SPECIFIC POLYSACCHARIDES OF THE LIPOPOLYSACCHARIDES OF GRAM-NEGATIVE BACTERIA

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This review generalizes the result of structural investigations of the polysaccharides of the O-specific side chains of the lipopolysaccharides (LPSs) of the most studied representations of the family Enterobacteriaceae. The O-specific polysaccharides are the serologically dominating part of the molecule that is responsible for the O-antigenic specificity of the LPSs. The structures of the specific polysacchrides from various Gram-positive bacteria are given. The serological specificity of the O-antigens is discussed and a connection is shown between the chemical structures of the polysaccharides and their serological affinity.

Lipopolysaccharides (LPSs) — component parts of the plastic layer of the cell wall of Gram-negative bacteria, where they are present in the form of a high-molecular-weight complex with protein and with phospholipids — represent a unique group of natural biopolymers with various biological properties [1-5]. They are the O-antigens of the bacteria, are powerful endotoxins, and frequently acts as receptors for bacteriophages, and they have therefore long attracted the attention of scientists for their comparative investigation in structural, biosynthetic, and genetic aspects.

A large number of investigations by specialists of various kinds has been devoted to the LPSs, but the results obtained have not always been unambiguous, which indicates the complexity and multilevel nature of the problem. Great advances have been achieved in the investigation of the structure of the LPSs [6-10]. They are forcing research workers to turn to the elucidation of the link between the structures of the endotoxins and their biological properties. The main difficulties in the solutions of this problem are connected with three circumstances: hitherto the complete structures of the endotoxins has remained unknown; different samples of LPS have different chemical and biological properties; and intra- and interspecies differences in the reaction of a host to an endotoxin exist. Consequently, the interest of research workers in the structural study of the LPSs is increasing from year to year.

In the present review we generalize the results of a study of the structures of the polysaccharides of the O-specific side chains of the LPSs of the most studied representatives of the family Enteriobacteriaceae.

ISOLATION AND STRUCTURAL DETERMINATION

The classical model for the LPSs is one originally suggested by Luderitz and Westphal [3] for the most studied smooth strains of the genus Salmonella. According to this model, the LPSs consist of three covalently bound fragments: the polysaccharide of the O-specific side chain, the oligosaccharide of the core, and lipid A, each of which has its own structure and fulfills a definite biological function [5].

The O-specific polysaccharide is the serologically dominating part of the molecule that is responsible for the O-antigenic specificity of an LPS. It is constructed of repeating oligosaccharide units having serological determinants. The O-specific polysaccharides from various smooth strains can be distinguished to a considerable extent with respect to their composition and structure in accordance with their serological specificity.

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TABLE 1. Structure of the O-Specific Polysaccharides of Salmonella

Serogroup*	Repeating unit**	Literature
C_2	Abep 1 ↓ 3 (on Manp); OAc2/3-α-D-Glcp 1 ↓ 3 (on Manp) →4)-β-L-Rhap (1→2)-α-D-Manp (1→2)-α-D-Manp (1→3)-β-D-Galp (1→	15
C_3	Abep 1 ↓ 3 (on Manp); OAc-D-Glcp 1 ↓ 4 (on Galp) →4)-β-L-Rhap (1→2)-α-D-Manp (1→2)-α-D-Manp (1→3)-β-D-Galp (1→	16
D_2	Tyvp 1 ↓ 3 (on Manp); α-D-Glcp 1 ↓ 4 (on Galp) →6)-β-D-Manp (1→4)-α-L-Rhap (1→3)-α-D-Galp (1→	17
E_1	D-Glcp 1 ↓ 4 (on Galp) →6)-β-D-Manp (1→4)-α-L-Rhap (1→3)-α-D-Galp (1→	18
E_2	D-Glcp 1 ↓ 4 (on Galp) →6)-β-D-Manp (1→4)-α-L-Rhap (1→3)-β-D-Galp (1→	19
E_4	α-D-Glcp 1 ↓ 6 (on Galp) →6)-β-D-Manp (1→4)-α-L-Rhap (1→3)-α-D-Galp (1→	20
B	Ac-2-α-Abep 1 ↓ 3 (on Manp); D-Glcp 1 ↓ 4 (on Galp) →2)-α-D-Manp (1→4)-β-L-Rhap (1→3)-α-D-Galp (1→	21

*C_2 — S. newport; C_3 — S. kentucky; D_2 — S. strasbourg; E_1 — S. muenster; E_2 — S. newington; E_4 — S. seftenberg; B — S. typhimurium.

**Abe — 3,6-dideoxy-D-xylohexose (abequose); Tyv — 3,6-dideoxy-D-arabinohexose (tyvelose); p — pyranose.

To obtain the O-specific polysaccharides, the LPSs are subjected to mild acid hydrolysis with dilute acetic acid followed by centrifugation to eliminate the lipid A and by gel filtration of the carbohydrate component on Sephadex G-50 [11].

Below we consider information on the structure of specific polysaccharides for the most studied LPSs.

Salmonella. The LPSs of salmonellas the most studied, since the Kauffmann-White scheme, in which hundreds of bacterial species belonging to the genus Salmonella are classified according to their serological and biochemical properties [12, 13] has served as the starting point and basis for all chemical and biochemical work on the LPSs of this genus of Gram-negative bacteria.

The majority of information on the O-specific polysaccharides of the side chains has been obtained by the classical methods of carbohydrate chemistry and of immunochemistry (for details, see the reviews [6-10]). However, it must be mentioned that structural investigations of LPSs have been considerably accelerated by the use of the method of chromato-mass spectrometry [14].

The results of structural investigations of the O-specific polysaccharides of salmonellas are given in Table 1.

As can be seen from the structures presented in Table 1, the O-specific polysaccharides of groups D_2, E_1, E_2, and E_4 are chemically related to one another. They have a common main

chain consisting of identical monosaccharide residues similarly bound to one another. However, in contrast to the specific polysaccharides of group E_1, in the specific polysaccharide of group D_2 the mannose residue bears a tyvelose residue.

Escherichia coli. About 150 different O-antigens are known for the colon bacillus E. coli [22].

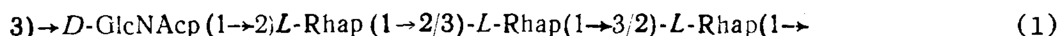
The repeating unit of the O-specific polysaccharides may contain up to six different monosaccharide residues. There are only two O-specific homopolysaccharides that have so far been described in the E. coli genus, and these are the O8- and O9-specific mannans.

For a long time it was considered that all the O-antigens of E. coli contained only neutral monosaccharides. LPSs have been isolated comparatively recently that contain acidic components such as glyceryl phosphate [23], hexuronic acids, neuraminic acid [24, 25], or glucolactylic acid [26].

The structures of the specific polysaccharides of E. coli that have been established are shown in Table 2.

As can be seen from Table 2, the structures of the O-specific polysaccharides of the various serovars of E. coli are fairly diverse in their monosaccharide compositions and structures.

Shigella. Until recently, the best studied of all Shigella groups was the species Sh. flexneri. In a structural study of the O-specific polysaccharides of this species [39-41] it was established that they each contain a main carbohydrate chain consisting of N-acetylglucosamine and rhamnose residues to which α-glucose or O-acetyl-α-glucose residues are attached. Furthermore, according to the structure of their main chains, the O-antigens of Sh. flexneri can be divided into two groups. The first group, including the serovars 1a, 2a, 5a, and variant Y, have a main chain consisting of the trisaccharide repeating units α-D-GlcNAc1 → 2L-Rha1 → 4L-Rha linked to one another by α-(1 → 6)-glycosidic bonds. The second group includes serovars 4a, 3a, 5b and variant X. The O-specific polysaccharide chains of these O-antigens contain α-D-GlcNAc1 → 3L-Rha1 → 4-L-Rha repeating units linked to one another by α-(1 → 4)-glycosidic bonds. Subsequent structural investigations [42, 43] using chromato-mass spectrometry enabled the structure of the repeating unit of the O-specific polysaccharides of Sh. flexneri to be refined. In contrast to the structures proposed initially [41], the repeating unit is the tetrasaccharide (1):



$$3)\rightarrow D\text{-GlcNAc}p(1\rightarrow 2)L\text{-Rha}p(1\rightarrow 2/3)\text{-}L\text{-Rha}p(1\rightarrow 3/2)\text{-}L\text{-Rha}p(1\rightarrow \qquad (1)$$

The monosaccharide compositions of the LPSs of Sh. sonnei (phase I and phase II) have been determined [44-46]. It was found that the main component of the LPS of Sh. sonnei (phase I) is a 2-amino-2-deoxyhexuronic acid with the L-altro configuration [47], while the specific fraction of Sh. sonnei (phase II) contains glucose, galactose, glucosamine, 2-keto-3-deoxyoctonic acid (KDO), and phosphorylethanolamine residues. The structure of the O-specific chains of the LPS of Sh. sonnei (phase I) has been established [48]. They consist of disaccharide repeating units containing a 2-amino-2-deoxy-L-altruronic acid and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose. The N-acetylated α-pyranosic uronic acid residue is substituted in position 4, and the β-pyranosic diaminosugar residue is substituted in position 3.

The greatest interest for the study for the structures of the O-specific polysaccharides is presented by the group Sh. dysenteriae, consisting of 10 serological variants with well-defined serological properties and the absence of group serological affinity. It might have been expected that the O-specific polysaccharide chains of the somatic antigens of this group would have different chemical structures [49-58]. In actual fact, it can be seen from Table 3 that the O-specific polysaccharides of the bacterium Sh. dysenteriae are complex heteropolypolysaccharides constructed of monosaccharides belonging to different classes. They are acidic hexosaminoglycans many of which contain labile 6-deoxyhexose residues. In determining the structures of such complex heteropolysaccharides, the investigators devoted great attention to the primary characterization polysaccharides by physicochemical methods (electrophoresis, ion-exchange chromatography, IR, and PMR spectroscopy), which enabled the most complete information to be obtained on the various functional groups and substituents. In addition, the new method of selective cleavage of hexosaminoglycans at the hexosaminide bonds, based on the N-decacetylation reaction with the subsequent cleavage of the modified polysaccharide with nitric acid or by acid hydrolysis was developed and introduced into

TABLE 2. Structures of the O-Specific Polysaccharides of E. coli

Sero-var	Repeating unit*	Liter-ature
08	→3) -α-*D*-Manp (1→2)-α-*D*-Manp (1→2) -α-*D*-Manp (1→	27
09	→3) -α-*D*-Manp (1→2) -α-*D*-Manp (1→2) -α-*D*-Manp (1→2) -α-*D*-Manp (1→	28
020	→4) -α-*D*-Galp (1→2) β-Ribf (1→ β-*D*-Manp 1 ↓ 4	29
075	→3) -α-*D*-GlcNAcp (1→3)-α-*D*-Galp (1→4)-α-*L*-Rhap (1→ α-*L*-Fucp 1 ↓ 2	30
086	α-*D*-Galp (1→3) β-*D*-Galp (1—3/4)-*D*-GalNAcp (1— α-Colp α-Colp 1 1 ↓ ↓ 6 4	31
0111	β-*D*-GlcNAcp (1→2) -*D*-Glcp (1 → 4)-*D*-Galp (1→ β-*D*-Glcp 1 ↓ 4	32
032	→4) β-*D*-GlcUAp (1→3) -*L*-FucNAcp (1→3)-*D*-GlcNAcp (1→6)-*D*-Galp (1→ α-*L*-RhaLA Ac 1 | ↓ ↓ 3 2/3	33
058	→3) -β-*D*-GlcNAc (1→4) -α-*D*-Man (1→3)-α-*D*-Man (1→ P-Glyc ↓	34
0100	→3) -Gal (1→4) -GlcNAc (1→2) -Rha (1→4) -Rha (1→ β-*D*-GlcLAp (1→6) -α-*D*-Glcp 1 ↓ 4	23
0124	→3) -β-*D*-GalNacp (1→3) -α-*D*-Galp (1→6) -β-*D*-Galf (1→ Rha GlcUA ↓ ↓	26
0141	→2/4) -GlcUA (1→2/6) Man (1→3) -Man (1→3) -*D*-GlcNAc→Man (1→ ↑ →3) -GlcNAc (1→ Rha	35
069	→3)-α-*D*-GlcNAcp (1→2) -α-*L*-Rhap (1→2) -α-*L*-Rhap (1→2) -β-*D*-Galp (1→ α-Colp (1→2) -β-*D*-Galp 1 ↓ 3	36
055	→3) -β-*D*-GlcNAcp (1→4) -*D*-GalNAcp (1→6) -α-*D*-GlcNAcp (1→	37
0114	→3) -α-*D*-GlcNAcp (1→4) -β-3-N- (SerNAc) -3,6-dd-*D*-Glcp (1→ →3) -β-*D*-Ribf (1→4)-β-*D*-Galp (1→	38

*Col — colitose (3,6-dideoxy-L-xylohexose); Rib — ribose; D-GalNAc — N-acetyl-D-galactosamine; D-GlcNAc — N-acetyl-D-glucosamine; L-FucNAc — N-acetyl-L-fucosamine; P-Glyc — glyceryl phosphate; D-Glc-UA — glucuronic acid; D-GlcLA — glucolactylic acid (4-O-(R)-1'-carboxyethyl-D-glucose); 3-N-(SerNAc)-3,6-dd-D-Glc — 3[(N-acetylseryl)amino]-3,6-dideoxy-D-g-glucose; f — furanose.

carbohydrate chemistry [59-91]. An analytical method of determining the relative amounts of glucosamine, galactosamine, and hexoses in the specific polysaccharides, based on the deamination reaction, was also proposed [62].

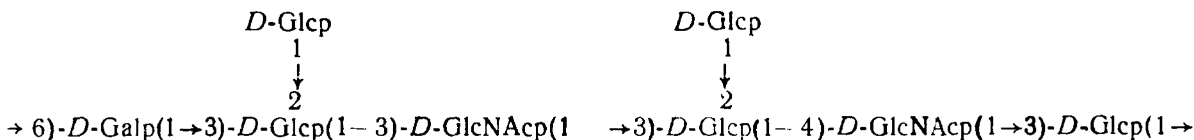
As a result of the investigations performed it was shown for the first time that the specific polysaccharide chains of all 10 serovars of Sh. dysenteriae had different chemical structures. Furthermore, in the course of the structural investigation of the specific

TABLE 3. Structures of the O-Specific Polysaccharides of Shigella dysenteriae

Serovar	Repeating unit*	Literature
1	→3)-α-L-Rhap(1→3)-α-L-Rhap(1→2)-α-D-Galp(1→3)-α-D-GlcNAcp(1→	49
2	α-D-GlcNAcp 1 ↓ 2 →3)-α-D-GalNAcp(1→3)-α-D-GalNAcp(1→4)-α-D-Glcp(1→4)-β-D-Galp(1→	50
3	β-D-GlcLA(1→6)-D-Glcp 1 ↓ 4 →3)-β-D-GalNAcp(1→3)-α-D-Galp(1→6)-β-D-Galf(1→	51
4	Ac-α-L-Fucp 1 ↓ 4 →3)-α-D-GlcNAcp(1→3)-α-D-GlcNAcp(1→4)-α-D-GlcUAp(1→3)-α-L-Fucp(1→	52
5	α-L-RhaLA Ac 1 | ↓ ↓ 3 →3)-β-D-GlcNAcp(1→4)-α-D-Manp(1→3)-α-D-Manp(1→	53
6	X 1 ↓ 4 →3)-β-D-GalNAcp(1→3)-α-D-Galp(1→6)-α-D-Glcp(1→	54
7**	GlcNAc, GalNUA, Gly (1:2:1)	55
8	β-D-GlcNAcp(1→4)-β-D-Glcp 1 ↓ 4 →3)-β-D-GalNAcp(1→4)-β-D-GlcUAp(1→3)-β-D-GalNAcp(1→ CH$_3$CCOOH Ac ∧ ↓ 4,6 2/3	56
9	→3)-β-D-Galp(1→4)-β-D-Manp(1→4)-α-D-Galp(1→3)-β-D-GlcNAcp(1→	57
10	→3)-α-D-ManNAcp(1→3)-α-L-Rhap(1→4)-α-D-GlcNAcp(1→2)-β-D-Manp(1→	58

*L-RhaLA — rhamnolactylic acid (3-O-[(R)-1'-carboxyethyl]-L-rhamnose).
**Explanation in the text.

polysaccharides of this group, new monosaccharides were discovered: 3-O-[(R)-1'-carboxyethyl]-L-rhamnose and 4-O-[(R)-1'-carboxyethyl]-D-glucose, each bearing a lactic acid residue attached to the monosaccharide by an ether bond. Their structures were shown and confirmed by independent synthesis [63, 64]. It is interesting to note that the specific polysaccharide of Sh. dysenteriae serovar 7 completely lacks neutral monosaccharide residues. Glucosamine, galactosaminuronic acid, and glycine have been detected in its composition. However, in 1982 the structure of the specific polysaccharide of Sh. dysenteriae serovar 7 (strain NCTC 519166) was established [65] and differed with respect to its monosaccharide composition from that described previously [53].

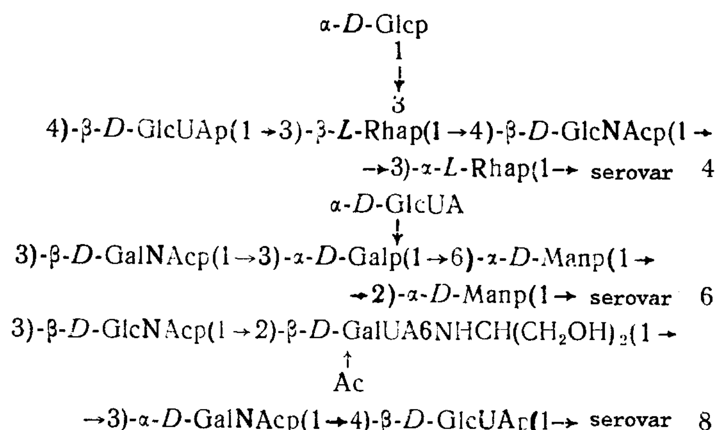


The structure of the specific polysaccharide of serovar 10 was confirmed completely with the aid of ^{13}C NMR spectroscopy [66]. At the present time, ^{13}C NMR spectroscopy has become one of the most important methods of investigating polysaccharides and other biopolymers [67].

The structures of the specific polysaccharides of the Sh. boydii group have been established for a number of serovars [68-70]:

TABLE 4. Structure of the O-Specific Polysaccharides of Klebsiella

Serovar	Repeating unit	Literature
1,2	→3)-α-*D*-Galp (1→	72
3	→2)-α-*D*-Manp (1→3) -α-*D*-Manp (1→3)-α-*D*-Manp (1→2)-α-*D*-Manp (1→2) -α-*D*-Manp (1→	73
4	→4) -α-*D*-Galp (1→2) -β-*D*-Ribf (1→	74
5	→3)-α-*D*-Manp(1→2)-α-*D*-Manp (1→3)-α-*D*-Manp(1→2)-α-*D*-Manp(1→2)-α-*D*-Manp (1→	75
7	→2) -α-*L*-Rhap (1→2)-β-*D*-Ribf (1→3)-α-*L*-Rhap (1→3) α-*L*-Rhap (1→	76
8	OAc ⋮ 2/4, OAc ⋮ 2, OAc ⋮ 2/6 →3)-α-*D*-Galp (1→3) -α-*D*-Galf (1→3) -α-*D*-Galp (1→ OAc ⋮ 6 α-*D*-Galp 1 ↓ 3	77
9	→3)-α-*D*-Galf (1→3)-α-*D*-Galp (1→3)-α-*D*-Galf (1→2) -α-*D*-Galp (1→ ⋮ 6 OAc	78
10	→3) -α-*L*-Rhap (1→3) -β-*D*-Ribf (1→ 3)-α-*L*-Rhap (1→3) -β-*D*-Ribf (1→4) -α-*L*-Rhap (1→	79



On comparing them with one another, it is not difficult to see considerable differences in structure.

Klebsiella. The Klebsiella genus includes 12 serological groups [71], which have been determined by Nimmich. The various O-specific polysaccharides (Table 4) are fairly simple in composition and structure. It is interesting to note that they contain a number of monosaccharide residues in the furanose form.

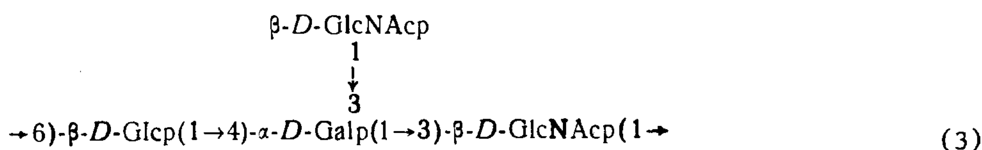
Thus, for example, the specific polysaccharides of serovars 1, 2, 8, and 9 are galactans (with partial O-acetylation in the last two serovars), while 3 and 5 are mannans.

The specific side chains of serovars 5 and 10 have terminal 3-O-methylmannose and 3-O-methylrhamnose residues, respectively. The presence of 3-O-methylmannose and 3-O-methylrhamnose residues, respectively. The presence of 3-O-methylmannose in the specific polysaccharides of three serovars of Klebsiella [73] and E. coli 08 [25] explains the cross-reaction between the LPSs of the species in spite of the difference in structure.

Seratia. The O-specific polysaccharides have been isolated from the LPSs of two strains of the genus Serratia (Bizio and 08) and have been studied. The O-specific polysaccharide of the Bizio (2) strain of Serratia consists of a disaccharide repeating unit containing D-glucose and L-rhamnose [80]

→6)-β-*D*-Glcp(1→2)-β-*L*-Rhap(1→ (2)

The specific polysaccharide of strain 08 [81] has a more complex structure (3). It consists of tetrasaccharide repeating units including D-glucose, D-galactose, and two D-glucosamine residues.



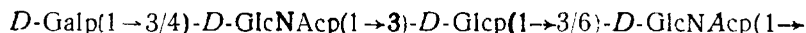
(3)

Proteus. The P mirabilis group constains 31 serovars which are divided on the basis of the monosaccharide composition into 11 chemotypes [82]. All the P. mirabilis serovars so far investigated have different characteristic features. They contain D-galacturonic acid residues, and D-glucuronic acid residues are also present in 8 serovars. The second feature of P. mirabilis distinguishing it from other representatives of the enterobacteria is the presence of D-glycero-D-mannopentose in 8 out of 11 chemotypes [83].

However, the most unusual property of P. mirabilis is the presence in 20 serovars of considerable amounts of L-lysine [81] which has been shown to be a component of the O-specific polysaccharides.

In a study of the O-specific polysaccharide of P. mirabilis strain 1959, Gromska and Mayer [84] established the structure of a disaccharide: D-GalUA(1 → 4)-GalNAc; the lysine residue is attached by its α-amino group to the carboxy group of the galacturonic acid residue of the disaccharide fragment obtained.

Comparatively recently, 1,4-anhydroorbital, formed on the acid hydrolysis of the LPSs, has been identified in the composition of two chemotypes of P. mirabilis (V and IX) [85]. A structural investigation of the O-specific polysaccharide chains of P. mirabilis (strain D-52) has shown that its polysaccharide chain consits of a repeating unit with the following structure [86]:


D-Galp(1→3/4)-*D*-GlcNAcp(1→3)-*D*-Glcp(1→3/6)-*D*-GlcNAcp(1→

It was found that ribitol phosphate was attached in the C-3 position of the galactose residue, while the glucose was substituted by ethanolamine phosphate at C-6.

A determination of the monosaccharide compositions of the LPSs of 20 serovars of the P. vulgaris group permitted them to be divided into 7 chemotypes. Common components for all of them are L- and D-glycero-D-mannopentoses, glucose, glucosamine, galacturonic acid, and KDO residues. In a number of cases glucuronic acid was also detected [87].

Yersinia pseudotuberculosis. The pseudotuberculosis microbe is the only representative among numerous species of microorganisms in which all the rare 3,6-dideoxyhexoses known in nature have been detected [88].

The structures of the O-specific polysaccharides of some serovars have recently been refined, and that of the polysaccharide of serovar VI has been established for the first time. It can be seen from Table 5 that a N-acetylhexosamine residue is present at the reducing end of the specific polysaccharide side chains of all the serovars investigated.

TABLE 5. Structures of the O-Specific Polysaccharides of <u>Yersinia pseudotuberculosis</u>

Serovar	Repeating unit*	Literature
IA	α-Parp (1→3) -β-6d-Hepp 1 ↓ 4 →3) -α-*D*-Galp (1→3)-β-*D*-GlcNAcp (1→	89
IB	β-Parf 1 ↓ 3 →2) -α-*D*-Manp (1→4) -α-*D*-Manp (1→3)-α-*L*-Fucp (1→3)-β-*D*-GlcNAcp (1→	90, 91
IIA	α-Abep (1→3) -α-6d-Hepp 1 ↓ 4 →3) -α-*D*-Galp (1→	92
IIB	Abep 1 ↓ 3 →2) -α-*D*-Manp (1→3) -α-*L*-Fucp (1→	93
III	β-Parp 1 ↓ 4 →2) α-*D*-Manp (1→3) -α-*L*-Fucp (1→3) -α-*D*-GalNAcp (1→	94
IVA	Tyvp 1 ↓ 6 →3)-α-*D*-Manp(1→3) -β-*D*-Manp (1→3)-α-*D*-Manp (1→3) -α-*D*-GalNAcp (1→	95
IV	-Tyvp (1→3)-α-6d-Hepp 1 ↓ 4 →3) α-*D*-Galp (1→	96
VA	Abep 1 ↓ 3 →2)-α-*L*-Fucp (1→3) -α-*D*-Manp (1→4) -α-*L*-Fucp (1→3) -α-*D*-GalNAcp (1→	97
VB	β-6d-Altf 1 ↓ 3 →2) -α-*L*-Fucp (1→3)-α-*D*-Manp(1→4)-α-*L*-Fucp (1→3)-α-*D*-GalNAcp(1→	98
VI	α-Colp (1→2) -β-Yerp 1 ↓ 3 →3) -β-*D*-GlcNAcp (1→6) -α-*D*-GalNAcp (1→3) -β-*D*-GalNAcp (1→	99

*Yer – yersiniose (3,6-dideoxy-4-C-(hydroxyethyl)-D-xylohexose [100]; Par – paratose; 6d-Hep – 6-deoxyheptose; 6d-Alt – 6-deoxyaltrose.

<u>Pseudomonas</u>. The constant interest in the LPSs of <u>P. aeruginosa</u> is due to the pathogenicity of the micrognanism because of its extremely high resistance to many antibiotics and its great ensitivity to EDTA [101]. However, up to the present time there has been no clear serological division of <u>P. aeruginosa</u>, and existing classification systems are fairly contradictory [102-105].

Of course, the identification of the serological variants of <u>P. aeruginosa</u> is acutely necessary. It is not excluded that the chemical structures of the corresponding LPSs may serve as molecular bases for reducing all the classification variants to a single common scheme.

The monosaccharide analysis of the O-specific polysaccharides of <u>P. aeruginosa</u> has shown that they basically consist of amino sugars [106-109], among which D-glucosamine, D-galactosamine, D- and L-fucosamines, quinovosamine, 2-amino-2-deoxy-L-galacturonic acid, and 2,4-diamino-2,4,6-trideoxy D-glucose have been identified. Unidentified amino sugars have also been detected [110, 111].

TABLE 6. Structures of the O-Specific Polysaccharides of Pseudomonas aeruginosa

Serovar	Repeating unit*	Literature
0:1 (Lanyi)	→4)-α-*D*-GalNAcp(1→4)-α-*D*-QuiNAcp(1→3)-α-*L*-Rhap(1→	116
0:3a, b (Lanyi)	→4)-β-*D*-ManImUp(1→4)-β-*D*-Man$(NAc)_2$U(1→3)-β-*D*-FucNAcp(1→	117
0:3a, d (Lanyi)	→4)-β-*D*-ManImUp(1→4)-β-*D*-Man$(NAc)_2$Up(1→3)-α-*D*-FucNAcp(1→	117
0:6 (Lanyi)	→4)-α-*D*-GalNAcp(1→4)-β-*D*-Glc$(NAc)_2$Ap(1→3)-α-*D*-FucNAcp(1→ →3)-α-*D*-QuiNAcp(1→	114
0:7 (Lanyi)	→3)-α-*L*-FucNAcp(1→3)-β-*D*-FucNAcp(1→2)-β-*D*-Glcp(1→	118

*D-ManImu = 2,3-(1-acetyl-2-methyl-2-imidazolino-5,4)-2,3-dideoxy-D-mannuronic acid; D-Man$(NAc)_2$U = 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid; D-QuinAc — N-acetyl-D-quinovosamine.

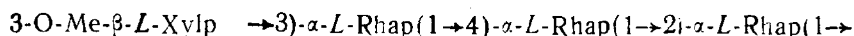
The gel filtration of "degraded" polysaccharides has shown that the O-specific polysaccharides of certain strains of P. aeruginosa are distinguished by a high degree of heterogeneity [112, 113].

The results of a study of the O-specific polysaccharides of P. aeruginosa are given in Table 6.

The complexity of the monosaccharide composition of the polysaccharides of P. aeruginosa creates considerable difficulties in their investigation. Attempts by workers to use ordinary acid hydrolysis in the determination of monosaccharide compositions lead to the identification mainly of well-known monosaccharides such as rhamnose, glucosamine, fucosamine, and quinosamine. Of the usual amino sugars it was possible to isolate and identify only 2,4-diamino-2,4,6-trideoxy-D-glucose [109]. Of course, the presence of monosaccharides with glucosidic bonds resistant to hydrolysis (amino uronic and diamino uronic acids) greatly distorts the results of the quantitative analyses of even ordinary monosaccharides [114]. To determine complete monosaccharide compositions, use was made of the method of selective cleavage of the polysaccharide molecules and, in particular, of solvolysis with hydrogen fluoride [115].

This method permitted, for example, the identification of 2,3-diamino-2,3-dideoxy-D-mannuronic acid derivatives — two new monosaccharides forming components of the specific polysaccharides of serovars 0:3a,b and 0:3a,d (Table 6) — without their isolation in the free state [117]. The identification of these monosaccharides was confirmed by independent synthesis.

Structural investigations of the O-specific polysaccharide of Ps. multophilia, strain NCTC 10257, showed [119] that its repeating unit consisted of the branched tetrasaccharide:



It must be mentioned that the species Ps. multophilia is the most remote from the other pseudomads and is far closer to the genus Xanthomonas. The microorganism Ps. multophilia does not show the sensitivity to EDTA that is characteristic for Ps aeruginosa and for other Pseudomonas species.

SEROLOGICAL SPECIFICITY OF THE O-ANTIGENS

The investigation of the structures of the O-antigens permitted an important principle of the immunodominance of the terminal monosaccharide in the O-specific polysaccharides to be formulated [1].

These results were obtained by comparing various monosaccharides and oligosaccharides as inhibitors of the precipitation of the complement-binding reaction with the use of factor-antifactor systems. It was found that, in the main, one of the monosaccharide residues of a specific polysaccharide chain is the best inhibitor. This monosaccharide residue was also

determined as the immunodeterminant monosaccharide [120]. The role of immunodominants is very frequently played by residues of 3,6-dideoxyhexose as components of O-specific polysaccharides. But, although this monosaccharide plays a very important role in the specificity of the antigenic determinant (the O factor), in itself it does not express complete specificity since one and the same immunodeterminant monosaccharide may be present in different determinants (O-factors).

The immunodeterminant monosaccharide residue is present as a component of the repeating unit of the polysaccharide chain and in many cases (but not always) forms the side chain of the polysaccharide. In such a position, the residue of the immunodeterminant monosaccharide proves to be more accessible for contact with immunocompetent cells.

The functional importance of the terminal mono- or disaccharide residue has been clearly shown by experiments in which a mono- or disaccharide is used as a hapten linked with a protein carrier. Experiments on the immunization of amino conjugate of immunodeterminant 3,6-dideoxyhexoses with protein led to the production of sera reacting with the corresponding LPS [1, 7, 120].

In a comparison of the chemical structures of LPSs differing in their serological specificity an important role of the second monosaccharide in the polysaccharide chain directly linked to its immunodominant unit was revealed. For example, in the O-antigen of Citrobacter 4 the presence of the terminal disaccharide Abe(1 → 3)-D-Rha is characteristic while the O-antigen of Salmonella C_2, differing from it with respect to serological specificity, possesses a terminal disaccharide with somewhat different composition: Abe-α-(1 → 3)-L-Rha. The absence of cross-reactions between the O-antigens of the salmonella groups B and C_2 is also explained by the different positions of the abequose residues in this group (Table 1) [3].

In addition, the specificity of an O-antigen may also be determined by the nature of the bond between the first and second monosaccharides in the polymeric chain. A good illustration of this is the inhibition of the serological reaction of the O-antigen of Salmonella 4 with the specific antiserum that is observed when various derivatives of the immunodominant monosaccharide — abequose — are added to the system [7]. The most active inhibitor proved to be p-nitrophenyl α-abequoside, the activity of which was greater than that of free abequose, since the latter did not have the configuration acquired by the molecule within the disaccharide. It was shown that abequose possesses serological activity only in the α-pyranose form. Consequently, it is precisely in this form that it is the immunodeterminant monosaccharide of the O-antigen.

The nature of the link of the second monosaccharide with the third may also exert a substantial influence on the specificity of a O-antigen. This can be well seen for the case of determinants having glucose as the immunodeterminant monosaccharide. These are, for example, the O-antigens of Salmonella 1, 6, 7, 12_2, 14, 19, and 34. Thus, the O-antigen of Salmonella 12_2 and the O-antigen of Salmonella 34 (Table 7) have a weak serological cross-reaction [13].

All the examples given above indicate what a fine tool for the recognition of the oligosaccharide antigenic determinant the active center of the antibody is. Examples of the recognition of glucose in the form of the immunodeterminant monosaccharide are of particular interest, since the glucose molecule and various compounds containing glucose residues circulate constantly in the human and animal organism. They do not inhibit the synthesis of "antiglucose" cell immunoglobulin since the receptors of the immunocompetent cells recognize not the glucose molecule itself but its concrete conformation determined by its position in the oligosaccharide molecule.

It follows from this that the question of the size of the antigenic determinant is very difficult to answer unambiguously. In some cases it may be considered that the second monosaccharide unit in the chain is a component part of the antigenic determinant, and in others that it determines only the conformation and position of the immunodominant monosaccharide, having an influence on the specificity of the O-antigen in this way.

Evidence in favor of a small size of the specific determinant of a O-antigen is the possibility of the formation of antibodies of two different specificities for one and the same monosaccharide [7] where the immunodominant monosaccharide is present in the main polysaccharide chain and is not a lateral branch. This is the case in the polysaccharide of Salmonella group E, where mannose is the immunodominant monosaccharide in the linear determinant (Table 8).

TABLE 7. Structure of Various O-Factors Containing Glucose as the Imunodeterminant Monosaccharide [3]

Salmonella group	Factor	Oligosaccharide
D	12_2	α-*D*-Glc 1 ↓ 4 α-*D*-Gal (1→2)-*D*-Man (1→4)-*L*-Rha
B	1_{12}	α-Glc 1 ↓ 2 α-*D*-Gal (1→2)-*D*-(Man 1→4) -*L*-Rha
E_4	19	α-Glc 1 ↓ 6 α-*D*-Gal (1→6)-*D*-Man (1→4)-*L*-Rha
E_4B	1	Glc 1 ↓ 6 *D*-Gal→
E_3	34	α-Glc 1 ↓ 4 β-*D*-Gal (1→6)-*D*-Man (1→4)-*L*-Rha
C_1	6_2	α-Glc 1 ↓ 3 *D*-Man→*D*-Man→*D*-Man→*D*-Man
	7	α-Glc 1 ↓ 3 *D*-Man
	14	α-Glc 1 ↓ 3 *D*-Man→*D*-Man (1→3)-*D*-GlcNAc

TABLE 8. Structures of Various O-factors of Salmonella Having the Immunodeterminant Monosaccharide in the Main Chain [3]

Salmonella group	Factor	Immunodominant monosaccharide	Oligosaccharide
E_1, E_4	3	α-*D*-Man	→6) -β-*D*-Man (1→4) -*L*-Rha (1→3) -α-*D*-Gal (1→
E_2, E_3	3	α-*D*-Man	→6) -β-*D*-Man (1→4) -*L*-Rha (1→3) -β-*D*-Gal (1→
E_2	15	β-*D*-Gal	→6) β-*D*-Gal (1→6) -*D*-Man (1→4) -*L*-Rha (1→
E_1	10	α-6-O-Ac-*D*-Gal	Ac-*D*-α-Gal (1→6) -*D*-Man (1→4) -L-Rha (1→
B	5	α-2-O-Ac-*D*-Gal	Ac ↓ *D*-Gal→*D*-Man→*L*-Rha→
		α-2-O-Ac-Abe	Ac-Abe 1 ↓ 3 *D*-Gal (1→2) -*D*-Man (1→4) -*L*-Rha→

The reaction of this antigen with the specific antiserum is inhibited equally by the oligosaccharides α-galactosylmannosylrhamnose, β-galactosylmannosylrhamnose, and mannosylrhamnose. The antiserum probably contains two types of antibodies, one of which recognizes the first of the trisaccharides mentioned and the other the second of them. The origin of

two types of antibodies is interpreted as if the specific receptors of the immunocompetent cells can "consider" the molecule from two different sides, recognizing different configurations of it.

As mentioned above, the absence of group serological affinity for the 10 serovars of Sh. dysenteriae is explained by a basic difference in the chemical structures of the O-specific polysaccharides. The chemical results obtained permitted an explanation for the first time at the molecular level of a number of immunochemical properties of the bacterium Sh. dysenteriae: 1) the absence of serological affinity within the group (absence of a group factor); 2) the retention of typical agglutinogenic properties of the bacteria of serovars 1 and 2 after heating (thermostability) and the thermolability of the antigens of serovars to 3-9 [51-57]; 3) the presence of serological affinity with some serovars of the genus Pneumococcus [121]; and 4) the complete serological identity with some "shigella-like" serovars of the genus E. coli [34, 26].

In actual fact, the absence of serological affinity within the Sh. dysenteriae group follows logically from the chemical structure of the specific polysaccharides, which have no common structural elements whatever. As can be seen from Table 4, the specific polysaccharides of Sh. dysenteriae form two groups: neutral and acidic. Serovars 1, 2, and 10 belong to the first group and serovars 3-9 to the second. The absence of acidic functions in the specific polysaccharides of the first group probably makes the antigen resistant to autohydrolytic cleavage, which explains the thermostability of the antigens of this group. The cells of the thermolabile serovars have acidic functions and labile bonds within their specific polysaccharides.

The existence of cross-reactions between Sh. dysenteriae of serovar 1 and Pneumococcus serovars 2 and 6 [121] is explained by the structural affinity between these antigens. Thus, the cross-reaction with Pneumococcus serovar 2 is observed because the specific polysaccharides have a common structural section — one 3-O-substituted α-L-rhamnopyranoside residue. The cross-reaction with Pneumonoccus (serovar 6) is stronger, since the structure of the specific polysaccharides of the two antigens contain similar blocks consisting now of three monosaccharide residues.

The question of the serological identity of some serovars of Sh. dysenteriae and E. coli is extremely important, since it shows the existence of intergenus links and the evolutionary closeness of the bacteria. As the results of a structural study of the specific polysaccharides of the above-mentioned species of bacteria [26, 34, 51, 53], direct proof has been obtained that the complete serological identity of Sh. dysenteriae 3 and E. coli 0124 and of Sh. dysenteriae 5 and E. coli 058 is due to the complete chemical identity of their specific polysaccharides.

Thus, the undiminishing interest in the study of the LPSs and of the O-specific polysaccharides present in them is due to the fact that the molecular and cellular bases of their biological activity have been studied to only a small extent and, moreover, the biological properties of the endotoxins continue to be of interest for practical medicine in connection with the development of effective measures for combating diseases of Man and animals caused by gram-negative bacteria.

LITERATURE CITED

1. O. Luderitz, A. M. Staub, and O. Westphal, Bacteriol. Rev., 30, 192 (1966).
2. H. Nikaido, Adv. Enzymol., 31, 77 (1968).
3. O. Luderitz, O. Westphal, A. M. Staub, and H. Nikaido, in: Microbial Toxins, (ed. G. Weinbaum, S. Kadis, and S. J. Ajl), Academic Press, New York, Vol. 4 (1971), pp. 145-233.
4. O. Luderitz, M. A. Freudenberg, C. Galanos, V. Lehmann, E. Th. Rietshel, and D. H. Shaw, Curr. Top. Membr. Transp., 17, 79 (1982).
5. I. Ya. Zakharova, and L. D. Varbanets, Carbohydrate-Containing Biopolymers of Bacterial Membranes [in Russian], Kiev (1983).
6. K. Jann and O. Westphal, in: The Antigens (ed. M. Sela), Academic Press, New York, Vol. 3 (1975), pp. 1-110.
7. S. G. Wilkinson, in: Surface Carbohydrates of the Prokaryotic Cell (ed. I. M. Sutherland), Academic Press, New York (1977), pp. 97-175.
8. I. Orskov, F. Orskov, B. Jann, and K. Jann, Bacteriol. Rev., 41, 667 (1977).
9. C. Galanos, O. Luderitz, E. Th. Rietschel, and O. Westphal, Int. Rev. Biochem., Biochem., Lipids, 14, 242-253 (1977).

10. D. A. R. Simmons, Bacteriol. Rev., 35, 117 (1971).
11. G. Schmidt, B. Jann, and K. Hann, Eur. J. Biochem., 10, 501 (1969).
12. F. Kauffmann, Die Bakteriologie der Salmonella Species, Munksgaard, Côphenhagen (1961).
13. F. Kauffmann, in: The World Problem of Salmonellosis (ed. E. Oge), Uitgeverij Dr. W. Junk, The Hague (1964), pp. 21-28.
14. H. Bjorndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, Angew Chem., 82, 643 (1970).
15. C. G. Hellerqvuist, B. Lindberg, S. Svensson, T. Holme and A. A. Lindberg, Carbohydr. Res., 14, 17 (1970).
16. C. G. Hellerqvist, J. Hoffman, A. A. Lindberg, B. Lindberg, and S. Svensson, Acta Chem. Scand., 26, 3282 (1972).
17. C. G. Hellerqvist, B. Lindberg, and A. Pillotti, Acta Chem. Scand., 24, 1168 (1970).
18. C. G. Hellerqvist, B. Lindberg, and J. Lonngren, Carbohydr. Res., 16, 289 (1971).
19. C. G. Hellerqvist, B. Lindberg, J. Lonngren, and A. A. Lindberg, Acta, Chem. Scand., 25, 939 (1971).
20. C. G. Hellerqvist, B. Lindberg, and A. Pillotti, Carbohydr. Res., 16, 297 (1971).
21. C. G. Hellerqvist, O. Larm, B. Lindberg, and A. A. Lindberg, Acta Chem. Scand., 25, 744 (1971).
22. F. Orskov, I. Orskov, V. Jann, E. Miller-Seitz, and O. Westphal, Acta. Pathol. Microbiol. Scand., 71, 339 (1967).
23. B. Jann, K. Jann, G. Schmidt, I. Orskov, and F. Orskov, Eur. J. Biochem., 15, 29 (1970).
24. F. Orskov, I. Orskov, B. Jann, and K. Jann, Acta Pathol. Microbiol. Scand., 79, 142 (1971).
25. K. Jann, Z. Physiol. Chem., 350, 666 (1969).
26. B. A. Dmitriev, V. L. L'vov, N. K. Kochetkov, and K. Jann, Eür. J. Biochem., 64, 491 (1976).
27. K. Reske and K. Jann, Eur. J. Biochem., 31, 320 (1972).
28. P. Prehm, V. Jann, and K. Jann, Eür. J. Biochem., 67, 53 (1976).
29. V. N. Vasil'ev, I. Ya. Zakharova, and E. A. Kovalenko, Zh. Mikrobiol., 42, 456 (1980).
30. C. Erbing, S. Svensson, and S. Hammarstrom, Carbohydr. Res., 44, 259 (1975).
31. P. P. Singh and G. A. Adams, Can. J. Chem., 48, 2500 (1970).
32. R. D. Edstrom and E. S. Heath, J. Biol. Chem., 242, 4125 (1967).
33. B. Jann, K. Jann, G. Schmidt, and F. Orskov, Eür. J. Biochem., 23, 515 (1971).
34. B. A. Dmitriev, Yu. A. Knirel, N. K. Kochetkov, B. Jann, and K. Jann, Eur. J. Biochem., 79, 11 (1977).
35. K. Jahn, B. Jann, F. Orskov, and I. Orskov, Biochem., Z., 346, 368 (1966).
36. C. Erbing, L. Kenne, B. Lindberg, G. Naumann, and W. Nimmich, Carbohydr. Res., 56, 371 (1977).
37. B. Lindberg, F. Lindh, I. Lonngren, A. A. Lindberg, S. Svensson, Carbohydr. Res., 97, 103 (1981).
38. B. A. Dmitriev, V. L. L'vov, N. V. Tochtamysheva, A. S. Shaskov, N. K. Kochetkov, B. Jann, and K. Jann, Eür. J. Biochem., 134, 517 (1983).
39. D. A. R. Simmons, Eür. J. Biochem., 11, 554 (1969).
40. D. A. R. Simmons, Immunology, 20, 17 (1971).
41. D. A. R. Simmons and R. Alastair, Eür. J. Biochem., 18, 53 (1971).
42. B. Lindberg, J. Lonngren, U. Ruden, and D. A. R. Simmons, Eür. J. Biochem., 32, 15 (1973).
43. L. Kenne, B. Lindberg, K. Petersson, E. Katzenellebogen, and E. Romanovska, Eur. J. Biochem., 91, 279 (1978).
44. E. Romanovska and M. Mulczyk, Biochim. Biophys. Acta, 136, 312 (1967).
45. E. Romanovska and V. Reinhold, Eür. J. Biochem., 36,160 (1973).
46. E. Romanovska and M. Mulczyk, Eur. J. Biochem., 5, 109 (1968).
47. T. Kontrohr, Carbohydr. Res., 489 (1977).
48. L. Kenne, B. Lindberg, K. Petersson, E. Katzenellenbogen, and E. Romanovska, Carbohydr. Res., 78, 119 (1980).
49. B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, Eur. J. Biochem., 66, 559 (1976).
50. B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, Eür. J. Biochem., 76, 433 (1977).
51. B. A. Dmitriev, V. L. L'vov and N. K. Kochetkov, Carboydr. Res., 56, 207 (1977).
52. B. A. Dmitriev, V. L. L'vov, N. K. Kochetkov, and I. L. Gofman. Bioorg. Khim., 3, 1226 (1977).
53. B. A. Dmitriev, L. V. Backinowsky, Yu. A. Knirel, and N. K. Kochetkov, Eur. J. Biochem., 78, 381 (1977).

54. B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, Carbohydr. Res., 44, 77 (1975).
55. B. A. Dmitriev, V. M. Dashunin, Yu. A. Knirel', and N. K. Kochetkov, Bioorg. Khim., 5, 77 (1979).
56. B. A. Dmitriev, Yu. A. Knirel', E. L. Ramos, N. K. Kochetkov, and I. L. Gofman, Bioorg. Khim., 4, 760 (1978).
57. B. A. Dmitriev, Yu. A. Knirel', E. V. Vinogradov, N. K. Kochetkov, and I. L. Gofman, Bioorg. Khim., 4, 40 (1978).
58. B. A. Dmitriev, Yu. A. Knirel', O. K. Sheremet, N. K. Kochetkov, and I. L. Gofman, Bioorg. Khim., 3, 1219 (1977).
59. B. A. Dmitriev, Yu. A. Knirel', N. K. Kochetkov, Carbohydr. Res., 28, 451 (1973).
60. B. A. Dmitriev, Yu. A. Knirel', N. K. Kochetkov, Carbohydr. Res., 30, 45 (1973).
61. B. A. Dmitriev, Yu. A. Knirel', and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., Nö. 2, 411 (1974).
62. B. A. Dmitriev, L. V. Backinowsky [Bakinovskii], V. L. L'vov, and N. K. Kochetkov, Eur. J. Biochem., 50, 539 (1975).
63. N. K. Kochetkov, B. A. Dmitriev, and L. V. Backinowsky [Nakinovskii], Carbohydr. Res., 51, 229 (1976).
64. N. K. Kochetkov, B. A. Dmitriev, and V. L L'vov, Carbohydr. Res., 54, 253 (1977).
65. T. K. Ghosh, C. C. V. N. Rao, A. N. Chakraborty, and S. G. Dastidar, Carbohydr. Res., 102, 241 (1982).
66. A. S. Shashkov, B. A. Dmitriev, Yu. A. Knirel', O. K. Sheremet, and N. K. Kochetkov, Bioorg. Khim., 5, 583 (1979).
67. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1975).
68. A. S. Shashkov, V. L. L'vov, N. V. Tokhtamysheva, B. A. Dmitriev, and N. K. Kochetkov, Biorg. Khim., 7, 729 (1981).
69. V. L. L'vov, N. V. Tokhtamysheva, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, Bioorg. Khim., 9, 60 (1983).
70. V. A. Dmitriev, L. V. Backinowsky [Bakinovskii], V. L. L'vov, Yu. A. Knirel, and N. K. Kochetkov, Carbohydr. Res., 41, 329 (1975).
71. W. Nimmich and G. Kôrten, Pathol. Microbiol., 36, 179 (1970).
72. H. Bjorndal, B. Lindberg, and W. Nimmich, Acta Chem. Scand., 25, 750 (1971).
73. M. Curvall, B. Lindberg, J. Lonngren, and W. Nimmich, Acta Chem. Scand., 27, 2645 (1973).
74. H. Bjorndal, B. Lindberg, J. Lonngren, K. Nilsson, and W. Nimmich, Acta Chem. Scand., 26, 1269 (1972).
75. B. Lindberg, J. Lonngren, W. Nimmich, and U. Ruden, Acta Chem. Scand., 27, 3787 (1973).
76. B. Lindberg, J. Lonngren, and W. Nimmich, Acta Chem. Scand., 26, 2231 (1972).
77. M. Curvall, B. Lindberg, J. Lonngren, U. Ruden, and W. Nimmich, Acta Chem. Scand., 27, 4019 (1973).
78. B. Lindberg, J. Lonngren, and W. Nimmich, Carbohydr. Res., 23, 47 (1972).
79. H. Bjorndal, B. Lindberg, and W. Nimmich, Acta Chem. Sci., 24, 34 (1970).
80. C. S. Wang and P. Alaupovic, Biochemistry, 12, 309 (1973).
81. L. Tarcacy, C. S. Wang, S.-C. Li, and P. Alaupovic, Biochemistry, 12, 1948 (1974).
82. K. Kotelko, I. Fromme, and Z. Sidorczyk, Bull. Acad. Pol. Sci., Ser. Sci. Biol., 23, 249 (1975).
83. G. Bagdian, W. Droge, K. Kotelko, O. Luderitz, and O. Westphal, Biochem. Z., 344, 197 (1966).
84. W. Gromska and H. Mayer, Eur. J. Biochem., 62, 391 (1976).
85. J. Gmeiner, H. Mayer, I. Fromme, K. Kotelko, and K. Zych, Eur. J. Biochem., 72, 35 (1977).
86. J. Gmeiner, Eür. J. Biochem., 74, 171 (1977).
87. Z. Sidorczyk, W. Kaca, and K. Kotelko, Bull. Acad. Pol. Sci. Ser. Biol., 23, 603 (1975).
88. D. A. L. Davies, Nature (London), 191, 43 (1961).
89. N. A. Komandrova, R. P. Gorshkova, V. V. Isakov, and Yu. C. Ovodov, Bioor. Khim., 10, 232 (1984).
90. S. V. Tomshich, R. P. Gorshkova, Yu. N. El'kin, and Yu. S. Ovodov, Eür. J. Biochem., 65, 193 (1976).
91. V. V. Isakov, R. P. Gorshkova, S. V. Tomshich, Yu. S. Ovodov, and A. S. Shashkov, Bioorg. Khim., 7, 559 (1981).
92. C. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. R. Brubaker, Acta Chem. Scand., 26, 1389 (1972).
93. C. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. R. Brubaker, Acta Chem. Scand., 26, 1394 (1972).
94. R. P. Gorshkova, N. A. Komandrova, A. I. Kalinovsky, and Yu. S. Ovodov, Eur. J. Biochem., 107, 131 (1980).

95. R. P. Gorshkova, V. A. Zubkov, V. V. Isakov, and Yu. S. Ovodov, Bioorg. Khim., 9, 1401 (1983).
96. K. Samuelsson, B. Lindberg, and R. R. Brubaker, J. Bacteriol., 117, 1010 (1974).
97. R. P. Gorshkova, N. I. Korchagina, and Yu. S. Ovodov, Eur. J. Biochem., 131, 345 (1983).
98. N. I. Korchagina, R. P. Gorshkova, and Yu. S. Ovodov, Bioorg. Khim., 8, 1666 (1982).
99. R. P. Gorshkova, V. A. Zubkov, V. V. Isakov, and Yu. S. Ovodov, Bioorg. Khim., 9, 1068 (1983).
100. R. P. Gorshkova, V. A. Zubkov, V. V. Isakov, and Yu. S. Ovodov, Carbohydr. Res., 126, 308 (1984).
101. S. G. Wilkinson, J. Bacteriol., 104, 1035 (1970).
102. I. Hals, Z. Hyg. Infect. Abt. Med. Microbiol. Immunol., 144, 218 (1957).
103. E. Verder and J. Evans, J. Infect. Dis., 109, 183 (1961).
104. B. Lanyi, Acta Microbiol. Acad. Sci. Hung., 13, 295 (1966-1967).
105. M. W. Fisher, H. B. Delvin, and F. J. Gnabasic, J. Bacteriol., 98, 835 (1969).
106. D. Horton, G. Rodemeyr, and R. Rodemeyr, Carbohydr. Res., 56, 128 (1977).
107. D. Horton, G. Rodemeyr, and T. H. Haskell, Carbohydr., Res., 55, 35 (1977).
108. S. G. Wilkinson and A. P. Welbourn, Biochem. J., 149, 783 (1976).
109. S. G. Wilkinson, Biochem. J., 161, 103 (1977).
110. I. R. Chester, G. W. Gray, and S. G. Wilkinson, Biochem. J., 126, 395 (1972).
111. D. T. Drewry, K. S. Symes, G. W. Gray, and S. G. Wilkinson, Biochem. J., 149, 93 (1975).
112. S. G. Wilkinson and L. Galbraith, Eur. J. Biochem., 52, 331 (1975).
113. I. R. Chester and P. M. Meadow, Eur. J. Biochem., 58, 273 (1975).
114. B. A. Dmitriev, N. A. Kocharova, Yu. A. Knirel, A. S. Shashkov, N. K. Kochetkov, E. S. Stanislavsky, and G. M. Mashilova, Eur. J. Biochem., 125, 228 (1982).
115. A. J. Mort and D. T. A. Lamport, Anal. Biochem., 82, 2899 (1977).
116. Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, B. S. Stanislavsky and G. M. Mashilova, Eur. J. Biochem., 125, 221 (1982).
117. Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, E. S. Stanislavsky and G. M. Mashilova, Eur. J. Biochem., 128, 81 (1982).
118. B. A. Dmitriev, Yu. A. Knirel, N. A. Kocharova, N. K. Kochetkov, E. S. Stanislavsky and G. M. Mashilova, Eur. J. Biochem., 106, 643 (1980).
119. D. J. Neal and S. G. Wilkinson, Eur. J. Biochem., 128, 143 (1982).
120. A. M. Staub, R. Tinelli, O. Luderitz, and O. Westphal, Ann. Inst. Inst. Pasteur, 96, 303 (1959).
121. M. Heidelberger, C. V. N. Rao, and D. A. L. Davies, Pathol. Microbiol., 28, 691 (1965).