UNUSUAL MONOSACCHARIDES IN THE O-FACTORS OF LIPOPOLYSACCHARIDES OF GRAM-NEGATIVE BACTERIA

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Information is generalized on the structure of the O-specific polysaccharides forming components of the lipopolysaccharides of Gram-negative bacteria in which residues of unusual monosacchrides rarely found in nature have been identified. A chemical substantiation of the immunological specificity of the O-factors of specific chains is given. The role of the unusual monosaccharides in the manifestation of O-factor specificity is discussed.

Lipopolysaccharides located on the surface of the cell membrane of Gram-negative bacteria take a direct part in the interaction of the microorganisms with the human or animal organism.

The lipopolysaccharides of enterobacteria (I) consist of three structural-functional regions: lipid A, on oligosaccharide core, and a polysaccharide of O-specific side chains which is constructed of oligosaccharide repeating units and is attached to the oligosaccharide core by a glycosidic bond. The immunological specificity of the bacteria is due to the chemical structure of this polysaccharide, which is called O-specific.

The link between O-factor typing and the structure of the O-specific polysaccharide chains has been studied best for bacteria of the genus Salmonella. It is therefore most convenient to consider the chemical substantiation of the immunological specificity of O-factors for the case of the specific polysaccharides from Salmonella. The first part of the present review is devoted to this question.

The monosaccharides D-glucose, D-galactose, D-mannose, L-fucose, L-rhamnose, glucosamine, and galactosamine are found most frequently as components of the O-antigens of various genera. The number of new unusual monosaccharides identified in structural sudies of the O-specific polysaccharides is rising every year. Many of these monosaccharides are rare and are characteristic only for a certain genus or species of bacteria. However, up to the present time there have been few studies connected with establishing their role in the manifestation of antigenic specificity. The following part of the review contains information on the structures of O-specific polysaccharides containing residues of unusual monosaccharides. And, in those cases where this has been established, the link is shown between O-factor typing and the chemical structure of the O-antigens.

CHEMICAL SUBSTANTIATION OF THE IMMUNOLOGICAL SPECIFICITY OF O-FACTORS OF SPECIFIC CHAINS

Long before structural investigations, in the immunochemical study of smooth strains of the genus Salmonella it was established that the polysaccharide chains of the lipopolysaccharides are bearers of the O-factors determining the immunological specificity of the O-antigens.

In the Kauffmann–White scheme developed for bacteria of the Salmonella genus, O-factor typing is based on the cross-absorption of sera by closely related strains. To each serovar corresponds a set of O-factors denoted by the numerals 1, 2, 3, etc.

Thanks to the development of this serological classification, Gram-negative bacteria of various species of the genus Salmonella have become the object of intensive investigations. Numerous structural and immunochemical studies on the O-specific polysaccharides have been well expounded in earlier reviews [1-7]. It has been established that the O-antigenic poly-

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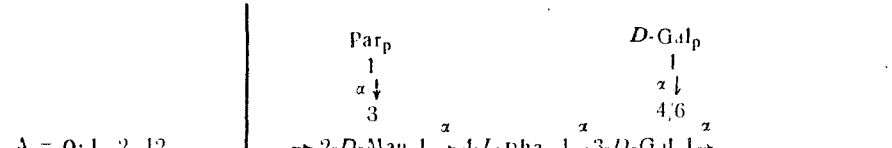
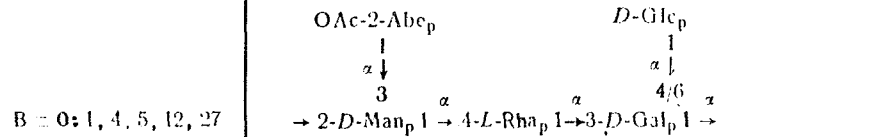
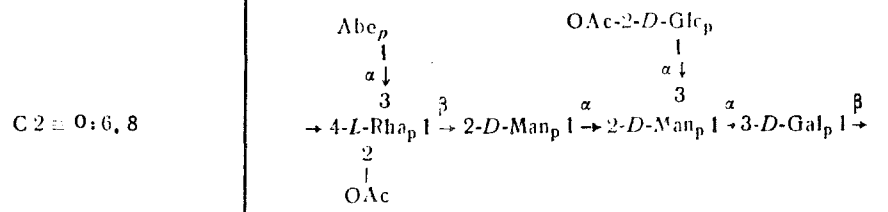
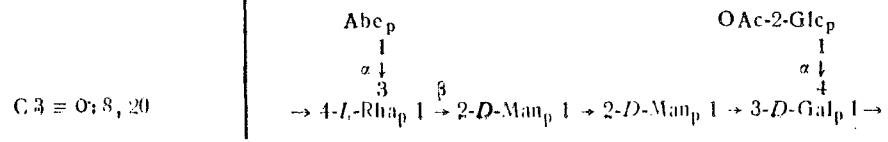
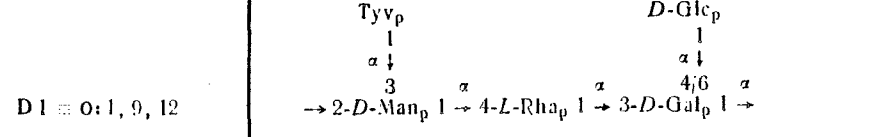
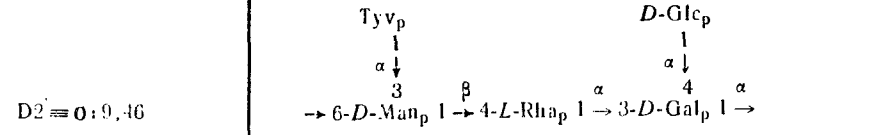
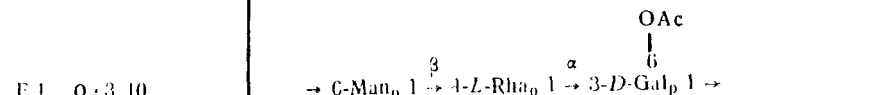
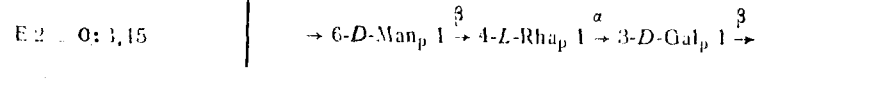
TABLE 1. Structures of the O-Antigenic Polysaccharides and O-Antigenic Immunodeterminants in Some Serogroups

Serogroup	Structure	Immunodeterminant structures established
A ≡ O:1, 2, 12		Par$_p$ 1 →$^{\alpha}$ 3-*D*-Man$_p$ ≡ O:2 *D*-Glc$_p$ 1 →$^{\alpha}$ 4-*D*-Gal$_p$ ≡ O:12$_2$ *D*-Glc$_p$ 1 →$^{\alpha}$ 6-*D*-Gal$_p$ ≡ O:1
B ≡ O:1, 4, 5, 12, 27		Abe 1 →$^{\alpha}$ 3-*D*-Man$_p$ ≡ O:4 2-OAc-Abe$_p$ ≡ O:5
C 2 ≡ O:6, 8		Abe$_p$ 1 →$^{\alpha}$ 3-*L*-Rha$_p$ ≡ O:8
C 3 ≡ O:8, 20		*D*-Glc$_p$ 1 →$^{\alpha}$ 4-*D*-Gal$_p$ ≡ O:20
D 1 ≡ O:1, 9, 12		Tyv 1 →$^{\alpha}$ 3-*D*-Man$_p$ ≡ O:9 *D*-Glc$_p$ 1 →$^{\alpha}$ 4-*D*-Gal$_p$ ≡ O:12$_2$ *D*-Glc$_p$ 1 →$^{\alpha}$ 6-*D*-Gal$_p$ ≡ O:1
D2 ≡ O:9,46		Tyv$_p$ 1 →$^{\alpha}$ 3-*D*-Man$_p$ ≡ O:9 *D*-Gal$_p$ 1 →$^{\alpha}$ 6-*D*-Man$_p$ 1 →$^{\beta}$ 4-*L*-Rha$_p$ ≡ O:46
E 1 ≡ O:3,10		OAc-6-*D*-Gal$_p$ ≡ O:10 *D*-Man$_p$ 1 →$^{\alpha}$ 4-*L*-Rha$_p$ ≡ O:3
E 2 ≡ O:3,15		*D*-Man$_p$ 1 →$^{\alpha}$ 4-*L*-Rha$_p$ ≡ O:3 *D*-Gal$_p$ 1 →$^{\beta}$ 6-*D*-Man$_p$ 1 →$^{\beta}$ 4-*L*-Rha$_p$ ≡ O:15

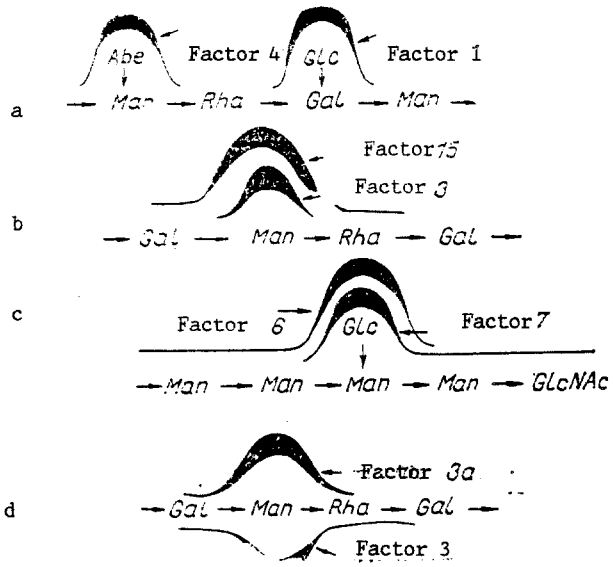


Fig. 1. Distribution of O-factors along a polysaccharide chain: a) factors corresponding to different immunodominant monosaccharides; b) family of factors corresponding to various sections of the antigenic determinant; c) factors located on both sides of the immunodominant monosaccharide; d) factors due to the "viewing" of the determinant from different sides.

saccharide chain is a polymer of an oligosaccharide repeating unit the chemical structure of which determines the O-antigenic specificity of the bacteria.

In work on establishing the structures of the repeating units of O-specific polysaccharides, wide use is made of the methods of methylation, periodate oxidation, and acid and alkaline degradation of the oligosaccharides obtained on the partial hydrolysis of the lipopolysaccharides or of the degraded polysaccharides. Particularly fruitful have been structural investigations of the specific polysaccharides by Lindberg's group [8] who have developed a chromato-mass spectrometric method of identifying partially methylated monosaccharides.

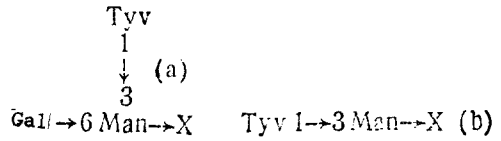
Some structures of O-specific polysaccharides of Salmonella (groups A, B, C, and E) are given in Table 1 [7].

A knowledge of the structures of the O-specific polysaccharides, and the possibility of using in immunochemical tests not only monosaccharides but also oligosaccharides has permitted the elucidation of the nature of the immunological O-factors.

On the basis of a comparison of the inhibiting activity of of monosaccharides, glycosides, and oligosaccharides in precipitation and complement-binding reactions it is possible to draw the conclusion that different O-factors are determined by one and the same polysaccharide molecule [1, 9, 10]. Furthermore, it has been found that most frequently one monosaccharide in an oligosaccharide chain is the best inhibitor. This monosaccharide has been called immunodominant [2]. As a rule, it is found in the terminal nonreducing position: for example, paratose in group A (factor O:2); abequose in group B (O:4); and tyvelose in group C (O:9) [7]. In certain cases, a monosaccharide residue located within the oligosaccharide chain may also be immunodominant [11], as is the case for galactose and mannose in factors O:15 and O:3, respectively, in group E. It has been established that the presence of of substituent in a monosaccharide residue, such as an O-acetyl group makes it immunodominant: 2-OAc-Abe_p (O:5) in group B, and 6-OAc-Glc_p (O:10) in group E [11] (Table 1).

Although the immunodominant monosaccharide plays an important role in the manifestation of specificity, by itself it does not express complete specificity, since one and the same monosaccharide may be present as a component of different O-factors. For example, factors O:4 $\equiv$ $Abe_p1 \xrightarrow{\alpha} 3Man_p$ and O:8 $\equiv$ $Abe_p1 \xrightarrow{\alpha} 3Rha_p$, which include terminal abequose residues are serologically different. This fact shows the substantial contribution of the second monosaccharide residue in the specificity of the O-antigen. A disaccharide better expresses the factorial specificity (antigenic determinant), since the complementarity between the antigen

and the antibody rises with an increase in the length of the oligomer. This has been confirmed [12] by a comparison of the inhibiting activity of the tri- and tetrasaccharides (a) and (b):



The tetrasaccharide exhibits great activity in immunochemical reactions. A very important role in the manifestation of specificity is played by the configuration of the glycosidic bond of the immunodominant monosaccharide. Using the inhibition of precipitation as an example, it has been shown [4] that the α-glycosides of abequose and tyvelose are more active inhibitors than the corresponding β-anomers or the free monosaccharides, which do not have the configuration assumed by the molecule when a disccharide fragment is present.

Th nature of the bond of the second monosaccharide with the third is also extremely important [13]. Thus, the O-antigen Salmonella 12_2 is determined by the trisaccharide α-Glc_p1 → 4-α-Gal_p1 → 2-α-Man_p, while O-antigen 34 differs by the type of bond between the galactose and the mannose residues: α-Glc_p1 → 4-α-Gal_p1 $\overset{\alpha}{\rightarrow}$ 6Man_p. The serological cross-reactions between these antigens are very weak.

Thus, on the basis of all the results obtained it has been established [1] that oligosaccharide determinants including several different monosaccharides (from 2 to 4 and more) are usually characterized by the presence of one immunodeterminant monosaccharide, but their serological specificity is also connected with the conformation of the molecule, which depends on the neighboring monosaccharides.

The immunological determinants (O-factors) can be oriented differently along the polysaccharide chain. Some factors determined by one and the same polysaccharide may contain different immunodominant monosaccharides. In this case, each O-factor is determined by different oligosaccharide fragments (Fig. 1a). However, they may contain one and the same immunodominant monosaccharide, and in this case three variants are possible:

1. The factors due to oligosaccharides of different dimensions are component parts of one and the same determinant group and, thus, form a family of factors.

2. The factors due to different determinant groups are located on the two sides of the immunodominant monosaccharide (Fig. 1c).

3. The factors correspond to different determinant oligosaccharides as a result of the "viewing" of the antigenic molecule from different sides (Fig. 1d).

The location of the barious determinants causing the formation of specific antibodies can be illustrated by the general scheme shown in Fig. 2. The tetrasaccharide illustrated can theoretically cause the formation of antibodies (I-IV) with different specificities, of which antibodies (I) and (II) are connected with one immunodominant monosaccharide (A); I and III with (B); II and IV with (D); and III and IV with (C). These theoretical hypotheses have been repeatedly confirmed by the immunochemical analysis of the transformation of one serotype of Salmonella into another by bacteriophages [1-4]. The O-antigenic factors can be classified in the following way [7]:

1. Major factors – these determine the O-antigenic group. Thus, for Salmonella, the O:8 factor characterizes group D.

2. Minor factors – a) these have a smaller or finely distinguishable value, since they are always linked with another factor. Thus, factor O:12 is always associated with O:2, O:4, or O:9 in groups A, B, and D;

b) they appear as the result of a chemical modification of a major antigen. For example, the O-antigen of the Salmonella group B possesses the factors O:4 and O:12 that are characteristic for this group. In addition, it may possess the following minor factors:

O:5, which is the result of the acetylation of the abequose responsible for the O:4, O:12 specificity; and:

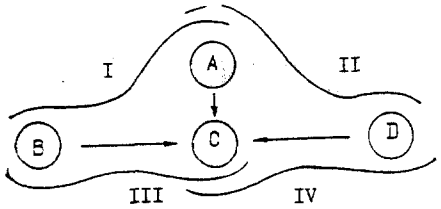


Fig. 2. General scheme of the arrangement in a tetrasaccharide of determinant groups connected with different antigens: (I) anti-1; (II) anti-2; (III) anti-3; (IV) anti-4.

O:1, which is formed on the glycosylation of galactose by glucose having an α-1,6-bond because of inversion by phage P22 (Table 1).

The structure of the immunodeterminants (O-factors) has also been established for other Salmonella serotypes [4].

The development of new physicochemical methods of investigations, paticularly ^{1}H and ^{13}C NMR spectroscopy and their use together with traditional methods has permitted a considerable intensification of the structural study of the O-antigenic polysaccharides of Gram-negative bacteria. As has been shown [14], all the serovars (apart from VI) of Salmonella flexneri contain O-specific polysaccharides closely lined with one another serologically. Their repeating units are based on a linear tetrasaccharide consisting of one N-acetylglucosamine and three (I, II, and III) rhamnose residues:

$$\underset{\text{I}}{\rightarrow 3)\text{-}\beta\text{-}D\text{-}Glc_p NAc\text{-}(1\rightarrow 2)\text{-}\alpha\text{-}L\text{-}Rha_p}\text{-}(1\rightarrow 2)\text{-}\underset{\text{II}}{\alpha\text{-}L\text{-}Rha_p}\text{-}(1\rightarrow 3)\text{-}\underset{\text{III}}{\alpha\text{-}L\text{-}Rha_p}\text{-}(1\rightarrow$$

The immunological differences between serovars are due to structural changes in the polysaccharide: the addition to one of the monosaccharides of the main tetrasaccharide of a short side chain in the form of α-D-glucopyranose or O-acetyl groups. A monosaccharide containing an O-acetyl group and α-D-glucopyranose are immunodominant determinant groups.

In the following section we give information on the chemical structures of the O-specific polysaccharides containing rare monosaccharides and those detected for the first time, and also information on their role in the O-antigenic determinants.

STRUCTURES OF O-SPECIFIC POLYSACCHARIDES CONTAINING UNUSUAL MONOSACCHARIDES

In the performance of a structural investigation of the O-specific polysaccharides of various Gram-negative bacteria several new monosaccharides have been identified the total number of which already exceeds 50 and is continuing to rise every year [6]. Among them are neutral monosaccharides, O-methyl and O-acetyl derivatives, deoxy-, amino-, and diaminosugars, uronic and aldulosonic acids, and branched monosaccharides. Some of them had been identified previously in natural polysaccharides and glycosides while others were found for the first time in the lipopolysaccharides of bacterial cell walls [15].

In fact, microorganisms find numerous pathways for the transformation of common monosaccharides into unusual ones [16]. This may possibly be explained by the fact that bacteria resistant to the action of antibiotics (such as Pseudomonas aeruginosa) or retaining a capacity for reproduction at a low temperature (Yersinia enterocolitica, Yersinia pseudotuberculosis) include as components many unusual monosaccharides not previously found in natural sources. The unusual monosaccharides and their abbreviations are given in Table 2.

The polysaccharide of Pseudomonas maltophilia N. C. I. B. 9204 contains the residues of two rare monosaccharides – 6-deoxy-D-talose, presumably O-acetylated in position 2 [17], and D-arabinose in the furanose form. The structure of the O-specific polysaccharide shows a trisaccharide repeating unit:

$$\begin{array}{l} \rightarrow 3\text{-GalNAc}\ (\beta 1\rightarrow 3)\text{-6d-}D\text{-Tal}\ (\alpha 1\rightarrow \\ \quad\ 2 \qquad\qquad\qquad\quad 2 \\ \quad\ \uparrow \qquad\qquad\qquad\quad | \\ \alpha\text{-}D\text{-Ara}_f\ 1 \qquad\qquad\quad OAc \end{array}$$

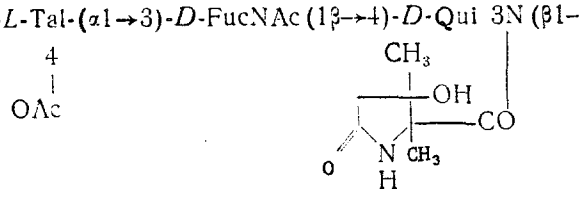
TABLE 2. Unusual Monosaccharides Forming Components of the O-Specific Polysaccharides of the Various Gram-Negative Bacteria

Types of monosaccharides	Abbreviations	Sources of the lipopolysaccharides
Deoxysugars		
4-deoxy-D-arabino-hexose	4-d-Ara	Citrobacter
6-deoxy-L-talose	6-d-Tal	Pseudomonas maltophilia, Pseudomonas fluorescens
6-deoxy-D-mannose (D-rhamnose)	Rha	Pseudomonas cepacia
6-deoxy-L-altrose	6-d-Alt	Yersinia pseudotuberculosis, Yersinia enterocolitica
3,6-dideoxy-L-xylo-hexose (colitose)	Col	Salmonella, Escherichia coli, Arizona, Yersinia pseudotuberculosis
3,6-dideoxy-D-xylo-hexose (abequose)	Abe	Salmonella, Citrobacter
3,6-dideoxy-L-arabino-hexose (ascarylose)	Asc	Yersinia pseudotuberculosis
3,6-dideoxy-D-arabino-hexose (tyvelose)	Tyv	Salmonella, Yersinia pseudotuberculosis
3,6-dideoxy-D-ribo-hexose (paratose)	Par	Salmonella, Yersinia pseudotuberculosis
6-deoxy-D-manno-heptose	6-*D*-Hep	Yersinia pseudotuberculosis
Ketoses		
D-threo-pent-2-ulose (xylulose)	*D*-Xlu	Pseudomonas diminuta, Yersinia enterocolitica
D-arabino-hexulose (fructose)	*D*-Fru	Vibrio cholerae
Amino sugars		
4-amino-4-deoxy-L-arabinose	Ara 4 N	Citrobacter spp.
2-amino-2-deoxy-D-mannose (mannosamine)	Man N	Salmonella spp., Escherichia coli, Citrobacter spp., Moraxella osloensis
2-amino-2,6-dideoxy-D-glucose (quinovosamine)	Qui N	Vibrio cholerae, Achromobacter spp., Salmonella, Pseudomonas, Brucella, Proteus vulgaris, Rhodopseudomonas viridis, Shigella boydii
2-amino-2,6-dideoxy-D-galactose (fucosamine)	Fuc N	Chromobacter violaceum, Salmonella, Citrobacter, Pseudomonas spp., Escherichia coli
2-amino-2,6-dideoxy-L-mannose (rhamnosamine)	Rha N	Escherichia coli
3-amino-3,6-dideoxy-D-glucose	Qui 3 N	Citrobacter, Salmonella spp., Escherichia coli, Pseudomonas synxantha
3-amino-3,6-dideoxy-L-glucose	Qui 3 N	Aeromonas
3-amino-3,6-dideoxy-D-galactose	Fuc 3 N	Xanthomonas campestris, Escherichia coli, Salmonella, Citrobacter spp., Pseudomonas fluorescens, Brucella melitensis

6-Deoxy-L-talose, the optical isomer of 6-deoxy-D-talose, has been identified in hydrolysates of some lipopolysaccharides of E. coli [4] and in a polysaccharide Pseudomonas fluorescens [18]. The repeating unit of the specific polysaccharide from Pseudomonas fluorescens includes residues of the rare monosaccharides 6-deoxy-L-talose O-acetylated in position 4, N-acetyl-D-fucosamine, and 3-amino-3,6-dideoxy-D-glucose with an unusual N-acyl substituent consisting of a residue of 3-hydroxy-2,3-dimethyl-5-oxoproline [18, 19].

TABLE 2 continued

Types of monosaccharides	Abbreviations	Sources of the lipopolysaccharides
4-amino-4,6-dideoxy-D-mannose (perosamine)	Rha 4 N	Vibrio cholerae
4-amino-4,6-dideoxy-D-glucose (viosamine)	Qui 4 N	Escherichia coli, Chromobacterium violaceum
4-amino-4,6-dideoxy-D-galactose (tomosamine)	Fuc 4 N	Escherichia coli
2,4-diamino-2,4,6-trideoxy-D-glucose (bacillosamine)	Bac N_2	Pseudomonas, Shigella sonnei
Uronic acids		
D-glucuronic	Glc A	Shigella boydii, Escherichia coli
D-galacturonic	Gal A	Xanthomonas, Shigella newcastle
Aminouronic acids		
2-amino-2-deoxy-D-galacturonic acid	Gal N A	Citrobacter intermedium, Rhodoapirelaceae, Pseudomonas aeruginosa,
2-amino-2-deoxy-L-alturonic acid	Alt N A	Shigella sonnei
2,3-diamino-2,3-dideoxyuronic acids:		
(D-gluco-)	Glc (2N 3N) A	Pseudomonas aeruginosa
(D-manno-)	Man (2N 3N) A	
(L-gluo-)	Gul (2N 3N) A	
2,3-diacetamido-2,3-dideoxy-D-glucuronic acid	Glc $(NAc)_2$ A	
2,3-diacetamido-2,3-dideoxy-L-guluronic acid	Gul $(NAc)_2$ A	
5-amino-3,5-dideoxynonulosonic acids (D-glycero-D-galacto-neuraminic acid)	Neu A	Salmonella Citrobacter, Arizona
5,7-diamino-3,5,7,9-tetradeoxy-D-glycero-L-galactononulosonic acid	Non (5N7N)	Pseudomonas aeruginosa
5,7-diacetamido-3,5,7,9-tetradeoxy-D-glycero-L-galactononulosonic acid	Non $(NAc)_2$	Shigella boydii
5-acetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-nonulosonic acid	NonN_5A	Shigella boydii
5,7-diamino-3,5,7,9-tetradeoxy-L-glycero-L-mannononulosonic (pseudaminic) acid	Pse (5N7N)	
5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-glycero-L-manno-nonulosonic acid	Pse (5NAc7NFm)	Pseudomonas aeruginosa



The disaccharide FucNAc(1→4)-Qui3NR is a component of the antigenic determinant of the polysaccharide, but the immunodominant monosaccharide is Qui3NR, and immunological reactions have shown the important role of the acyl substituent – 2-acylamino-1,3-dihydroxypropane – obtained on the periodate oxidation of Qui3NR. 4-Deoxy-D-arabino-hexose was first detected in the lipopolysaccharides of Citrobacter, serovars O:4, O:23, O:27, and O:36 [20]. A structural investigation of the O-specific polysaccharide of serovar O:36 showed [21] that it was a linear homopolymer constructed of (β1→2)-bound 4-deoxy-D-arabino-hexopyranose residues. The configuration of the glycosidic bonds established by measurements of the optical activity

of the polysaccharide was confirmed by immunochemical reaction. Thus, the inhibiting activity of methyl 4-deoxy-β-D-arabino-hexoside in the precipitation reaction proved to be almost twice as great as the inhibiting activity of the corresponding α-anomer.

3,6-Dideoxyhexoses. Four representatives of this class of monosaccharides were first identified as components of Salmonella O-antigens, where they play the role of immunodominant species-specific monosaccharides [2]. Three of them were 3,6-dideoxy-D-ribohexose (Paratose) in group A; 3,6-dideoxy-D-xylohexose (abequose) in group B; and 3,6-dideoxy-D-arabinohexose (tyvelose) in group D. The structures of the O-specific polysaccharides of these groups of Salmonella are given in Table 1. The O-antigens of Salmonella greenside, group Z, contain the fourth such monosaccharide, 3,6-dideoxy-L-xylohexose (colitose), as a component of the repeating unit of the specific polysaccharide [22]:

$$\begin{array}{l}\rightarrow 3)\text{-}D\text{-}\mathrm{Gal}_p\,(\alpha 1\rightarrow 3)\text{-}D\text{-}\mathrm{Gal}_p\mathrm{NAc}\,(\beta 1\rightarrow 6)\text{-}D\text{-}\mathrm{Glc}_p\mathrm{NAc}\,(\beta 1\rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 3 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\mathrm{Col}_p\,(\alpha 1\rightarrow 2)\text{-}D\text{-}\mathrm{Gal}_p\end{array}$$

An identical structure has been established for the O-specific polysaccharide of E. coli O 55 [23]. Two α-colitose residues attached to α-D-glucose are present in the repeating unit of the O-antigens of E. coli O 111 and S. adelaide, which also have identical structures [22, 24]

$$\begin{array}{l}\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\alpha\text{-}\mathrm{Col}_p\,1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 3 \\ \rightarrow 3)\text{-}D\text{-}\mathrm{Glc}_p\mathrm{NAc}\,(\beta 1\rightarrow 4)\text{-}D\text{-}\mathrm{Glc}_p\,(\alpha 1\rightarrow 4)\text{-}D\text{-}\mathrm{Gal}_p\,(\alpha 1\rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 6 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\alpha\text{-}\mathrm{Col}_p\,1\end{array}$$

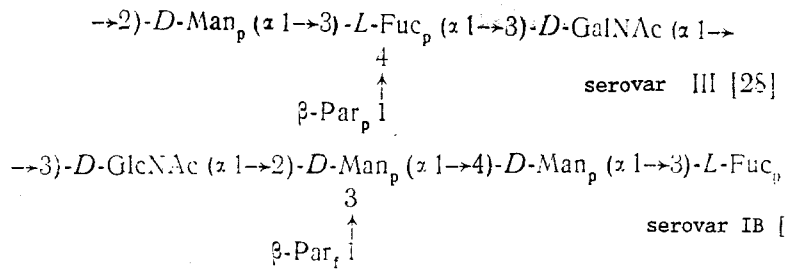
All the above-mentioned 3,6-dideoxyhexoses have also been detected in the lipopolysaccharides of Yersinia pseudotuberculosis. Characteristic for them, as also for Salmonella, is the presence of this class of monosaccharides in side-chains. The polysaccharides of serovars IIA and IIB, the structures of which have not been definitively established, contain abequose:

$$\begin{array}{lll}\qquad\qquad\qquad\qquad\rightarrow 3)\text{-}D\text{-}\mathrm{Gal}_p\,(\alpha 1\rightarrow & & \\ \qquad\qquad\qquad\qquad\qquad\quad 4 & & \\ \qquad\qquad\qquad\qquad\qquad\quad \uparrow & & \\ \qquad\qquad\mathrm{Abe}_p\,(\alpha 1\rightarrow 3)\text{-}6\text{b-}\mathrm{Hep}_p\,1 & \text{serovar} & \text{IIA [25]} \\ \rightarrow 2)\text{-}D\text{-}\mathrm{Man}_p\,(\alpha 1\rightarrow 3)\text{-}L\text{-}\mathrm{Fuc}_p\,(\alpha 1\rightarrow & \text{serovar} & \text{IIB [26]} \\ \qquad\quad 3 & & \\ \qquad\quad \uparrow & & \\ \mathrm{Abe}_p\,1 & & \end{array}$$

The immunodominant groups in both O-antigens are disaccharide fragments consisting of α-abequopyranose residues attached in the C3 position of 6-deoxy-D-mannopyranose or α-D-mannopyranose [22].

The complete structures of the O-specific polysaccharides of the pseudotuberculosis microbe have been established by Yu. S. Ovodov et al. It has been shown that paratose in the pyranose form is a component of the polysaccharides of serovars IA and III, while its furanose form has been determined in the structure of serovar IB:

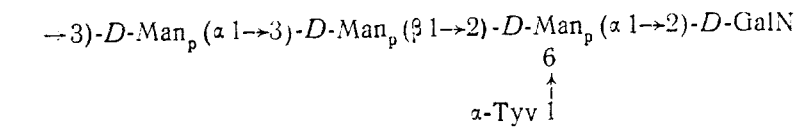
$$\begin{array}{lll}\rightarrow 3)\text{-}D\text{-}\mathrm{GlcNAc}\,(\beta 1\rightarrow 3)\text{-}D\text{-}\mathrm{Gal}_p\,(\alpha 1\rightarrow & & \\ \qquad\qquad\qquad\qquad\quad 4 & & \\ \qquad\qquad\qquad\qquad\beta\uparrow & & \\ \mathrm{Par}_p\,(\beta 1\rightarrow 3)\text{-}6\text{d-}\mathrm{Hep}_p\,1 & \text{serovar} & \text{IA [27]}\end{array}$$



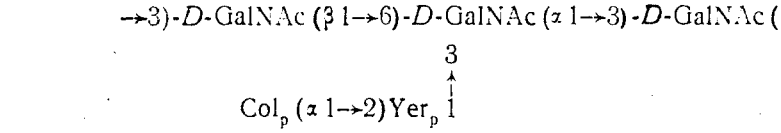
serovar III [28]

serovar IB [29]

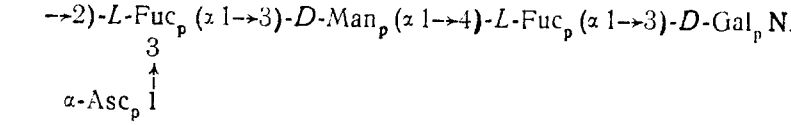
Characteristic for the specific polysaccharides of serogroup IV is the presence of α-tyvelose residues [30]. In the polysaccharide chain of serovar IVA, the terminal tyvelose group is attached in position 6 to an α-mannose residue having a 1,2-bond [31]:



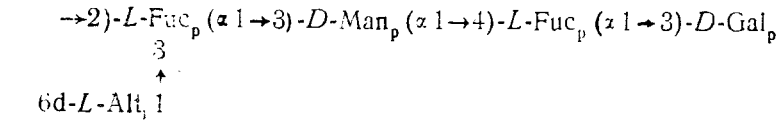
Colitose residues having the α-configuration are included in the polysaccharide chain of serovar VI [32]:



The fifth representative of the class of 3,6-dideoxyhexoses – ascarylose (3,6-dideoxy-L-arabinohexose) – is present in the repeating unit of the polysaccharide of serovar VA [33]:

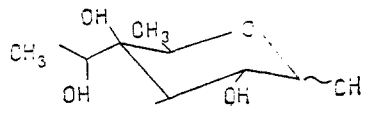


In the structure of the polysaccharide of serovar VB the terminal position is occupied by the rare monosaccharide 6-deoxy-L-altrose in the furanose form [34]:



The combination in one serogroup of serovars VA and VB having different terminal monosaccharide residues (ascarylose and 6-deoxy-L-altrose) is due to their identical linear polymeric chains. The cross serological reactions of the lipopolysaccharides of Y. pseudotuberculosis with O-factor sera of Salmonella [2, 35] – of serovar II with O-factors 4 and 27; of serovar IV with O-factors of 9 and 46 and also of serovar VI with E. coli O-group 55 [36] – are due to common immunodominant monosaccharides: abequose, tyvelose, and colitose, respectively. It is striking that the pseudotuberculosis microbe is the only representative of Gram-negative bacteria that contains all the 3,6-dideoxyhexoses that have been detected in Nature. In addition, the O-antigens of this species of bacteria contain two other rate monosaccharides. One of them – 6-deoxy-D-mannoheptose [21, 24] – forms a component of the repeating unit of the polysaccharides of serovars IA and IIA.

It must be mentioned that the D-manno-configuration of the 6-deoxyheptose was suggested on the basis of the immunological cross interaction of the lipopolysaccharide of serovar IIa with a group B Salmonella containing a D-mannose residue and was confirmed by comparison with a synthetic sample [37]. In the polysaccharide of serovar VI a new branched monosaccharide has been detected [32] which has been called yersiniose A (YerA) and is 3,6-dideoxy-4-C-(1-hydroxyethyl)-D-xylohexose [38].

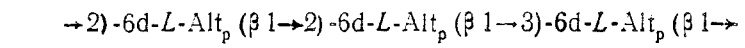


Later, a new representative of the class of branched octoses was detected as a component of the specific polysaccharides of Y. enterocolitica 0:4:32 and Y. intermedia 0:4:32 — yersiniose B — which differed from yersiniose A by the configuration of the asymmetric center at C1' [39].

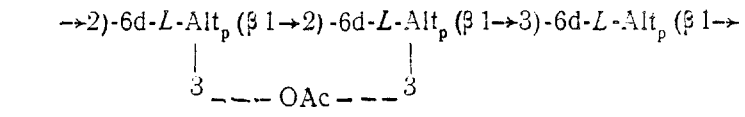
The presence of 6-deoxy-L-altrose and 6-deoxy-D-gulose has proved to be characteristic for a number of specific polysaccharides of Y. enterocolitica.

The specific polysaccharides of Y. enterocolitica, serovars O:1, 2a, 3 and O:2a, 2b, 3 are homopolymers consisting of trisaccharide repeating units with 1,2- and 1,3-bound 6-deoxy-L-altrose residues.

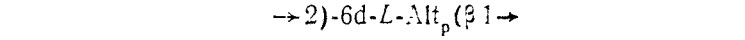
Y. enterocolitica 0:1,2a, 3 [40]



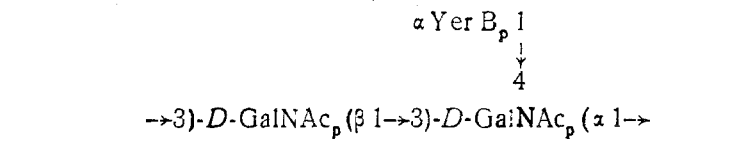
Y. enterocolitica 0:2a,2b,3 [40]



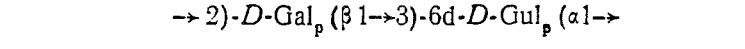
Y. enterocolitica 0:3 [40, 41]



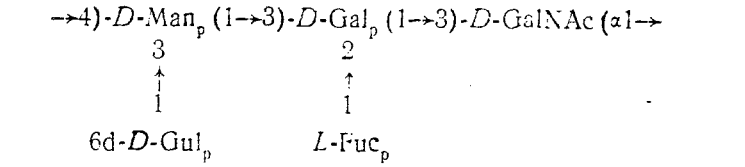
Y. intermedia 0:4.33 (strain 1476) [42]



Y. enterocolitica 0:6.31 [43]

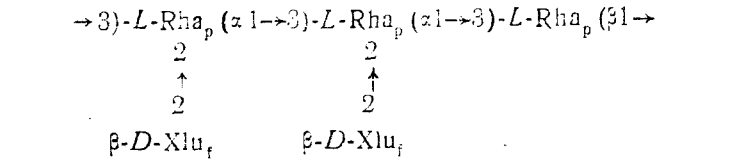


Y. enterocolitica 0:8 [44]

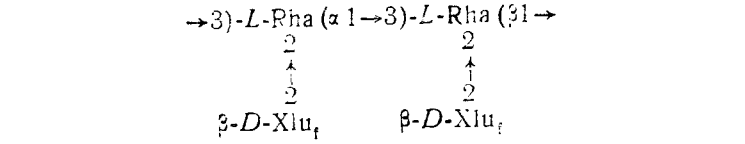


In the lipolysaccharides of Y. enterocolitica, serovars 0:5 and 0:5:27, an acid-labile ketose (D-threo-pent-2-ulose) has been identified [45]; it had been found previously as a component of a bacterial polymer from Pseudomonas diminuta [46].

The repeating unit of the specific polysaccharides from Y. enterocolitica, serovars 0:5 and 0:5:27 is a pentasaccharide the linear chain of which is constructed of L-rhamnose residues while branches contain D-threo-pent-2-ulose residues in the furanose form [45]:



Later the following structure of the tetrasaccharide repeating unit was suggested for the polysaccharide of Y. enterocolitica serovar 0:5:27 [47]:

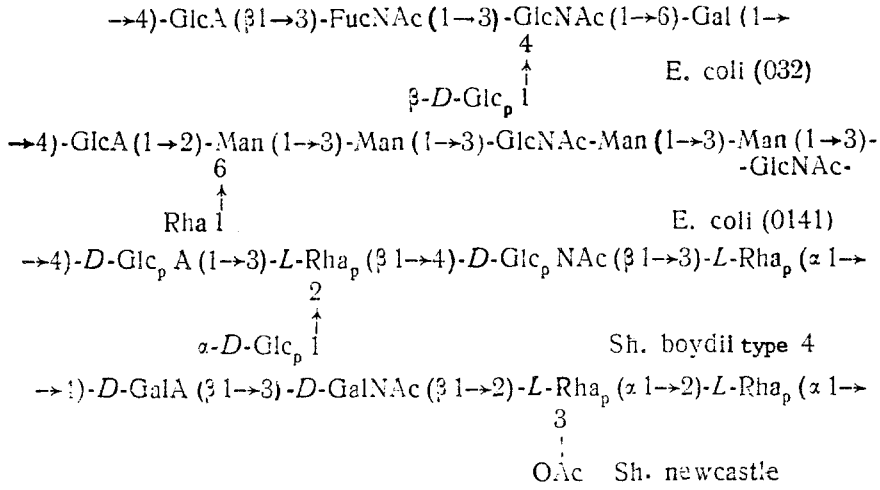


The inhibition of the direct hemagglutination reaction by various fragments of the side chains of Y. enterocolitica, serovars 0:5 and 0:5.27 showed that the determinant groups connected with factor 5 corresponded to α-L-rhamnose residues glycosylated by D-threo-pent-ulose [48].

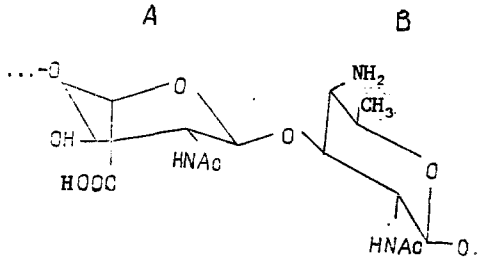
A lipopolysaccharide was isolated from the phenol obtained in the treatment of Y. enterocolitica 0:9 the specific polysaccharide of which consisted of a homopolymer constructed of 1,2-bound 4-formamido-4,6-dideoxy-α-D-mannopyranose residues [49]. Polysaccharides of identical structure have also been isolated from Brucella abortus 19-BA and B. melitensis 565 [50]. Earlier, a homopolymer of similar structure composed of 2-O-bound 4-amino-4,6-dideoxy-α-D-mannopyranose residues N-acylated with 3-deoxy-L-glycero-tetronic acid had been isolated from the O-antigenic lipopolysaccharide of Vibrio cholerae [51].

Thus, the existence of cross serological reactions of Y. melitensis and B. abortus with Y. enterocolitica and V. cholerae is due to the presence of the common structural fragment 4-amino-4,6-dideoxy-α-D-mannopyranose within the O-specific homopolysaccharide chains of the lipopolysaccharides.

It was previously considered [3] that the presence of acidic monosaccharides in lipopolysaccharides showed their contamination with capsular polysaccharides. However, it has now been established that O-specific polysaccharides may contain acidic components such as glucuronic and galacturonic acid residues. This has been observed for a number of strains of E. coli [49, 52], Shigella dysenteriae type 4 [53], Shigella boydii [54], and Shigella newcastle [55]. Some polysaccharide side-chain structures containing hexuronic acid residues are given below.



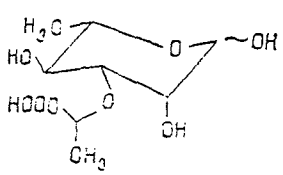
Two unusual monosaccharides have been identified in the O-antigens of Shigella sonnei: 2-acetamido-2-deoxy-L-altruronic acid (A) and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (B), which are components of the biological repeating unit [56]:



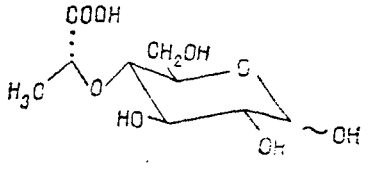
New, not previously encountered monosaccharides – 3-O-(1-carboxyethyl)-L-rhamnose [57] and 4-O-(1-carboxyethyl)-D-glucose – have been detected in the lipopolysaccharides of Shigella dysenteriae, serovars 3 and 5 [58]. The monosaccharides identified form a new class of acidic monosaccharides for which the workers concerned proposed the trivial name "glycolactylic acids." At the present time, two such acids are known: glucolactylic (GlcLA) and rhamnolactyoic (RhaLA):

TABLE 3. Structures of Pseudomonas aeruginosa Polysaccharides [65]

Serogroup	Literature source	Structure of the repeating unit
O : 1	[66]	→4)-*D*-Glc(NAc)$_2$A-(β1→3)-*D*-FucNAc-(α1→3)-*D*-QuiNAc-(α1→ →4)-*D*-GalNAc-(α1→
O : 2a, b	[67—69]	→4)-*D*-Man(2NAc3N)A-(β1→4)-*D*-Man(NAc)$_2$A-(β1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-(β1→
O : 2a, b, e		→4)-*D*-Man(2NAc3N)A-(β1→4)-*D*-Man(NAc)$_2$A-(β1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-(β1→ | OAc
O : 2(a), c		→4)-*D*-Man(2NAc3N)A-(β1→3)-*L*-Gul(NAc)$_2$A-(α1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-(β1→
O : 2a, d		→4)-Man(2NAc3N)A-(β1→4)-*D*-Man(NAc)$_2$A-(β1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-(α1→
O : 2a, d, e		→4)-*D*-Man(2NAc3N)A-(β1→4)-*L*-Gul(NAc)$_2$A-(α1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-α1→
O : 2 (a), d, f		→4)-*D*-Man(2NAc3N)A(β1→4)-*D*-Man(NAc)$_2$A-(β1→ 3 | $CH_3-C=NH$ →3)-*D*-FucNAc-(α1→ 4 | OAc
O : 3a, b	[70, 71]	→4)-*L*-GalNAcA-(α1→3)-*D*-Bac(2NAc4N)-(β1→2)-*L*-Rha-(α1→ 4 | 3 | (S)-$CH_3CH(OH)CH_2CO$ AcO →6)-*D*-GlcNAc-(α1→
O : 3a, b, c		→4)-*L*-GalNAcA-(α1→3)-*D*-Bac(2NAc4N)-(β1→2)-*L*-Rha-(α1→ 3 | 4 \ 3 | OAc (S)-$CH_3CH(OH)CH_2CO$ OAc →6)-*D*-GlcNAc→(α1→
O : 3a, d		→4)-*L*-GlcNAcA-(α1→3)-*D*-Bac(NAc)$_2$-(β1→3)-*L*-Rha-(α1→ →6)-*D*-GlcNAc-(α1→
O : 4a, b	[72]	→2)-*L*-Rha-(α1→3)-*L*-FucNAc-(α1→3)-*L*-FucNAc-(α1→ →3)-*D*-QuiNAc-(β1→
O : 4a, c		→2)-*L*-Rha-(α1→3)-*L*-FucNAc-(α1→3)-*L*-FucNAc-(α1→ →3)-*D*-FucNAc-(β1→
O : 6	[73]	→3)-*L*-Rha-(α1→4)-*D*-GalNAcA-(α1→4)-*D*-GalNFmA-(α1→ 3 | 6 AcO NH_2 →3)-*D*-QuiNAc-(α1→ NH_2 6 | →2)-*L*-Rha-(α1→4)-*D*-GalNAcA-(α1→4)-*D*-GalNFmA-(α1→ 6 | 3 NH_2 OAc →3)-*D*-QuiNAc-(α1→



(RhaLA)



(GlcLA)

TABLE 3 Continued

Serogroup	Literature source	Structure of the repeating unit
O:6a, c		→3)-*L*-Rha-(α1→4)-*D*-GalNAcA-(α1→4)-*D*-GalNFmA-(α1→ 6| NH_2 →3)-*D*-QuiNAc-(α1→
O:6a, d		→3)-*L*-Rha-(α1→4)-*D*-GalNAcA-(α1→4)-*D*-GalNFmA-(α1→ 3| 6| 6 OAc NH_2 NH_2 →3)-*D*-QuiNAc-(β1→
O:7a, b, c	[74]	→3)-*D*-FucNAc-(β1→4)-Pse (5N7NFm)-(α2→4)-*D*-Xyl-(β1→ | 4 5 OAc $OCCH_2CH(OH)CH_3$-(R)
O:7a, b, d		→3)-*D*-FucNAc-(β1→4)-Pse (5NAc7NFm)-(α2→4)-*D*-Xyl-(β1→
O:7a, b		→3)-*D*-FucNAc-(β1→4)-Pse (5NAc7NFm)-(α2→4)-*D*-Xyl-(β1→
O:9a, b	[75]	→3)-*D*-QuiNAc-(β1-O | (R)-CH_3CHCH_2CO | Pse (5NAc7N)-(β2→4)-*D*-FucNAc-(α1→ 4| OAc
O:9a		→3)-*D*-QuiNAc-(β1-O | (R)-CH_3CHCH_2CO | Pse (5NAc7N)-(β2→4)-*D*-FucNAc-(α1→
O:10a, b	[76]	→4)-*L*-GalNAcA-(α1→3)-*D*-QuiNAc-(α1→3)-*L*-Rha-(α1→ 2| OAc
O:10a, c		→4)-*L*-GalNAcA-(α1→3)-*D*-QuiNAc-(α1→3)-*L*-Rha-(α1→
O:11a, b (O:11a, c)	[77]	→3)-*L*-FucNAc-(α1→3)-*D*-FucNAc-(β1→2)-*D*-Glc-(β1→
O:12	[78]	→3)-*D*-QuiNAc-(α1→8)-Non (NAc)$_2$-(α2→3)-*L*-FucN-(α1→ | CH_3—C=NH
O:13a, b	[79]	→4)-*D*-GalNAcA-(α1→3)-*D*-QuiNAc-(β1→2)-*L*-Rha-(α1→ 3| OAc
O:13a, c		→3)-*L*-Rha-(α1→ →4)-*D*-GalNAcA-(α1→3)-*D*-QuiNAc-(β1→2)-*L*-Rha-(α1→ 3 ↑ α-*D*-Glc 1 →3)-*L*-Rha-(α1→
O:14	[80]	→4)-*L*-Rha-(α1→3)-*D*-ManNAc-(β1→

They have also been detected in the lipopolysaccharides of shigella-like strains of E. coli O 124 and O 58 which cross-react in immunochemical reactions with Sh. dysenteriae, serovars 5 and 3, respectively. It is interesting to note that the two species of bacteria cause the same type of disease, namely dysentery. An investigation of the O-antigens of these species of bacteria has permitted the substantiation of the existence of immunochemical cross-reactions between them at the level of the structure of the O-specific polysaccharides. It has been shown that the polysaccharides of E. coli O 58 and O 124 [59, 60], just like the polysaccharides of Sh. dysenteriae, serovars 5 and 3 [61, 62] have identical structures of their repeating units:

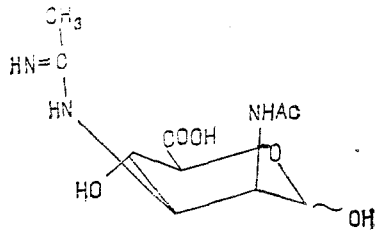
→3)-GlcNAc (α 1→4)-Man (α 1→4)-Man (β1→ E. coli 058=
3 2 Sh. dysenteriae type 5
↑ ↓
α-RhaL.A 1 OAc

→3)-Gal$_p$NAc (β 1→3)-Gal$_p$ (α 1→6)-Gal$_f$ (β 1→ E. coli 0124=
Sh. dysenteriae type 3
↑
GlcLA (β 1→6) Glc$_p$ α 1

This is an example of the interspecies identify of surface antigens which is also reflected in the occurrence of identical mechanisms of the pathogenic states caused by the bacteria. An analogue of N-acetylneuraminic acid was detected for the first time as a component of the specific polysaccharide of Shigella boydii Type 7: 5-acetamido-3,5,7,9-tetradeoxy-7-[(3R)-3-hydroxybutyramido]-L-glycero-L-manno-nonulosonic acid ($NonN_2a$) [63]. The O-specific polysaccharide is constructed of a pentasaccharide repeating unit [64]:

$$\rightarrow 2)\text{-}Gal_f\ (\beta 1\rightarrow 3)\ GlcNAc_p\ (\alpha 1\rightarrow 8)\ NonN_2A\ (\beta 2\rightarrow 6)\ Gal_p\ (\alpha 1\rightarrow 6)\ Glc_p\ (\alpha 1\rightarrow$$
$$\begin{array}{c} 4 \\ | \\ Ac \end{array}$$

The O-antigens of P. aeruginosa, which are the most studied among Gram-negative bacteria outside the family of Enterobacteriaceae, are characterized by a great diversity of unusual, including unique, monosaccharides. The structures of the O-specific polysaccharides of the majority of known serovars of P. aeruginosa have been established by the work of N. K. Kochetkov and his colleagues (Table 3). Many polysaccharides contain residues of acidic monosaccharides: aminouronic and diaminouronic acids. Among them are a N-acetyl-N-galactosaminouronic acid and two of its unusual derivatives: the N-formyl derivative (GalNFmA) and the primary amide (GalNAcA) which are components of serogroup O:6 [73]. 2,3-Diamino-2,3-dideoxyuronic acids – as the di-N-acetyl derivatives with the D-gluco-, D-manno-, and L-gulo- configurations – and also the branched monosaccharide:



have been detected in the polysaccharides of P. aeruginosa, serogroup O:2 for the first time [67, 68].

An unusual sialic acid, a derivative of 5,7-diamino-3,5,7,9-tetradeoxy-L-glycero-L-manno-nonulosonic (pseudaminic) acid, is present in the polysaccharide chains of P. aeruginosa, serogroup O:9 [75] and of Sh. boydii [64]. The cross immunochemical reactions between these O-antigens are connected with the presence in them of only one common monosaccharide – the pseudaminic acid. The specific polysaccharides of almost all the serovars of P. aeruginosa contain rarely found 6-deoxyhexosamines: D-quinovosamine and D- and L-fucosamines. 2,4-Diamino-2,4,6-trideoxy-D-glucose (bacillosamine) N-acylated by a (S)-3-oxobutyryl group has been detected in the polysaccharide of serovar O:3 [71].

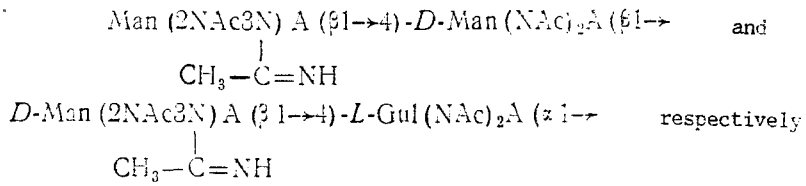
In establishing the interrelationship between the immunochemical specificity of the O-antigens of P. aeruginosa and the determined structures of their polysaccharide chains, it was shown [81] that the role of the group O-factors is played not by the terminal monosaccharides but by the common oligosaccharide fragments of the main chain. For example, the cross precipitation reactions between the lipopolysaccharides of serovars O:2a, b and O:2(a), c or O:2a, d and O:2a, d, e are due to the presence of the common specific determinants

$$D\text{-}FucNAc\ (\beta 1\rightarrow 4)\text{-}Man\ (2NAc3N)\ A\ (\beta 1\rightarrow$$
$$\begin{array}{c} | \\ CH_3\text{—}C\text{=}NH \end{array}$$

and

$$D\text{-}FucNAc\ (\alpha 1\rightarrow 4)\text{-}D\text{-}Man\ (2NAc3N)\ A\ (\beta 1\rightarrow,$$
$$\begin{array}{c} | \\ CH_3\text{—}C\text{=}NH \end{array}$$

and those between the lipopolysaccharides of O:2a, b and O:2a, d or O:2(a), c and O:2a, d, e to the determinant



and

respectively

The immunochemical affinity of the antigens of serovars O:10a, b and O:10a, c is explained by the common structural fragment →4)L-GalNAcA(α1→3)QuiNAc(α1→, in which the N-acetylgalactosaminouronic acid residues are of great importance for the manifestation of the group-specific factor O:10a (this is shown by the absence of inhibiting activity in the polysaccharide reduced at the carboxy groups [81].

The absence of serological affinity between the O-antigens of different serogroups (O:1, O:2, O:3, etc.) is due to the absence of common structural elements in their polysaccharide chains.

A comparison of the repeating units of the O-specific polysaccharides of Pseudomonas aeruginosa, serovars O:10a, b and O:10a, c shows that the differences between them consist in the presence in the polysaccharide of serovar O:10a,b of O-acetyl groups acylating the rhamnose residue in position 2 [75] (Table 3). A confirmation of the link of the O-acetyl group with the factor responsible for the immunochemical difference of the serovars was obtained from the results of the inhibition of passive hemagglutination. The polysaccharide of O:10a,b possesses a high inhibiting activity in the hololog0us test system LPS O:10a,b/anti-O:10a,b serum and exhibits no activity in a heterologous system. After deacetylation it it loses activity in the homologous test system but becomes active in the heterologous test system LPS O:10a,c/anti-O:10a,c serum [81]. On the basis of the results obtained, the hypothesis was put forward that the O:10b factor is connected to the presence of a rhamnose residue O-acetylated in position C-2, and factor O:10c with an unacetylated rhamnose residue, the latter factor being masked by the O-acetyl group in the O:10a,b polysaccharide. The O-deacetylation of this antigen changes its immunochemical specificity, causing a transformation of factor O:10b into O:10c [81].

It has also been established that from the point of view of the structure of the O-specific polysaccharides the division of serogroup O:11 into two O-serovars appears unsubstantiated – the repeating units of the O:11a,b and O:11a,c polysaccharides proved to be identical [77, 81].

Thus, unusual monosaccharides and their derivatives play an important role in the manifestation of the O-antigenic specificity of Gram-negative bacteria. In the majority of cases they are the immunodominant monosaccharides in the structures of the O-antigenic determinants [6].

Nevertheless, at the present time the chmemical nature of only a few O-factors has been established, and therefore a further study of the structures of O-specific polysaccharides and the revelation of the interrelationship with the O-factor typing of Gram-negative bacteria is necessary.

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COMPARATIVE STRUCTURAL STUDY OF THE LIPOPOLYSACCHARIDES OF Y. enterocolitica SEROVARS O:7.8 AND O:19.8

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UDC 579.842.23+577.114.5+543.544.45+51

The lipopolysaccharides of Yersinia enterocolitica, serovars O:7.8 (strain 106) and O:19.8 (strain 842), isolated from the microbial mass by phenol-water extraction, contained residues of L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, D-glucose, D- and L-glycero-D-mannoheptoses, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, and 2-keto-3-deoxyoctonic acid (KDO). The polysaccharides obtained by mild acid hydrolysis of the lipopolysaccharides followed by gel filtration on Sephadex G-50 were a mixture of the O-specific polysaccharide and the core, which could not be separated even by repeated rechromatography because of the comparability of their molecular masses. On the basis of the results of monosaccharide analysis, methylation, Smith degradation, and partial hydrolysis, a structure has been suggested for the repeating unit of the O-specific polysaccharides of the lipopolysaccharides of Y. enterocolitica of the serovars studied.

We have previously suggested a structure for the repeating unit of the O-specific polysaccharide of the Y. enterocolitica, serovar O:8 (strain 161) [1]. The present work is a continuation of the structural study of the lipopolysaccharides (LPS) of the microorganism causing yersiniosis, Y. enterocolitica.

The LPS were isolated from a dry acetone powder of the microbial mass of Y. enterocolitica of serovars O:7.8 (strain 106) and O:19.8 (strain 842) by phenol-water extraction according to Westphal [2]. To determine their monosaccharide compositions, the LPS were subjected to acid hydrolysis, and the monosaccharides were identified with the aid of paper chromatography (PC), GLC, and chromato-mass spectrometry (GLC-MS) in the form of polyol acetates [3]. The LPS investigated had the same monosaccharide composition and contained residues of L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, D-glucose, D- and L-glycero-D-manno-heptoses, D-glucosamine, D-galactosamine, and 2-keto-3-deoxyoctonic acid (KDO) (Table 1).

The polysaccharides (PS) obtained by mild acetic-acid hydrolysis of the LPS followed by gel filtration on Sephadex G-50 and G-25 consisted of the O-specific polysaccharide attached to the core, as in the case of the polysaccharide of serovar O:8 [1], and contained all the monosaccharide residue of the initial LPS with the exception of the KDO (see Table 1). This can probably be explained by the assumption that the O-specific chains and core have comparable molecular masses and are eluted together on gel filtration. The lability of the 6-deoxy-D-glucose bond must be mentioned, since even under the conditions of mild acid hydrolysis a considerable amount of it was split out and was isolated in the pure form. In view of this, the PS were distinguished by a lower content of this sugar.

To establish the link between the monosaccharide residue, the LPS of Y. enterocolitica, serovar O:7.8 and O:19.8 and of the PS obtained from them were methylated by Hakomori's method [4] followed by methanolysis (or hydrolysis). The mixture of partically methylated monosaccharides so obtained was analyzed in the form of methyl glycosides and polyol acetates with the aid of GLC [5] and of GLC-MS [3, 6]. It is obvious from the results on the methyla-

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