of fraction C2c was treated with α-galactosidase, 116 mμmoles of galactose were recovered. These findings confirm that oligosaccharide C2c is a disaccharide and indicate that it is an α-D-galactosyl-D-galactose. In solvent III (Simmons *et al.* 1965*a*) it was indistinguishable from an authentic sample of *O*-α-D-galactosyl-(1→3)-galactose kindly given by Professor W. T. J. Morgan, The Lister Institute, London.

Oligosaccharide C2a. On microanalysis oligosaccharide C2a (Table 1) contained galactose, galactosamine and glucosamine in the proportions 2:1:1 and chromatographically it behaved as a tetrasaccharide. On borohydride reduction, chromatographic evidence indicated that the glucosamine was reduced. When fraction C2a was heated at 100° for 7 min. in 1% (w/w) tetraborate (as in the Morgan–Elson reaction) and the resulting products were analysed chromatographically after the removal of borate by high-voltage electrophoresis, three products were detected with silver nitrate. These were eluted. The first was chromatographically indistinguishable from fraction C2a itself and appeared to be undegraded starting material. The second product, which behaved like a disaccharide in solvent III, was chromatographically indistinguishable from disaccharide C2c and, like it, yielded only galactose on acid hydrolysis. The third product gave a direct Morgan–Elson reaction, i.e. a positive colour reaction with *p*-dimethylaminobenzaldehyde without pretreatment with borate. The elimination of the galactose disaccharide under these conditions proves that the two galactose residues are bound together and indicates that the disaccharide is (1→3)-linked to the adjacent *N*-acetylgalactosamine, which is in turn linked to the 3-position of the terminal reducing *N*-acetylglucosamine.

From the above evidence the most probable structure of oligosaccharide C2a is galactosylgalactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetylglucosamine.

Oligosaccharides C1 and E3. On *N*-acetylation

fraction E3 gave a product that was chromatographically indistinguishable from fraction C1, and on microanalysis (Table 1) both contained galactose, galactosamine, glucosamine and fucose in the proportions 2:1:1:1. The chromatographic mobility of fraction C1 in solvent I and the electrophoretic mobility of fraction E3 in buffer A are consistent with the view that both substances are pentasaccharides. On borohydride reduction of a sample of fraction C1 the fucose content fell from 39 to 1 mμ-mole, and fucitol was demonstrable chromatographically among the reaction products. Thus the fucose residue is at the reducing end of the pentasaccharide. In the Morgan–Elson reaction oligosaccharide C1 reacted strongly. After *N*-acetylation the colour yield of unhydrolysed fraction E3 in the Morgan–Elson test was 1·3 times that of the hydrolysed substance despite the fact that all the *N*-acetylhexosamine residues are glycosidically linked. This reaction indicates once again that both the *N*-acetylhexosamine residues are 3-*O*-substituted, which agrees with the findings for oligosaccharides C4 and C2b. Again we assume that the reducing fucose is partially split off and destroyed under the conditions of the Morgan–Elson reaction, as with fraction C2 of *S. friedenau* lipopolysaccharide (Simmons *et al.* 1965*a*).

When a sample of oligosaccharide C1 containing 310 mμmoles of galactose was treated with β-galactosidase, only 10 mμmoles of galactose could be recovered. The inability of this enzyme to utilize oligosaccharide C1 (which contains 2 residues of galactose/mol.) as substrate contrasts sharply with its marked action on oligosaccharide C4 (which contains only 1 residue of galactose/mol.). This suggests that the terminal galactose of oligosaccharide C1 is bound by an α-linkage. This conclusion is supported by the quantitative conversion of oligosaccharide C2c into galactose by α-galactosidase and by the liberation of galactose from *S. milwaukee* lipopolysaccharide by the same α-galactosidase system.

On periodate oxidation 1 mol. of pentasaccharide C1 reduced 2·8 mol. of periodate and yielded 1·7 mol. of formic acid, 0·4 mol. of formaldehyde and no acetaldehyde. After 24 hr. there was a slight linear increase in the reaction products, which was attributed to overoxidation. The values given were derived by extrapolation to zero time. Precise interpretations of these results are difficult. Despite these difficulties, however, the low reduction of periodate and the correspondingly low yield of reaction products indicate that the molecule is highly protected, which suggests that the penultimate galactose and both *N*-acetylhexosamine residues are probably 3-*O*-substituted. This conclusion is in agreement with the results obtained with oligosaccharide C2b. The quantitative periodate oxidation studies, and especially the absence of acetaldehyde formation, favour 4-*O*-substitution of the fucose. The oxidation of fucose in *S. milwaukee* lipopolysaccharide on treatment with periodate contraindicates 3-*O*-substitution of this residue, and the behaviour of oligosaccharide C1 in the Morgan–Elson reaction excluded 2-*O*-substitution (see Foster & Horton, 1959).

From these results the structure of oligosaccharide C1 is considered to be α-D-galactosyl-(1→3)-β-D-galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetylglucosaminyl-(1→4)-L-fucose.

Oligosaccharide C5. On microanalysis oligosaccharide C5 contained glucosamine and fucose in the ratio 1:1 and its chromatographic behaviour in solvent I indicated that it was a disaccharide. After borohydride treatment the fucose residue was reduced to fucitol. The latter was demonstrated chromatographically as a hydrolysis product of the reduced disaccharide. In the Morgan–Elson test oligosaccharide C5 reacted weakly, the colour yield of the unhydrolysed disaccharide being 0·3 times that of the hydrolysed substance. From the evidence available the structure of oligosaccharide C5 is considered to be *N*-acetylglucosaminylfucose.

Oligosaccharide C3. On microanalysis oligosaccharide C3 contained equal amounts of glucose and galactose. It behaved chromatographically as a disaccharide and was indistinguishable in solvents I and III (Simmons *et al.* 1965*a*) from the C3 oligosaccharides isolated from *Salmonella* group G and N lipopolysaccharides. On borohydride reduction the galactose was reduced to dulcitol. The structural sequence of oligosaccharide C3 is therefore considered to be glucosylgalactose.

Fraction E1. Fraction E1 and an authentic sample of 3-deoxy-2-oxo-octonate were indistinguishable when compared chromatographically in solvent I and electrophoretically in buffer A.

Fraction E7. Fraction E7 contained free glucosamine and galactosamine only.

Complement-fixation inhibition. Of the sugars tested in the homologous *S. milwaukee* complement-fixation system 1·0 μmole of methyl α-D-galactoside gave a weak but reproducible 5% inhibition. All the other sugars tested (see Simmons *et al.* 1965*a*) failed to produce significant reactions. This result may indicate the presence of terminal α-galactosidic linkages in *S. milwaukee* lipopolysaccharide.

DISCUSSION

Partial acid hydrolysis of *S. milwaukee* (group U) lipopolysaccharide yields a number of oligosaccharides, most of which contain one or both of the 'special sugars' of chemotype VI, namely *N*-acetylgalactosamine and fucose. The structural relationship of the oligosaccharides in this series is shown

C1 α-D-Gal-(1→3)-β-D-Gal-(1→3)-GalNAc-(1→3)-D-GlcNAc-(1→4)-L-Fuc
C2a D-Gal ⟶ β-D-Gal-(1→3)-GalNAc-(1→3)-D-GlcNAc
C2b β-D-Gal-(1→3)-GalNAc-(1→3)-D-GlcNAc-(1→4)-L-Fuc
C2c α-D-Gal-(1→3)—D-Gal
C4 β-D-Gal-(1→3)-GalNAc-(1→3)-D-GlcNAc
C5 D-GlcNAc⟶L-Fuc
C6 GalNAc-(1→3)-D-GlcNAc

Scheme 1. Structural relationship of oligosaccharides obtained by partial acid hydrolysis of *Salmonella* group U lipopolysaccharide. Gal-, galactosyl; GalNAc-, *N*-acetylgalactosaminyl; GlcNAc-, *N*-acetylglucosaminyl; GlcNAc, *N*-acetylglucosamine; Fuc, fucose.

D-Glc
↓
G β-D-Gal-(1→3)-GalNAc-(1→3)—GalNAc-(1→4)-L-Fuc
D-Glc
↓
N β-Glc-(1→3)-GalNAc-(1→4)-L-Fuc
U α-D-Gal-(1→3)-β-D-Gal-(1→3)-GalNAc-(1→3)-D-GlcNAc-(1→4)-L-Fuc

Scheme 2. Largest oligosaccharide units isolated from *Salmonella* group G, N and U lipopolysaccharides. Gal-, galactosyl; GalNAc-, *N*-acetylgalactosaminyl; Glc-, glucosyl; GlcNAc-, *N*-acetylglucosaminyl; Fuc, fucose.

in Scheme 1. This series of oligosaccharides suggests that the structural unit of the *S. milwaukee* specific side chain is most probably the pentasaccharide α-D-galactosyl-(1→3)-β-D-galactosyl-(1→3)-*N*-acetylgalactosaminyl-(1→3)-*N*-acetyl-D-glucosaminyl-(1→4)-L-fucose. This structure offers a possible explanation for the known blood-group B activity of *Salmonella* group U species (Springer, Williamson & Brandes, 1961) in that it possesses a common sequence with α-D-galactosyl-(1→3)-β-D-galactosyl-(1→3)-*N*-acetylglucosamine, which plays a role in determining blood-group B activity (Rege, Painter, Watkins & Morgan, 1963).

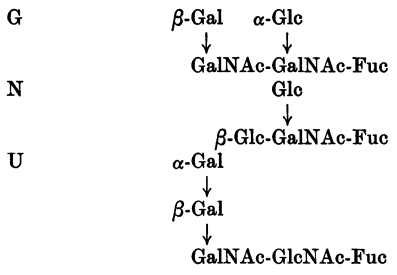
A further oligosaccharide isolated from *S. milwaukee* lipopolysaccharide was found to be structurally unrelated to the repeating pentasaccharide unit. It was a glucosylgalactose indistinguishable from that previously isolated from the lipopolysaccharides of *S. godesberg* and *S. urbana* (Simmons *et al.* 1965*b*).

The oligosaccharides shown in Scheme 2 are the largest units isolated from *Salmonella* group G, N and U lipopolysaccharides. We believe that these oligosaccharides represent repeating units in the respective lipopolysaccharide where they form the specific side chains. These structures may be incomplete, owing to the hydrolysis of acid-labile sugars in the same way that 3,6-dideoxyhexoses are split from the repeating units of *Salmonella* group B and D antigens. This seems particularly probable with the group G repeating unit, where terminal non-reducing fucose might be expected from the known blood-group H (O) activity of the parent lipopolysaccharide (Springer *et al.* 1961) and from the role of fucose in determining H (O) activity (Morgan, 1964; Schiffmann, Kabat & Thompson, 1964). The complement-fixation inhibition results do reveal some non-reducing fucose in the lipopolysaccharide but, from its quantitative analysis, it is evident that only a few repeating units can carry non-reducing fucose. The 'chemical repeating unit' liberated by acid hydrolysis is determined by the acid-lability of the fucose linkages in these antigens. It is not necessarily identical with the 'biological repeating unit', which could contain non-reducing fucose. In this case the determinant side chains (but not every repeating unit), would each be terminated by a non-reducing fucose residue. The structures that are essential to the serological specificities of the above group G, N and U lipopolysaccharides have not yet been defined, nor is it known how the repeating units are linked together. Preliminary results of complement-fixation inhibition studies favour the view that they are bound as branching structures in which the hexoses are present as short side chains linked to the main O-specific chain. These hexose side chains would then be end-group determinants as indicated in Scheme 3. The Morgan–Elson reaction performed before and after hydrolysis of the *Salmonella* chemotype VI oligosaccharides was a useful guide in the elucidation of their structures. According to Foster & Horton (1959), chromogen formation in the Morgan–Elson reaction is increased by 3-*O*-substitution, unaffected by 6-*O*-substitution and prevented by 4-*O*-substitution. During chromogen production 3-*O*-substituents are eliminated but 6-*O*-substituents are not. The behaviour of *Sal-*

monella chemotype VI oligosaccharides in the Morgan–Elson reaction is shown in Table 2 and supports the view that all the *N*-acetylhexosamine residues are 3-*O*-substituted. Chromogen formation is decreased but not eliminated when the reducing group of an *N*-acetylhexosamine residue is linked to fucose because the latter is partially degraded under the conditions of the Morgan–Elson reaction.

Although the *Salmonella* species of groups G, N and U all belong to one chemotype (Kauffmann *et al.* 1960), further classification on the basis of 'basal' (core) sugars and 'specific' (side-chain) sugars is now possible as follows: Group G: 'basal sugars' + fucose, *N*-acetylgalactosamine, glucose and galactose. Group N: 'basal sugars' + fucose, *N*-acetylgalactosamine and glucose. Group U: 'basal sugars' + fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine and glucose. (The 'basal sugars' are 3-deoxy-2-oxo-octonate, heptose, glucose, galactose and *N*-acetylglucosamine.) This chemical reclassification shows that those lipopolysaccharides that, according to the older classification, all belong to chemotype VI are distinct with respect to the sugar composition of their O-specific side chains. This refined classification accounts for the absence of serological cross-reaction between the three *Salmonella* groups within this chemotype. It would now be of great interest to elaborate a scheme of *Salmonella* chemotypes based on the composition of the specific side chains of many selected O-antigens. Such a scheme would greatly increase our knowledge and understanding of the biochemistry, immunochemistry and genetics of this important genus and elucidate the role of different sugars in O-antigenic structure. It is already possible to conclude from the above findings that one sugar may play different roles. *N*-Acetylglucosamine, for example, is always a constituent of both the lipid component and the basal structure of *Salmonella* O-antigens and, in some lipopolysaccharides, is also found in the specific side chains. Glucose, to cite another example, is a constituent of the basal structure but also occurs in the specific side chains of certain lipopolysaccharides.

In recent years the biochemical analysis of *Salmonella* R-mutants has thrown much light on the biosynthesis of these mutant antigens. From the work of Nikaido (1962*a*,*b*) and Osborn, Rosen, Rothfield & Horecker (1962) it is known that the R-lipopolysaccharides are synthesized by the sequential addition of glucose, galactose and *N*-acetylglucosamine residues to a polyheptose phosphate 'backbone'. These studies, together with the



Scheme 3. End-group determinants of *Salmonella* group G, N and U lipopolysaccharides. Gal-, galactosyl; GalNAc-, *N*-acetylgalactosaminyl; Glc-, glucosyl; GlcNAc-, *N*-acetylglucosaminyl; Fuc, fucose.

Table 2. *Behaviour of* Salmonella *chemotype VI oligosaccharides in the Morgan–Elson reaction*

Gal-, galactosyl; GalNAc-, *N*-acetylgalactosaminyl; GalNAc, *N*-acetylgalactosamine; Glc-, glucosyl; GlcNAc-, *N*-acetylglucosaminyl; Fuc, fucose. The colour ratio is the colour intensity given by the oligosaccharide before hydrolysis to that given after hydrolysis by using the Morgan–Elson reaction as described in the text.

Salmonella serogroup	Oligosaccharide designation	Sugar sequence	Colour ratio
G	C4	Gal-GalNAc-GalNAc	1·9
G	C2	Gal-GalNAc-GalNAc-Fuc	1·1
G	C1	Glc+ Gal-GalNAc-GalNAc-Fuc	0·1
G	C6	GalNAc-GalNAc	0·9*
N	C5	Glc-GalNAc	2·3
N	C6	GalNAc-Fuc	low
N	C4	Glc-GalNAc-Fuc	0·3
U	C6	GalNAc-GlcNAc	0·9
U	C4	Gal-GalNAc-GlcNAc	1·8
U	C2b	Gal-GalNAc-GlcNAc-Fuc	1·2
U	C2a	Gal-Gal-GalNAc-GlcNAc	high
U	C1	Gal-Gal-GalNAc-GlcNAc-Fuc	1·3
U	C5	GlcNAc-Fuc	0·3

* This value assumes that the contaminating disaccharide *N*-acetylgalactosaminylfucose gives a low colour yield.

(I) GlcNAc-Glc-Gal-Glc-
(II) Glc-Gal-
(III) GlcNAc-Glc-

Scheme 4. Structure of oligosaccharides from the side chains attached to the polyheptose phosphate 'backbone' of *Salmonella* core polysaccharides. GlcNAc-, *N*-acetylglucosaminyl; Glc-, glucosyl; Gal-, galactosyl.

analysis of the lipopolysaccharides of RII mutants (Sutherland, Lüderitz & Westphal, 1965), have led to the elucidation of the structure of *Salmonella* RII antigen that is generally believed to represent the complete 'core polysaccharide' of O-antigens. The structure of the side chains that are attached to the polyheptose phosphate 'backbone' to give the complete core is shown (I) in Scheme 4. Two core oligosaccharides (II and III) have now been isolated from the different chemotype VI S-lipopolysaccharides and these have proved to be indistinguishable from the analogous oligosaccharides isolated from RII-mutant lipopolysaccharide. The isolation of these oligosaccharides from S-lipopolysaccharides provides strong evidence for the presence of common structures in these serologically distinct antigens and indicates that the common core is probably identical with RII-mutant lipopolysaccharide.

Although there is some similarity in the general structure of chemotype VI repeating units, their differences are too great to be dependent on single enzyme changes in the respective biosynthetic pathways. None of these oligosaccharides could function in a relatively simple way as the precursor of the others, as with *Salmonella* group E antigens (Robbins & Uchida, 1962), nor can one postulate a common precursor for all three units. This suggests that the relationship between *Salmonella* specific side chains is more complex than that which exists between the underlying 'rough' structures, which represent one-step biochemical lesions in the biosynthesis of the *Salmonella* lipopolysaccharide core.

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