INTERNATIONAL JOURNAL OF
SYSTEMATIC BACTERIOLOGY

Vol. 20, No. 4

October 1970

pp. 383-406

Copyright 1970, Iowa State University Press

LIPOPOLYSACCHARIDE IN THE TAXONOMY OF
ENTEROBACTERIACEAE

Hiroshi Nikaido*

Biochemical Research Laboratory
Massachusetts General Hospital
Boston, Massachusetts 02114

ABSTRACT. Review of the literature on the lipopolysaccharide of Enterobacteriaceae leads us to the following conclusions: (1) The structure of the peripheral portion, i.e. "O side chains," varies extensively within each "genus." (2) Although the structure of the central portion, i.e. "R core," tends to be rather similar within each "genus," there are cases of two Escherichia coli strains which synthesize the same O side chains and, at the same time, different R cores.

- - - - -

It is well known that the cell wall of Gram-negative bacteria usually contains four components: peptidoglycan, proteins, phospholipids, and lipopolysaccharide (LPS). The composition and the structure of peptidoglycan appear to vary little among a wide range of Gram-negative bacteria (Tipper, this symposium), and thus are of little use as taxonomic characters. Practically nothing is known on cell wall proteins. There have been some efforts to use cell wall phospholipids as taxonomic markers (Kates, 1964). However, the lipid composition appears to be rather constant among Gram-negative bacteria (Kates, 1964; Cho and Salton, 1966). Environmental factors, furthermore, are known to induce a great deal of change both in fatty acid composition of phospholipids and in the relative abundance of different classes of phospholipids (Marr and Ingraham, 1962; Kanfer and Kennedy, 1963). Thus, except for such cases as extreme halophiles where most of the phospholipid consists of an unusual glycerophospholipid containing polyisoprenoid side chains linked to glycerol through ether linkage (Kates et al., 1965), the prospect of utilizing phospholipids as taxonomic characters for Gram-negative bacteria does not seem particularly bright.

In contrast with these three components, the structure of LPS exhibits enormous variability from one type to another, and this is a very useful taxonomic character. Long before the structure of LPS was elucidated, taxonomists have indeed recognized its variability and have used one expression of LPS structure - immunological specificity - extensively for the classification of Gram-negative bacteria (Kauffmann, 1966). LPS is called "O antigen", when recognized by immunological methods. A group where immunological analysis was carried out in

* Present address: Department of Bacteriology and Immunology,
University of California, Berkeley, California 94720.

detail is Salmonella, where each O antigen is subdivided, by cross-absorption of various antisera, into O-antigenic factors. In this case, certain types which share common antigenic factor(s) can be regarded as synthesizing LPS of related structure. But, as will be seen later, the conclusions drawn from immunological data alone can be misleading, and in order to understand the relationship between various organisms, one must study the composition and chemical structure of LPS.

LPS can be extracted easily, in most cases, by using hot aqueous phenol (Westphal et al., 1952), and purified by ultracentrifugation (see Westphal and Jann, 1965). Mild acid hydrolysis, e.g. 30 minutes to one hour at 100° at pH 3.4 (Osborn, 1963), splits LPS into a lipid, called lipid A, and a polysaccharide. Although extensive comparative studies have not yet been performed on lipid A, the lipids from several Salmonella serotypes and an Escherichia coli strain appeared to be very similar, if not identical (Gmeiner et al., 1969). The polysaccharide portion, however, shows great variability and carries all the O antigen specificities. We shall thus concentrate on the polysaccharide portion of LPS.*

I. LPS from Salmonella

1. Sugar composition.

The monosaccharide composition of Salmonella LPS was studied, mainly by Westphal's group, by acid hydrolysis and paper or column chromatography (Table I). Each Salmonella strain is seen to belong to one of the various "chemotypes", defined on the basis of the sugar composition of LPS. As expected, there is a good correlation between serological specificities of O antigens and sugar composition. Thus organisms which belong to one serogroup always belong to one chemotype. The reverse is not always true, and organisms which belong to the same chemotype frequently synthesize O antigens with no detectable serological cross-reaction (for example serogroups G, N, and U belonging to chemotype VI, and serogroups B and C₂ belonging to chemotype XIV); this incidentally points out the danger of relying too heavily on the sugar composition alone in the classification of bacteria.

Several points should be noted in Table I. First, it is apparent that many rare sugars are present. Examples are an aldohexose (L-glycero-D-mannoheptose), an eight carbon sugar acid (2-keto-3-deoxyoctonic acid, KDO), various dideoxyamino sugars, and a group of 3,6-dideoxyhexoses (colitose, abequose, paratose, and tyvelose). The existence of the last group of sugars in nature had not been known until Westphal's group and Staub's group discovered them in these LPS preparations (for review see Westphal and Lüderitz, 1960).

Another point one can see in Table I is the presence of five "basal" sugars, i.e. KDO, heptose, D-glucose, D-galactose, and N-acetyl-D-glucosamine, in all LPS preparations. This finding will be discussed more thoroughly in the next section.

* Because of the limitation of space, we shall describe only the results on Enterobacteriaceae. For other families of Gram-negative bacteria the excellent review by Lüderitz et al. (1968) is recommended. More recently Adams et al. (1969, 1970) found a striking correlation between LPS structure and other taxonomic characters in Neisseria-Moraxella-Acinetobacter group.

TABLE I

SUGAR COMPOSITION OF *Salmonella* LIPOPOLYSACCHARIDES

Clonotype	3-Amino-3,6-dideoxyglucose	3-Amino-3,6-dideoxygalactose	2-Amino-2,6-dideoxyglucose	D-Mannosamine	D-Galactosamine	D-Glucosamine	2-Keto-3-deoxyoctonate	L-Glycero-D-mannoheptose	D-Galactose	D-Glucose	D-Mannose	L-Fucose	L-Rhamnose	D-Ribose	Colitose	Abequose	Paratose	Tyvelose	Serogroups
I						x	x	x	x	x									V, X, Y
II					○	x	x	x	x	x									L, P, 51
III						x	x	x	x	x		○							C ₁ , C ₄ , H
IV					○	x	x	x	x	x		○							K, R
V						x	x	x	x	x			○						W
VI					○	x	x	x	x	x		○							G, N, U
VII						x	x	x	x	x			○						59
VIII					○	x	x	x	x	x			○						53, 57
XXV						x	x	x	x	x				○					52
IX					○	x	x	x	x	x				○					56
X						x	x	x	x	x					○				0
XI					○	x	x	x	x	x					○				Z
XII					○	x	x	x	x	x	○	○							I
XIII						x	x	x	x	x	○		○						E, F, 54
XIV						x	x	x	x	x	○		○			○			B, C ₂ , C ₃
XV						x	x	x	x	x	○		○				○		A
XVI						x	x	x	x	x	○		○					○	D ₁ , D ₂
XXXIV				○		x	x	x	x	x									J
XXXV				○		x	x	x	x	x			○						T
XXXVI			○			x	x	x	x	x									58
XXXVII			○			x	x	x	x	x	○								S
XXXVIII		○			○	x	x	x	x	x									55
XXXII	○				○	x	x	x	x	x			○						M (28 ₁ , 28 ₂)
XXXIX	○				○	x	x	x	x	x				○					M (28 ₁ , 28 ₂)
XL	○				○	x	x	x	x	x	○	○							Q

This table was taken from Lüderitz *et al.* (1968).

2. Overall structure of LPS

We have just seen that there are five "basal" sugars which always exist in any LPS preparation from Salmonella, in contrast with various other sugars which are present only in some particular serotypes. This suggested that LPS was composed of two regions, one "invariant" region containing the five "basal" sugars and a "variable" region frequently containing other sugars.

This hypothesis was strongly supported by the studies of S-R mutation. From "smooth" wild type strains one can usually get "rough" mutants which form colonies with rough, granular surface. Since the appearance of colonies was found to be occasionally misleading, the symbols S and R were substituted for smooth and rough, and R mutants were defined as mutants lacking the characteristic O antigenic specificities of the parent S-forms. Westphal, Lüderitz, and collaborators investigated the sugar composition of LPS from many R mutants, and arrived at a clear-cut conclusion that all of the mutants examined contained only the five basal sugars regardless of the composition of the LPS of the parent S-form (Lüderitz *et al.*, 1960). They interpreted these results as an indication that the "invariant" portion of LPS, containing five basal sugars, comprises the central core of LPS, and that the variable portion, frequently containing other sugars and carrying all of O-antigenic specificities, exists as peripheral side chains sticking out from the central core. The central core came to be known as R core, and the peripheral side chains as O side chains. According to this hypothesis, R mutants are the mutants which are defective in the biosynthesis of O side chains; they thus synthesize only the central portion of LPS, that is, the R core containing the five basal sugars.

It is hardly possible to overemphasize the influence this hypothesis has had on subsequent studies on LPS. This was the first clue on the overall organization of LPS, and the hypothesis was later found to be correct in almost all aspects. On the basis of this hypothesis, LPS from R mutants has been used for the study of structure of R core region. Furthermore, biosynthetic work using cell-free system also provided a powerful tool for the elucidation of the structure of this region. On the other hand, the discovery of Robbins and Uchida (1962) that O side chains of group E₁ Salmonella were made up of oligosaccharide repeat units has later been extended to other serogroups and has greatly helped our understanding of the structure of O side chains in general. Finally, the mode of linkage of O side chains to the R core has been elucidated at least in S. typhimurium LPS (Nikaido, 1969). Thus we now have a fairly complete picture of the structure of some Salmonella LPS. An example is shown in Fig. 1.

We have so far assumed that R mutants derived from a single S form parent are all similar. This is an oversimplification. As is clear from the structure shown in Fig. 1, mutants defective in the biosynthesis of O side chains will synthesize a complete R core. These mutants are those which were found to contain the five "basal" sugars in their LPS. On the other hand, there are mutants which are defective in one of the steps of the biosynthesis of R core. In these mutants, the biosynthesis proceeds normally up to the point of the metabolic block; thus the composition of LPS varies according to the position of the block (See Fig. 1), but all the mutants are similar in that their LPS cannot accept O side chains owing to the lack of proper attachment sites. Stocker and his associates made an important genetic study on R mutants of S. typhimurium, and found that these two types of mutants

Figure 1. The structure of LPS from *S. typhimurium*. This scheme shows LPS from a 12₂-negative form lacking O antigen 1 (O antigen: 4,5,12₁,12₃). Several laboratories contributed to the elucidation of this structure. In addition to the papers cited in text, the works of Staub's group (Stocker *et al.*, 1960), Lindberg's group (Hellerquist *et al.*, 1968), Osborn's group (Osborn *et al.*, 1964), and Lüdderitz's group (Sutherland *et al.*, 1965; Drüge *et al.*, 1968) are especially important. For details consult recent reviews (Lüdderitz *et al.*, 1968; Nikaido, 1968).

have genetic lesions at different places in the chromosome (Subbaiah and Stocker 1964). The former group, called rough B or rfb, maps between his and metG, and the latter, called rough A or rfa, maps in the region close to ilv and xyI. Subsequent studies revealed that all the genes which are involved in O side chain synthesis are clustered together within rfb region (exceptions are noted below) (Nikaido *et al.*, 1966), and that eight genes so far known to be involved in the biosynthesis of R core all map in the general area close to ilv and xyI (Mäkelä and Stocker, 1969). There are two kinds of genes which are involved in O side chain synthesis but map outside the rfb gene cluster. The first consists of a gene, called rfc, which is assumed to code for an enzyme which catalyzes the polymerization of repeat units in groups B and D, Salmonella (Naide *et al.*, 1965). There is no evidence, however, that such a gene maps outside rfb cluster in group C₁ (Mäkelä, 1966). The second kind includes genes coding for those enzymes with dual functions; for example, UDP-glucose 4-epimerase and Phosphomannoisomerase, which have important catabolic functions in addition to their roles in O side chain synthesis, are coded for by the genes located outside the rfb cluster (see Mäkelä and Stocker, 1969).

Genes in rfa, rfb, and rfc region are not the only genes involved in LPS synthesis, because LPS can be further modified by a variety of "modification enzymes". These enzymes catalyze acetylation and glucosylation of various sugar residues in LPS, and are frequently coded for by genes in the prophage genome. The genes for these enzymes - and the prophages - map at different places all over the chromosome (Mäkelä and Stocker, 1969).

3. Structure of O side chains

The structure of O side chains varies, of course, from one serotype to another. As was noted earlier, the immunological specificity alone or the sugar composition by itself may not give us sufficient information about the relationship between different serotypes, but in many cases the structure of O side chains gives us the most valuable data on this point. Let us take, as an example, the group G, N, U, and L Salmonella studied by Lüderitz, Simmons, and their collaborators. Their antigenic formulae are 13, 22; 30; 43; and 21. In other words, there is no serological cross reaction between these groups, and, from these data alone, one would not expect that they are related in any manner. In contrast, if one looks at the sugar composition of LPS, the first three groups all belong to the same chemotype, VI, and the last group is distinguished from the first three only by the absence of L-fucose (Table II). Thus from these data alone, one would expect an extremely close relationship among these groups. The true relationship is revealed, however, only when one looks at the structure of O side chain repeat units (Table II). It is of course clear that the structure of repeat unit is different from one group to the other, but at the same time one can see some structural relationship. For example, the sequence β -Gal-(1 $\rightarrow$ 3)-GalNAc-(1 $\rightarrow$ 3)-GalNAc is present both in group G and in group L. In group U, the second GalNAc is substituted by GlcNAc. If we know whether sugars occurring as branches (e.g. glucose in group N and galactose in group U) are added by modifying enzymes, and which linkage is formed by the polymerization enzyme, it would not be difficult to calculate how many enzymes must be added and deleted to

Table II. Structure of O side chains in *Salmonella* of serogroups L, G, U, and N.

Serogroup	O-antigen	Chemotype	Repeat unit of O side chain	
			Gal	GlcNAc
			↓ α	↓ α
L	21	II	→	→
			β1,3 Gal →	1,3 GalNAc → GalNAc →
G	13,22	VI	→	→
			Gal	Glc
			β1,3 Gal →	↓ GalNAc → 1,3 GalNAc → 1,4 Fuc
U	43	VI	→	→
			Gal	α1,3 ↓ β1,3 Gal →
				1,3 GalNAc → 1,4 GlcNAc → Fuc
N	30	VI	→	→
				Glc
			β1,3 Glc →	↓ 1,4 1,4 GalNAc → Fuc

These data were taken from Simmons *et al.* (1965) and Lüderitz *et al.* (1966).

Abbreviations: Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine;

GlcNAc, N-acetyl-D-glucosamine; Fuc, L-fucose;

Glc, D-glucose.

convert one type to another. This will give us an index on the degree of relatedness between different groups of Salmonella, and it will be most interesting to compare these data with other indexes, such as DNA homology. Unfortunately, nothing is known on the biosynthesis of O side chains in these serogroups.

In some cases, differences in the structure of O side chains can be explained in a simple manner. When modification enzymes are involved in the biosynthesis of O side chains, and when these enzymes are determined by bacterial genes, there may exist mutant types which have lost modification enzyme(s). Thus S. typhimurium var. copenhagen, which lacks antigen factor 5 and the O-acetyl group located at C-2 of abequose in the O side chains, can be assumed to have derived from the usual S. typhimurium by the mutational loss of the acetylase.

Mutational loss of an enzyme determined by an rfb gene usually leads to the production of R-phenotype, but in rare cases this might not happen. Matsushashi has found that the donor of a 3,6-dideoxyhexose, CDP-tyvelose, is synthesized from the CDP-derivative of another 3,6-dideoxyhexose, CDP-paratose, by a reaction catalyzed by a single enzyme. Now Salmonella paratyphi A (O antigen 1, 2, 12; H antigen a:1, 5) and Salmonella sendai (O antigen 1, 9, 12; H antigen a:1, 5) are similar in fermentation patterns, serological specificity of H antigens, and clinical characteristics. They are also similar in O antigens except that factor 9 (determined by tyvelose) in S. sendai is substituted by factor 2 (determined by paratose) in S. paratyphi A. In fact, according to the recent studies by B. Lindberg's group in Stockholm, S. paratyphi A and several organisms belonging to group D₁, of which S. sendai is a member, were found to synthesize O side chain repeat units of exactly the same structure, except that in S. paratyphi A paratose replaced tyvelose found in group D₁ (Table III). From these results Matsushashi suggested that S. paratyphi A may be a single step mutant of S. sendai which has lost the enzyme catalyzing the conversion of CDP-paratose into CDP-tyvelose (Matsushashi and Strominger, 1965). This is an extremely attractive hypothesis, although it is not known whether S. sendai would so easily utilize CDP-paratose in the absence of CDP-tyvelose. In any case, the close structural relationship among the O side chains of group A, B, and D₁ Salmonella is quite obvious from Table III.

In some cases, lysogenization with temperate phages can result in an extensive alteration of the structure of O side chains. This phenomenon, known as lysogenic conversion, was studied in detail by Robbins, Uchida, and their colleagues. Their conclusion is shown in Table IV. When S. anatum (O antigen=3,10) is lysogenized by a phage called ϵ^{15} , the phage genome supplies three functions so that the structure of O side chains is altered. These functions are 1) inhibition of a polymerase which unites repeat units together through α -Gal-(1 $\rightarrow$ 6)-Man linkage; 2) production of a new β polymerase which produces β -Gal-(1 $\rightarrow$ 6)-Man linkage; and 3) repression of the production of a modification enzyme, O-acetylase. Thus the phage changes the serotype of the host bacterium from S. anatum to S. newington (O antigen =3,15). The latter strain can further be lysogenized with a second phage, ϵ^{34} , which supplies a new modification enzyme, glucosylase, so that O side chains now will contain glucose branches on all galactose residues. These examples illustrate at least one way by which the structure of

Table III. O side chain repeat units of some *Salmonella* serotypes containing 3,6-dideoxyhexoses.

Serogroup	Organism	O Antigen	Repeat Unit
A	<i>S. paratyphi</i> A	1,2,12	<div> <div>Par</div> <div> <div>1,3</div> <div>↓</div> </div> </div> <div> <div>1,4</div> <div>→</div> </div> <div> <div>Man</div> </div>
			<div> <div>OAc</div> <div>↓</div> </div> <div> <div>1,3</div> <div>→</div> </div> <div> <div>Rha</div> </div>
B	<i>S. typhimurium</i>	1,4,5,12	<div> <div>1,6</div> <div>↓</div> </div> <div> <div>1,2</div> <div>→</div> </div> <div> <div>Gal</div> </div>
			<div> <div>OAc</div> <div>↓</div> </div> <div> <div>1,3</div> <div>→</div> </div> <div> <div>Rha</div> </div>
D ₁	<i>S. typhi</i>	9,12	<div> <div>1,4</div> <div>→</div> </div> <div> <div>1,3</div> <div>→</div> </div> <div> <div>Gal</div> </div>
			<div> <div>OAc</div> <div>↓</div> </div> <div> <div>1,3</div> <div>→</div> </div> <div> <div>Rha</div> </div>

The most precise structures shown here were finally established by B. Lindberg's group by the technique of methylation—gas chromatography—mass spectrometric analysis (see Hellerqvist et al. 1968). One should not forget, however, that the main features of these structures were actually established by A.-M. Staub's group through the ingenious combination of chemical (periodate oxidation and partial acid hydrolysis) and immunochemical techniques.

Abbreviations: Par, paratose (3,6-dideoxy-D-glucose); Man, D-mannose; Rha, L-rhamnose; Abe, abequose (3,6-dideoxy-D-galactose); Tyv, tyvelose (3,6-dideoxy-D-mannose); OAc, O-acetyl; for others see Table II.

Table IV. Alteration of the structure of O side chains by converting bacteriophages in group E *Salmonella*.

Serotype	O-Antigen	Repeat unit of O side chain			
				OAc	
<i>S. anatum</i>	3,10	$\alpha 1,4$	1,3		$\alpha 1,6$
		Man	→ Rha	→ Gal	→
+ ϵ^{15}					
<i>S. newington</i>	3,15	$\alpha 1,4$	1,3		$\beta 1,6$
		Man	→ Rha	→ Gal	→
+ ϵ^{34}					
				Glc	
<i>S. minneapolis</i>	3,(15),34	$\alpha 1,4$	1,3	+ $\alpha 1,4$	$\beta 1,6$
		Man	→ Rha	→ Gal	→

This table is based on the results of Robbins & Uchida (1962) and Uchida *et al.* (1963). Abbreviations: See Tables II and III.

of O side chains can be altered. When the converting phages are not defective as in the example above, it is of course easy to detect such phages; however if the converting phage becomes extensively defective in its phage functions, we may not have any way to recognize that certain enzymes are coded for by the genes in the (defective) phage genome. This could have happened many times during the course of evolution.

4. Structure of R core

The original hypothesis on the overall structure of LPS proposed by Westphal's group assumed that the structure of a core is the same in all *Salmonella* serotypes, mainly on the basis of the sugar composition of the R core. We have seen, however, that the sugar composition is a poor evidence for deciding whether two given polysaccharides are identical or not.

There are two lines of more recent studies concerning this point. First, Beckman, Lüderitz and others have compared LPS preparations from R mutants of various *Salmonella* serotypes by the use of hemagglutination inhibition reaction. These preparations all appeared immunologically identical under these conditions, and thus it seems certain that the structure of R core is at least rather similar among various serotypes. It must be mentioned, however, that hemagglutination inhibition is the reaction which detects only the predominant antigenic determinant. We thus cannot exclude the possibility that differences exist in the deep interior of the core or that differences are too small to affect the outcome of hemagglutination inhibition reaction.

Another line of evidence comes from the chemical studies on the structure of R core. Table V shows the sequence of sugars determined by Lüderitz's group and by Osborn's group. Apparently there is a difference in the number of KDO residues and in the linkage of heptose to KDO. These differences could be due to the fact that these two groups used R mutants from different Salmonella serotypes, but they are more likely to be due to uncertainties caused by technical difficulties. Lüderitz's group, for example, found more recently that the linkage between heptose and KDO was 1 → 5 even in the LPS from S. typhimurium R mutants.

In the course of a different line of investigation, we stumbled upon an evidence which suggests that the R core of group C₂ Salmonella may be different from that of group B or C₁ Salmonella. When the R cores of groups B and C₁ are oxidized with periodate and reduced with sodium borohydride, one of the glucose residues of the R core remains unchanged while the other is converted into smaller fragments; the amount of glucose, therefore, decreased to about one-half after this treatment. In contrast, the amount of material which reacts as glucose in the hexokinase glucose-6-P dehydrogenase assay has increased greatly after the periodate-borohydride treatment of R cores from group C₂ Salmonella. We do not yet know which component of group C₂ R core is converted into "glucose", but these results suggest that this R core might be slightly different from the others.

II. LPS of groups other than Salmonella

1. Overall structure of LPS

For the LPS of many groups of Enterobacteriaceae, it has not yet been established that they are also composed of O side chains and R core. Some workers indeed suggested that such distinction cannot be made for the LPS of certain Escherichia coli serotypes. On the other hand, the gene locus corresponding to rfb has been found for other serotypes of E. coli (Ørskov and Ørskov, 1962), and a series of R mutants (corresponding to rfa?) producing progressively more deficient LPS have been isolated from E. coli O111 (Witkinson et al., 1968). These latter mutants, however, have not been genetically mapped. In the case of Shigella, structural studies indicated the presence of a distinct O side chain region (Simmons, personal communication) and an R core region (Johnston et al., 1967). A series of R mutants blocked at different steps of R core synthesis have been isolated (Johnston et al., 1967), although the affected genes have not been mapped. In this presentation we shall tentatively assume that LPS of most Gram-negative enteric bacteria consists of peripheral O side-chains and a central R core.

2. Structure of O side chains

Various rare sugars occur in the LPS, and probably in the O side-chains, from these groups. Table VI lists the sugars which have so far been found in the LPS from various groups. Of course a single E. coli strain, for example, cannot contain all the sugars listed in this Table under E. coli; it contains between five to seven sugars found in this list. Although E. coli LPS appears to be as complex as Salmonella LPS from this Table, this is undoubtedly due, at least in

Table V. Simplified structure of R core.

Organism	R core		Reference
<i>Salmonella typhimurium</i>	GlcNAc ↓ 1,2 1,2 Glc → Gal →	Gal ↓ 1,6 1,3 1,3 1,3 1,7 Glc → Hep → Hep → KDO	Osborn <i>et al.</i> 1964 Cherniak & Osborn, 1966
<i>Salmonella minnesota</i>	GlcNAc ↓ 1,2 1,3 Glc → Gal →	Gal ↓ 1,6 1,3 1,3 1,3 1,5 Glc → Hep → Hep → KDO → (KDO) ₂	Sutherland <i>et al.</i> 1965 Droge <i>et al.</i> 1968
<i>Shigella flexneri</i> 4b	GlcNAc ↓ 1,4 1,3 Glc → Gal →	Glc ↓ 1,4 1,3 Glc → (Hep?)	Johnston <i>et al.</i> 1967
<i>Escherichia coli</i> 0111*		Glc ↓ 1,7 1,4 1,3 1,3 1,8Glc → GlcN → Hep → Hep → KDO	Fuller <i>et al.</i> 1969

This table shows only the simplified structure of R core. Actually there are phosphate groups and phosphoethanolamine groups attached to heptose and KDO residues, and it is assumed that these chains are cross-linked through phosphodiester linkages. The structure shown here, furthermore, is linked to lipid A through KDO.

Glucosyl (1→2) galactose linkage in the structure of *S. typhimurium* R core is based on Nikaido (1969). N-acetylglucosaminyl (1→2) glucose linkages in *Salmonella* R cores are based on unpublished results from Lüdertitz's and Nikaido's laboratories.

*There are two other kinds of sequence. See text.

Table VI. Occurrence of various sugar components in LPS from some groups of *Enterobacteriaceae*.

Sugar	Taxonomic Group				
	<i>Salmonella</i>	<i>E. coli</i>	<i>Arizona</i>	<i>Shigella</i>	<i>Proteus</i>
Glucuronic Acid					+
Galacturonic Acid					+
3-Amino-3,6-dideoxyglucose	+	+			
3-Amino-3,6-dideoxygalactose	+	+	+		
2-Amino-2,6-dideoxyglucose	+		+		+
2-Amino-2,6-dideoxymannose		+			
2-Amino-2,6-dideoxygalactose		+			
Mannosamine	+	+	+		
Galactosamine	+	+	+		
Glucosamine	+	+	+	+	+
2-keto-3-deoxyoctonic Acid	+	+	+	+	+
L-Glycero-D-mannoheptose	+	+	+	+	+
D-Glycero-D-mannoheptose					+
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Mannose	+	+	+	+	+
Fucose	+	+	+		
Rhamnose	+	+	+	+	
6-Deoxytalose		+			
Ribose	+	+			
Colitose	+	+	+		
Abequose	+				
Paratose	+				
Tyvelose	+				

This table is based on the data presented in Lüderitz et al. (1968). Although groups outside of *Enterobacteriaceae* are not included in this table, it is interesting to note that L-glycero-D-mannoheptose, ubiquitous in *Enterobacteriaceae*, is absent in LPS from *Xanthomonas* species and *Pseudomonas aeruginosa* (Volk 1966; Gray and Wilkinson 1965). In a blue-green algae, it is replaced by a 2-amino-D-glyceroheptose (Weise 1968).

part, to the large number of E. coli strains examined, and to the heterogeneity of E. coli as a taxonomic group. A small and relatively homogeneous group like Shigella has LPS of relatively similar composition, and consequently the number of sugars found in Shigella LPS is limited. In the case of a group like Proteus, which is not closely related to E. coli - Shigella - Salmonella groups on the basis of GC-content (Falkow et al., 1962) and of homology (Brenner et al., 1969) of DNA, the LPS is also very different from those of the latter group; one finds uronic acids and the "second heptose" (D-glycero-D-mannoheptose) which never occur in the LPS of E. coli - Shigella - Salmonella group.

It has been known that organisms belonging to different groups (or genera) sometimes possess cross-reacting O antigens. In some cases the serological cross-reaction is complete, and no difference is found by cross-absorption test. In several such combinations, the sugar composition of LPS has been examined by Westphal's group (Table VII) and was found to be the same. It is regrettable that no detailed structural studies on O side chains has been performed so far on the LPS of any of these cross-reacting organisms; however, common sugar composition and complete serological cross-reaction suggest strongly that the structure of O side chains may be identical or at least very similar among each cross-reacting combination. It is indeed interesting that O side chains of similar structure, for whose synthesis probably more than a dozen genes are needed, are shared by organisms which belong to different "genera". These organisms presumably differ in hundreds of other characters. It is to be regretted, again, that DNA homology studies have not yet been carried out among these cross reacting combinations; however, it may be possible that the genetic information on the biosynthesis of O side chains was transferred from one species to another quite different species across the boundary of "genus". The clustering together of all the genes involved in O side chain synthesis within the small rfb area would be ideally suited for such an exchange of information.

In these cross-reacting combinations, one cannot yet rule out the possibility that each organism has independently developed the ability to synthesize a polysaccharide of similar structure. If true, this would serve as a good example of evolutionary convergence. In this case the structure of O side chains is likely to be similar but not exactly the same in each group; for this reason the structural studies on these LPS are eagerly waited for. An interesting case in this connection is the cross-reaction of human blood group B substance and LPS from group U Salmonella and E. coli O 86. As seen in Table VIII, the bacterial O side chains contain sequences which are similar to, yet somewhat different from, the sequence seen in the human blood group substance.

3. R core

The structure of R core from Shigella flexneri 4b has been established by Simmons and his coworkers (Table V.) It is different from, yet obviously related to, that of Salmonella. The main chain sequence of glucosyl-galatosyl-glucose has been retained, although the glucosyl-galactose linkage has been changed from (1 → 2) to (1 → 3).

Heath and coworkers have studied the structure of R core in two strains of E. coli belonging to serotype O111. Here the results are

Table VII. Sugar composition of serologically cross-reacting LPS preparations from different genera.

Organism	O-Antigen	Sugars found in LPS*							
		GalN	GlcN	Hep	Gal	Glc	Man	Fuc	Rha
<i>Salmonella weslaco</i>	42		+	+	+	+			+
<i>Arizona</i>	15		+	+	+	+			+
<i>E. coli</i>	31		+	+	+	+			+
<i>Salmonella inverness</i>	38	+	+	+	+	+			
<i>Arizona</i>	16	+	+	+	+	+			
<i>E. coli</i>	21	+	+	+	+	+			
<i>Salmonella whittingfoss</i>	16	+	+	+	+	+	+	+	
<i>Arizona</i>	25	+	+	+	+	+	+	+	
(<i>E. coli</i>)	11	+	+	+	+	+	+	+	
<i>Salmonella aberdeen</i>	11		+	+	+	+	+		+
<i>Arizona</i>	17		+	+	+	+	+		+
(<i>E. coli</i>)	75		+	+	+	+	+		+

This table is based on the results of Westphal *et al.* (1960). Three strains grouped together have *identical* O antigens on the basis of serological tests. The strains in parentheses strongly cross-react with others in the same group, yet their O antigens appear to be slightly different.

*The presence of KDO was not investigated, but this compound is most probably present in all LPS preparations.

Table VIII. Structure of O side chains which cross-react with human blood group B antigen.

	Structure	Reference
	Fuc	
Group U <i>Salmonella</i>	$ \begin{array}{ccccccc} & & \downarrow & & & & \\ \alpha 1,3 & & \beta 1,3 & & 1,3 & & 1,4 \\ \text{Gal} \rightarrow & \text{Gal} \rightarrow & \text{GalNAc} \rightarrow & \text{GlcNAc} \rightarrow & & & \end{array} $	Simmons <i>et al.</i> 1965
	Fuc	
<i>E. coli</i> 086	$ \begin{array}{ccccccc} & & \downarrow \alpha & & & & \\ \alpha 1,3 & & \beta 1,3 \text{ or } 4 & & & & \\ \text{Gal} \rightarrow & \text{Gal} \rightarrow & & \rightarrow & \text{GalNAc} \rightarrow & & \end{array} $	Springer <i>et al.</i> 1966
	Fuc	
Human Blood Group B	$ \begin{array}{ccccccc} & & \downarrow 1,2 & & & & \\ \alpha 1,3 & & \beta 1,3 \text{ or } 4 & & & & \beta 1,3 \\ \text{Gal} \rightarrow & \text{Gal} \rightarrow & & \rightarrow & \text{GlcNAc} \rightarrow & \text{Gal} \rightarrow & \end{array} $	Watkins, 1966

rather complicated. Even when they used UDP-glucose 4-epimerase-less mutants, which produce LPS consisting of a very incomplete and simplified R core, three kinds of sequences were found in one strain. One of them was identical with the sequence found in S. typhimurium R core, but another was very different and contained a glucosamine residue with a free amino group (Table V). However, in another strain of E. coli O111, such an unusual sequence was not present. Thus the R core in at least one strain of E. coli O111 contains a sequence which is not identical with the R core of Salmonella. These results are also interesting in that two strains belonging to the same O-serotype, and thus producing the same O side chains, are shown to synthesize R cores of different structure.

Jann's group studied rfb mutants from two E. coli strains belonging to the O-8 serotype (Schmidt et al., 1969). Serologically, the LPS produced by the mutants derived from one strain were completely different from the LPS of the mutants originated from the other strain. Neither of these cross-reacted with the R core from S. minnesota. Quantitative sugar analysis also indicated that the R core from one strain contained galactose, glucose, glucosamine, and heptose in a molar ratio of 2:4:1:4; while the R core of the other strain contained these components in the ratio of 2:3:0:3. These results thus show that both of these E. coli strains synthesize R cores which are different from the R core of Salmonella, and that the structure of R core can be different even among those strains synthesizing the O side chains of the same structure.

Qualitative sugar composition of E. coli LPS, determined by Westphal's group, further indicates that in at least four serotypes galactose is completely absent even in the S-forms. Since galactose is an integral component of R core in Salmonella, these E. coli serotypes must produce R cores with a different structure (see Lüderitz et al., (1968).

More work is needed concerning the structure of R cores in groups other than Salmonella. It is already clear, however, that some E. coli serotypes and Shigella flexeri produce R cores which are different from that found in Salmonella. Even among E. coli strains, the structure of R core may vary from one strain to another.

III. Conclusion

As we have seen, comparative studies on the structure of LPS in Gram-negative bacteria have not yet developed very far. But even at this stage, we can draw the following tentative conclusions. (1) The composition and structure of O side chains vary extensively within each group or "genus". (2) The structure of the R core tends to be rather similar within each group; this is not true, however, with an apparently heterogeneous group such as E. coli. (3) The structure of R core appears to be different from one group to another. (4) O side chains of the same or very similar structure sometimes occur in several organisms each belonging to a different "genus".

Why does the structure of O side chains show so much variation? In order to answer this question, we must speculate on the possible functions LPS and O side chains are performing for the bacterial cell. One of the most striking facts about O side chains is that they are

completely dispensable when the bacteria are cultured in test tubes. Indeed it is widely known that, in liquid media, S-form organisms tend to be overgrown by R mutants during prolonged period of incubation. In contrast to this, O side chains are absolutely necessary for the survival of these bacteria in their natural habitat, as is seen from the fact that R mutants have only very rarely been isolated from nature. Thus the function of O side chains must be related to the reaction of these bacteria toward their natural environment. In the case of pathogenic organisms such as Salmonella, it is perhaps easy to see how O side chains can contribute to the survival of these bacteria. When O side chains are present, that is, in the case of S forms, phagocytes cannot engulf these bacteria unless specific antibodies against O side chains are provided (see, for example, Fauve, 1964). Furthermore, the presence of O side chains protect these bacteria against the bactericidal action of "normal" serum (Nelson and Roantree, 1967). Host animals can attack these bacteria by producing antibodies against O side chains, but bacteria synthesizing O side chains of an unusual structure might escape this host response, since the immune system of the host species might have difficulties in producing effective antibody molecules against these O side chains (Roantree, 1967). This reasoning probably explains, at least in part, the observation that the most virulent Salmonella serotypes usually belong to chemotypes XIV, XV and XVI, and synthesize O side chains of rather complicated structure containing one of the unusual 3,6-dideoxyhexoses.*

Even in those cases when the host has succeeded in producing good anti-O antibodies, the bacteria will be able to escape the attack of phagocytes if they could produce variants with different O side chains. This selective advantage undoubtedly explains why such a variety of mechanisms are available for altering the structure of O side chains. We have already discussed mutations in the genes controlling modification enzymes, and lysogenic conversion. There are still other mechanisms. One of them is called "form variation" (Kauffmann, 1940); as an example of this variation, an antigen called 12₂ may or may not be present in the LPS of Salmonella of groups A, B, and D₁. There is a

*There are, however, serotypes which synthesize O side chains containing other rare sugars and yet are not very virulent. This suggested, to some investigators, that O side chains must have some specific, positive function in the interaction of these bacteria with the host animal, in addition to the rather non-specific function of covering up the R core. This may be true, although we cannot yet exclude the possibility that those O side chains containing 3,6-dideoxyhexoses contribute greatly to virulence because they are extremely efficient in covering up the R core owing to their conformation and other physicochemical properties. A decisive experiment on this point would consist in determining the virulence of mutants whose R core is largely exposed, yet carries a small number of O side chains. If O side chains had specific functions other than that of the blocking agent, these mutants should be more virulent than the complete R mutants. Such a result has indeed been reported (Nelson and Roantree, 1967), although under different set of conditions mutants of this type behaved exactly like complete R mutants (Nakano and Saito, 1969).

frequent interconversion between 12_2 -positive and 12_2 -negative "forms", so that even if one inoculates broth with a single 12_2 -negative colony, one will get a mixture of 12_2 -positive and -negative cells after overnight culture. This variation, which is similar to the phase variation of flagellar antigens, may possibly confer distinct selective advantage on these pathogenic bacteria. A more striking phenomenon, which is somewhat reminiscent of form variation, is the synthesis of so-called T1 side chains. Many S-form strains of *Salmonella* appear to produce, in addition to the O side chains of the proper structure, a very small amount of T1 side chains which consists of galactofuranose and ribose residues (M. Berst and O. Lüderitz, personal communication). We may speculate that under certain conditions - probably when the synthesis of O side chains becomes impossible - the mutants, in which the genes involved in T1 side chain synthesis have become more active, survive natural selection and that in these mutants LPS containing substantial amounts of T1 side chains is produced (Sarvas, 1967).* It is not yet known whether the minute amounts of T1 side chain produced in normal cells play any physiological role, but if they do not, it is indeed remarkable that genes for such "emergency" functions have been conserved without modification in various serotypes of *Salmonella*.

What can we say about non-pathogenic groups like *E. coli*? Here we must realize that we know very little about the ecology of these organisms. According to Florey (1933), non-pathogenic organisms do not induce the migration of phagocytes into intestinal lumen; thus phagocytosis does not appear to be the major factor against the survival of these organisms. A few authors have claimed that many bacteria are killed by intestinal secretions, but practically nothing is known on the nature of the active principle(s). If this process is like the killing of "normal" antiserum, O side chains might have a protective effect.

These mechanisms, however, cannot affect the survival of the many saprophytic Gram-negative bacteria. In this case (and probably also in the case of non-pathogenic organisms found in the intestinal lumen) one may propose that the natural enemy of these bacteria is the microorganisms which phagocytize them, and that O side chains again protect them against such an attack. Gerisch *et al.* (1967) have indeed found that the amoeboid form of *Dictyostelium discoideum* could phagocytize the R-forms, but not S-forms with O side chains, of *Salmonella milwaukee* in suspension culture. Furthermore, it is obvious that O side chains will protect bacteria against virulent bacteriophages which adsorb to the R core.

One fact which illustrates our ignorance in this area is that we do not yet know any organism or enzyme which is able to hydrolyze the carbohydrate backbone of LPS. (Many reports on the "degradation" of LPS with mammalian enzymes have been published; however, in view of the methods used - ultracentrifugation, Ouchterlony immunodiffusion, etc. - most, if not all, workers appear to have observed merely the dissociation of LPS micelles under the influence of various components of the "enzyme" preparations). LPS must be degraded by some organisms; otherwise this world would become a pile of LPS. We thus need much more work on this aspect of the ecology of these organisms.

*It must be noted, however, that at least in one laboratory the conversion of non-T1-form into T1 mutant has not been observed (Sarvas, 1967).

In view of the arguments presented so far, it will not be difficult to understand why the structure of the R core appears to be less variable. R core is buried underneath the O side chains, and apparently is not exposed to the selective pressure of the environment in the manner O side chains are. Furthermore, at least the inner portion of the R core seems to be important for the membrane-like functions of the outer layer of the cell wall, since the growth of "deep" *rfa* mutants is inhibited by a variety of dyes, and since mutants lacking the innermost portion of the R core have never been isolated despite numerous attempts. There might thus exist a selective pressure which tends to conserve the structure of at least the inner portion of R core.

A similar argument can be used in order to explain the constancy of the structure of the peptidoglycan layer in Gram-negative bacteria. In most Gram-positive bacteria, the peptidoglycan layer is exposed on the surface of bacteria. In contrast, the lipopolysaccharide-phospholipid-protein layer completely covers the peptidoglycan layer in Gram-negative cell wall, and the latter, therefore, is not exposed to the selective pressure from the environment.

What special values do the structure of LPS have as a taxonomic character? One advantage is its enormous diversity. It is obvious that characters which are common among most bacteria are useless as a taxonomic character. In contrast, in the case of LPS, the interaction of bacteria with their environment has always favored the diversification of LPS structure during evolution. We must note that at the level of individual characters, evolutionary forces usually tend to favor the achievement of higher or lower efficiency, and only very rarely diversification as such. In the former case, characters are useful in classification only at their extremes (for example, rapid utilization versus very slow utilization of lactose). In the latter, characters are always useful for classification. Another advantage is that structure of LPS can frequently tell us the degree of relationship between various bacteria. This is not possible with most other taxonomic characters except DNA homology; one can never say that the β -galactosidase of a strongly lactose-positive species will be more related to that of an intermediate lactose-positive species than to that of a weakly lactose-positive species. In order to get an idea about the relationship between species, one normally would have to isolate specific enzymes from each species and determine amino acid sequences of these enzymes, which is of course a formidable task. In contrast, in the case of LPS we are dealing with a product of enormous complexity, and consequently we can determine the relationship between species by looking at the structure of the polysaccharides, without going into the trouble of determining the amino acid sequences of enzymes.

Both of these advantages are related to the fact that the structure of LPS is a product of collaboration of several dozens of genes, in contrast with many "biochemical" taxonomic characters each of which is determined by a single gene, or at most by a few genes. Thus the amount of genetic information which finds its expression in the structure of LPS is much larger than that involved in the expression of individual "biochemical" characters.

When one considers these advantages, it is hardly surprising that Kauffmann has put so much emphasis on O antigens in the classification of Enterobacteriaceae. Of course there are many pitfalls. We have

seen that two strains which are otherwise quite different can occasionally synthesize O side chains of the same structure, and that the structure of O side chains can be altered by a number of mechanisms; it is thus clear that we must utilize all other available characters for classification. Yet if we are aware of the presence of these pitfalls, understand the genetic basis of alterations of LPS, and rely on the structure of LPS- and ultimately on the mode of biosynthesis of LPS and its genetic control - rather than on the immunological specificity alone, no one will deny that LPS is a very good taxonomic character which gives us a surprisingly large amount of information.

ACKNOWLEDGEMENT

The research in the author's laboratory has been supported by a U.S. Public Health Service Grant AI 05729 and a grant from the American Cancer Society (Massachusetts Division). The author is indebted to Dr. Matti Sarvas for many illuminating discussions and criticisms.

BIBLIOGRAPHY

- Adams, G.A., C. Quadling, M. Yaguchi and T. Tornabene. 1970. The chemical composition of cell-wall lipopolysaccharides from Moraxella duplex and Micrococcus calco-aceticus. Canad. J. Microbiol. 16:1.
- _____, T.G. Tornabene and M. Yaguchi. 1969. Cell-wall lipopolysaccharides from Neisseria catarrhalis. Canad. J. Microbiol. 15:364.
- Beckman, I., O. Lüderitz and O. Westphal. 1964. Zur Immunchemie der somatischen Antigene von Enterobacteriaceae. IX. Serologische Typisierung von Salmonella-R-Antigenen. Biochem. Z. 339:401-415.
- Brenner, D.J., G.R. Fanning, K.E. Johnson, R.V. Citarella and S. Falkow. 1969. Polynucleotide sequence relationships among members of Enterobacteriaceae. J. Bacteriol. 98:637-650.
- Cho, K.Y. and M.R.J. Salton. 1966. Fatty acid composition of bacterial membrane and wall lipids. Biochim. Biophys. Acta 116:73-79.
- Dröge, W., O. Lüderitz and O. Westphal. 1968. Biochemical studies on lipopolysaccharides of Salmonella R mutants. 3. The linkage of the heptose units. European J. Biochem. 4:126-133.
- Falkow, S., I. Ryman and O. Washington. 1962. Deoxyribonucleic acid base composition of Proteus and Providence organisms. J. Bact. 83:1318-1321.
- Fauve, M.R. 1964. Résistance cellulaire à l'infection bacterienne. II. Comportement de macrophages de souris entretenus, in vitro, dans un milieu sans sérum en présence de S. typhimurium d'inégale virulence. Ann. Inst. Pasteur 107:472-483.
- Florey, H.W. 1933. Observations on the functions of mucus and the early stages of bacterial invasion of the intestinal mucosa. J. Pathol. Bacteriol. 37:283-289.
- Fuller, N.A., R.G. Wilkinson and E.C. Heath. 1969. Unique oligosaccharide isolated from cell wall lipopolysaccharide of a mutant of Escherichia coli O111:B4. Bacteriol. Proc. p. 47.
- Gerisch, G., O. Lüderitz and E. Ruschmann. 1967. Antikörper fördern die Phagozytose von Bakterien durch Amöben. Z. Naturforsch. 11b: 109.

- Gmeiner, J., O. Lüderitz and O. Westphal. 1969. Biochemical studies on lipopolysaccharides of *Salmonella* R mutants. 6. Investigations on the structure of the lipid A component. *European J. Biochem.* 7:370-379.
- Gray, G.W. and S.G. Wilkinson. 1965. The effect of ethylenediamine-tetraacetic acid on the cell walls of some Gram-negative bacteria. *J. Gen. Microbiol.* 39:385-399.
- Hellerqvist, C.G., B. Lindberg, S. Svensson, T. Holm and A.A. Lindberg. 1968. Structural studies on the O-specific side-chains of the cell wall lipopolysaccharide from *Salmonella typhimurium* 395MS. *Carbohydrate Res.* 8:43-55.
- Johnston, J.M., R.J. Johnston and D.A.R. Simmons. 1967. The immunochemistry of *Shigella flexneri* O-antigens. The biochemical basis of smooth to rough mutation. *Biochem. J.* 105:79-87.
- Kanfer, J. and E.P. Kennedy. 1963. Metabolism and function of bacterial lipids. I. Metabolism of phospholipids in *Escherichia coli* B. *J. Biol. Chem.* 239:2919-2922.
- Kates, M. 1964. Bacterial lipids. *Advances in Lipid Res.* 2:17-90.
- _____, L.S. Yengoyan and P.S. Sastry. 1965. A diether analog of phosphatidyl glycerophosphate in *Halobacterium cutirubrum*. *Biochem. Biophys. Acta* 98:252-268.
- Kauffmann, F. 1966. The Bacteriology of *Enterobacteriaceae*. Munksgaard, Copenhagen.
- _____. 1940. Zur Serologie des I-Antigens in der *Salmonella*-Gruppe. *Acta Pathol. Microbiol. Scand.* 17:134-144.
- Lüderitz, O., C. Galanos, H.J. Risse, E. Ruschmann, S. Schlecht, G. Schmidt, H. Schulte-Holthausen, R. Wheat, O. Westphal and J. Schlosshardt. 1966. Structural relationships of *Salmonella* O and R antigens. *Ann. N.Y. Acad. Sci.* 133:349-374.
- _____, K. Jann and R. Wheat. 1968. Somatic and capsular antigens of Gram-negative bacteria. In: *Comprehensive Biochemistry*, M. Florkin and E. Stotz (Eds.), Vol. 26A, pp.105-228. Elsevier, Amsterdam.
- _____, F. Kauffmann, H. Stierlin and O. Westphal. 1960. Zur Immunchemie der O-Antigene von *Enterobacteriaceae*. II. Vergleich der Zuckerbausteine von *Salmonella* S-, T-, und R-Formen. *Zentr. Bakteriол. Parasitenk. Abt.I. Orig.* 179:180-186.
- Mäkelä, P.H. 1966. Genetic determination of the O antigens of *Salmonella* groups B (4, 5, 12) and C₁ (6, 7). *J. Bact.* 91:1115-1125.
- _____, and B.A.D. Stocker. 1969. Genetics of polysaccharide biosynthesis. *Ann. Rev. Genetics*
- Marr, A.G. and J.L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bact.* 84:1260-1267.
- Matsushashi, S. and J.L. Strominger. 1965. Reversible 2-epimerization of CDP-paratose and CDP-tyvelose. *Biochem. Biophys. Res. Comm.* 20:169-175.
- Naide, Y., H. Nikaido, P.H. Mäkelä, R.G. Wilkinson and B.A.D. Stocker. 1965. Semirough strains of *Salmonella*. *Proc. Natl. Acad. Sci. U.S.* 53:147-153.
- Nakano, M. and K. Saito. 1969. Chemical components in the cell wall of *Salmonella typhimurium* affecting its virulence and immunogenicity in mice. *Nature* 222:1085-1086.
- Nelson, B.W. and R.J. Roantree. 1967. Analysis of lipopolysaccharides extracted from penicillin-resistant, serum-sensitive *Salmonella* mutants. *J. Gen. Microbiol.* 48:179-188.

- Nikaido, H. 1968. Biosynthesis of cell wall lipopolysaccharide in Gram-negative enteric bacteria. *Advances in Enzymol.* 31:77-124.
- _____. 1969. Structure of cell wall lipopolysaccharide from *Salmonella typhimurium*. I. Linkage between O side chains and R core. *J. Biol. Chem.* 244:2835-2845.
- _____, K. Nikaido and P. H. Mäkelä. 1966. Genetic determination of enzymes synthesizing O-specific sugars of *Salmonella* lipopolysaccharides. *J. Bacteriol.* 91:1126-1135.
- Ørskov, F. and I. Ørskov. 1962. Behavior of *Escherichia coli* antigens in sexual recombination. *Acta Pathol. Microbiol. Scand.* 55:99-109.
- Osborn, M. J. 1963. Studies on the Gram-negative cell wall. I. Evidence for the role of 2-keto-3-deoxyoctonate in the lipopolysaccharide of *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. U.S.* 50:499-506.
- _____, S. M. Rosen, L. Rothfield, L. D. Zeleznick and B. L. Horecker. 1964. Lipopolysaccharide of the Gram-negative cell wall. *Science.* 145:783-789.
- Roantree, R. J. 1967. *Salmonella* O antigens and virulence. *Ann. Rev. Microbiol.* 21:443-466.
- Robbins, P. W. and T. Uchida. 1962. Studies on the chemical basis of the phage conversion of O-antigens in the E group *Salmonella*. *Biochemistry.* 1:323-335.
- Sarvas, M. 1967. Inheritance of *Salmonella* T1 antigen. *Ann. Med. Exptl. Biol. Fenniae (Helsinki)* 45:447-471.
- Schmidt, G., B. Jann and K. Jann. 1969. Immunochemistry of R lipopolysaccharides of *Escherichia coli*. I. Different core regions in the lipopolysaccharides of O group 8. *European J. Biochem.* 10: 501-510.
- Simmons, D. A. R., O. Lüderitz and O. Westphal. 1965. The immunochemistry of *Salmonella* chemotype VI O antigens. *Biochem. J.* 97:807-826.
- Springer, G. F., E. T. Wang, J. H. Nichols and J. M. Shear. 1966. Relations between bacterial lipopolysaccharide structures and those of human cells. *Ann. N.Y. Acad. Sci.* 133:566-579.
- Stocker, B. A. D., A. M. Staub, R. Tinelli and B. Koppacka. 1960. Étude immunochimique sur les *Salmonella*. VI. Étude de l'antigène 1 présent sur deux *Salmonella* des groupes B et E. *Ann. Inst. Pasteur.* 98:505-523.
- Subbiah, T. V. and B. A. D. Stocker. 1964. Rough mutants of *Salmonella typhimurium*. I. Genetics. *Nature.* 201:1298-1299.
- Sutherland, I. W., O. Lüderitz and O. Westphal. 1965. Studies on structure of polysaccharides of *Salmonella minnesota* and *Salmonella typhimurium* R strains. *Biochem. J.* 96:439-448.
- Sutherland, I. W., O. Lüderitz and O. Westphal. 1965. Studies on the structure of polysaccharides of *Salmonella minnesota* and *Salmonella typhimurium* R strains. *Biochem. J.* 96:439-448.
- Uchida, T., P. W. Robbins and S. E. Luria. 1963. Analysis of the serologic determinant groups of the *Salmonella* E group O antigens. *Biochemistry.* 2:663-668.
- Volk, W. A. 1966. Cell wall lipopolysaccharides from *Xanthomonas* species. *J. Bacteriol.* 91:39-42.

- Watkins, W.M. 1966. Blood group substances. *Science* 152:172-181.
- Weise, G. 1968. Dissertation, University of Freiburg.
- Westphal, O. and K. Jann. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further application of the procedure. In *Methods in Carbohydrate Chemistry*, R. L. Whistler *et al.* (Eds.) vol. 5, pp. 83-91. Academic Press: New York.
- _____, F. Kauffmann, O. Lüderitz and H. Stierlin. 1960. Zur Immunchemie der O-Antigene von *Enterobacteriaceae*. III. Analyse der Zuckerbausteine Kreuzreagierender *Salmonella*-, und *Escherichia* - O-Antigene. *Zentr. Bakteriол. Parasitenk. Abt. 1.* Orig. 179:336-342.
- _____, and O. Lüderitz. 1960. 3, 6-dideoxyhexosen. *Chemie und Biologie. Angew. Chem.* 72:881-891.
- _____, O. Lüderitz and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. *Z Naturforsch.* 7b:148-155.
- Wilkinson, R. G., N. A. Fuller, A. C. Lazen and E. C. Heath. 1968. Structure and composition of the "core portion of the cell wall lipopolysaccharide of *Escherichia coli*". *Bacteriol. Proc.* p. 63.

Note added in proof.

Recently Adams and Singh (*Biochim. Biophys. Acta* 202:557, 1970) found that the oligosaccharide backbone of a lipid A from an *Escherichia coli* strain consisted of two D-glucosamine residues linked together through $\beta(1 \rightarrow 4)$ linkage, in contrast with the oligosaccharide from *Salmonella* lipid A which contains $\beta(1 \rightarrow 6)$ linkage.