Occurrence of 3-Amino-3,6-Dideoxyhexoses in *Salmonella* and Related Bacteria

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Several species of *Salmonella*, *Citrobacter*, and *Arizona* were examined for the presence of 3-amino sugars, which were isolated from lipopolysaccharide hydrolysates by cation exchange chromatography and identified by paper and cation exchange chromatography in several systems and by specific colorimetric procedures. 3-Amino-3,6-dideoxyglucose was identified in *C. freundii* 8090, *C. freundii* 869, *S. halle*, *S. telaviv*, *S. dakar*, *S. wandsworth*, and *S. champaign*; 3-amino-3,6-dideoxygalactose was found in *S. tranoroa* and *Arizona* 24. Evidence suggests that these 3-amino sugars occur in lipopolysaccharides as the *N*-acetyl derivatives. The composition of the lipopolysaccharides containing the 3-amino sugars was determined, and the lipopolysaccharides were compared serologically in order to correlate chemotypes with serotypes. The lipopolysaccharides containing 3-amino-3,6-dideoxyglucose reacted with serogroups 28 or 39; those containing 3-amino-3,6-dideoxygalactose were found to react with group 55 antisera. The preparation of a lipopolysaccharide from the phenol phase of the 44% aqueous phenol extraction procedure is also reported.

Ashwell and Volk (2) first reported the isolation and characterization of crystalline 3-acetamido-3,6-dideoxy-D-galactose, obtained from a phenol-soluble lipopolysaccharide prepared from *Xanthomonas campestris*, a member of the *Pseudomonadaceae*. Raff and Wheat (18) found a different 3-amino-3,6-dideoxy sugar in phenol-soluble cell wall lipopolysaccharide of the enterobacterium, *Citrobacter freundii* ATCC 8090. Jann et al. (Nature, *in press*) and Müller Seitz (Thesis, University of Freiburg, Germany) independently identified 3-amino-3,6-dideoxy-D-galactose and 3-amino-3,6-dideoxyglucose in *Escherichia coli* serotypes.

The present work describes the tentative identification of 3-amino sugars found in the serologically cross-reactive *C. freundii* 8090, *Citrobacter* 869, *Salmonella dakar*, *S. telaviv*, *S. champaign*, *S. wandsworth*, *S. tranoroa*, and *Arizona* 24.

MATERIALS AND METHODS

Growth of cells. *C. freundii* ATCC 8090 was grown in 10-liter lots in 5-gal (18.9-liter) carboys at 25°C for 24 hr with constant aeration. The medium used was as follows, in grams per liter: yeast extract, 3; K₂PHO₄, 6.6; KH₂PO₄, 1.7; (NH₄)₂SO₄, 2.5; MgSO₄, 0.05; and glucose (autoclaved separately), 10. Cells were harvested with a DeLaval Gyrotect centrifuge,

washed with distilled water, and frozen or acetone-dried.

The other organisms used in this study were grown as previously described (13).

Preparation of lipopolysaccharides. The lipopolysaccharides and phenol-soluble fractions analyzed in this paper (except for *C. freundii* 8090) were derived from cultures obtained from F. Kauffmann, Statens Serum Institute, Copenhagen, and they are identical with those described in a previous paper (13).

The lipopolysaccharide of *C. freundii* 8090, found in the phenol-soluble fraction, was obtained by using the phenol fractionation procedure of Westphal, Lüderitz, and Bister (24). The phenol phase was dialyzed to remove phenol, and the dialyzed fractions were then centrifuged at low speed to remove water-insoluble residues. The water-soluble portion was then concentrated and the lipopolysaccharide freed of soluble components and ribonucleic acid by successive ultracentrifugation at 100,000 × *g*, after which neither 260-mμ absorption nor ribose could be detected. Lipopolysaccharide containing the 3-amino sugar was obtained from both the phenol layer and the phenol-water interphase layer. Yields of lipopolysaccharide from the aqueous layer were small and erratic.

Reference compounds. Crystalline *N*-acetyl-3-amino-3,6-dideoxy-D-galactose and *N*-acetyl-3-amino-3,6-dideoxy-D-mannose were gifts of Gilbert Ashwell of the National Institutes of Health, Bethesda, Md. The free amino sugars were prepared by 2 N HCl hydrolysis as described below. 3-Amino-

3,6-dideoxy-L-glucose was a gift of A. C. Richardson, University of Bristol. 3-Amino-3,6-dideoxy-D-mannose was also prepared from amphotericin B (7) as described below.

Isolation of amino sugars. Samples were hydrolyzed in 2 N HCl for 6 hr at 100 C in vacuo. The acid was removed under vacuum, and the amino sugars were then purified on a Dowex 50 H⁺ column (1 by 50 cm) prepared according to Gardell (8). Elution was with 0.33 N HCl, and fractions were assayed for cationic reducing sugar by the Park-Johnson ferricyanide assay (17).

In an alternative method, 35 mg of lipopolysaccharide was hydrolyzed in 3 ml of 2 N HCl for 3 hr in a sealed tube. The acid was removed as above, and the hydrolysate was submitted to paper electrophoresis (3,000 v, 60 min at 10 C). A guide strip was cut and stained with the silver-NaOH reagent (23, 25), and the area where free glucosamine was found was eluted with 0.05 N HCl. This basic fraction was subsequently separated by paper chromatography in solvent C (see below). Staining of a side strip revealed the presence of a spot migrating like glucosamine, one spot migrating like galactosamine (except for *Arizona* 24 lipopolysaccharide from which galactosamine is absent), and a third spot of unknown nature. The former amino sugars had been expected, since they had been identified earlier in these lipopolysaccharides. The area of the unknown was eluted with 0.05 N HCl; the eluate, after drying in vacuo, was taken up in 0.05 ml of water. This solution was kept frozen until analyses were performed.

Paper electrophoresis and paper chromatography. High-voltage electrophoresis on paper (Macherey and Nagel MN 214) was performed as previously described (14) with solvent D. Paper chromatography (Whatman no. 1) was performed with solvents A, B, and C. To avoid losses of the amino sugars, the papers, after paper electrophoresis and paper chromatography, were dried at room temperature. The following solvents were used: (A) pyridine-*n*-butanol-water (4:6:3); (B) acetic acid-*n*-butanol-water (1:5:2); (C) ethyl acetate-pyridine-acetic acid-water (25:25:5:15) as solvent, and ethyl acetate-pyridine-water (40:11:6) to saturate the atmosphere; (D) pyridine-acetic acid-water (100:40:860). Thin-layer chromatography (cellulose powder of Macherey and Nagel, MN 300G or Avicel, F.M.C. Corp., American Viscose Division) was used for quick identification of amino sugars with solvent systems B and C.

Analytical methods. Reducing sugar was determined by the Park-Johnson method (17). The Morgan-Elson method as modified by Reissig et al. was used for *N*-acetyl-2-amino-2-deoxy sugars (19). 2-Amino sugar that was not *N*-acetylated was determined by the method of Rondle and Morgan (20). The assay of Ashwell, Brown, and Volk was used for 3-amino sugars (1). Cation exchange procedures used were those of Boas (4), Gardell (8), and the modified amino acid analysis system of Brendel et al. (Anal. Biochem., *in press*). All methods were adapted to micro amounts, i.e., to quantities of sugars in the range of 0.01 to 0.05 μ mole in a final volume of 0.8 ml for reading in the spectrophotometer. The sulfuric

acid-cysteine reaction for identification of 6-deoxyhexoses was performed according to Dische and Shettles (6).

Nonacetylated amino sugars were also analyzed by the method of Strominger et al. (22). Preparative *N*-acetylation of free amino sugars was achieved as follows: 1 μ mole of sugar in 50 μ liters of water, 200 μ liters of saturated NaHCO₃, and 200 μ liters of water containing 10 μ liters of acetic acid anhydride were kept at room temperature for 60 min, heated (2 min, 100 C), and dried in vacuo over P₂O₅. The residue was taken up in 50 μ liters of water and submitted to paper electrophoresis for 30 min. The neutral fraction representing the acetylated amino sugar was then eluted. Alternatively, the *N*-acetylation mixture was treated with Dowex-1-HCO₃ (20 to 50 mesh) to remove anions before drying. Standard acetylamine sugars were deacetylated by treatment with 1 N HCl for 2 hr at 100 C to obtain the free amino sugar.

Acetaldehyde was determined on a microscale (e.g., 0.05 μ mole of rhamnose) after periodate oxidation by use of either Conway diffusion dishes (5) or the method described by Kabat and Mayer (11). For qualitative determinations, a gas diffusion train apparatus (16) was used. Hexoses, which give rise to formaldehyde, do not interfere.

Serological methods. Tube agglutination was performed with boiled bacterial suspensions and serial dilutions of serum. Passive hemagglutination was performed with erythrocytes sensitized with either the supernatant fluid of a boiled bacterial suspension or with lipopolysaccharides pretreated with NaOH (3). The *Salmonella* sera used in this study were a gift from E. H. Kampelmacher, Rijks Instituut voor de Volksgezondheid, Utrecht, Netherlands, and L. Le Minor, Institut Pasteur, Paris, France.

RESULTS

The amino sugars from the lipopolysaccharides were isolated by acid hydrolysis, followed by paper electrophoresis and paper or cation exchange chromatography.

Isolation of and identification of glucosamine and galactosamine. Cation exchange chromatography by the procedure of Gardell (8) indicated that the lipopolysaccharides examined of groups 28 and 39 contained both glucosamine and a cationic reducing sugar (elution ratio, RGlcN, 1.36) as previously reported for *C. freundii* 8090 (18). In addition, except for *Arizona* 24, the other preparations examined contained galactosamine (elution ratio, RGlcN, 1.18) as previously reported (13, 15). The galactosamine identity of the latter was confirmed by ninhydrin degradation (21), which yielded only lyxose (detected on thin-layer cellulose plates with alkaline silver nitrate) with solvent A, run twice.

Isolation and identification of 3-amino sugars. The unknown 3-amino sugars were isolated as outlined above. Portions of the isolated materials were *N*-acetylated. According to *R_F* values in

three different solvents, the free and corresponding *N*-acetylated unknown amino sugars fall into two distinct groups (Table 1). Group I includes those from *Salmonella* groups M and Q and *Citrobacter* 869 and 8090; group II includes those from *S. tranoroa* and *Arizona* 24. Table 2 shows the R_F values of 3-amino-3,6-dideoxyglucose, -galactose, and -mannose, as obtained by amino acid analyzer chromatography.

For the sake of brevity, the unknown amino sugars derived from antigens 28, 39, and *Citrobacter* 869 (which behave chromatographically like 3-amino-3,6-dideoxyglucose) are designated as group I amino sugars; those derived from antigen 55 and *Arizona* 24 (which migrate like 3-amino-3,6-dideoxygalactose) will be referred to as group II amino sugars.

If ninhydrin color is developed at room temperature, the free amino sugars of group II as well as 3-amino-3,6-dideoxygalactose give a spot of grayish color; other amino sugars yield violet.

The free amino sugars as well as their *N*-acetylated derivatives gave negligible color yield in the Morgan-Elson reaction as performed according to Strominger et al. (22) or Reissig et al. (19), respectively. Thus, the structure of a 2-amino hexose or pentose could be excluded.

When the Morgan-Elson reaction was performed on the acetylated amino sugars after controlled periodate oxidation according to the procedure of Ashwell et al. (1) for *N*-acetyl-3-amino sugars, all of the unknown amino sugars reacted strongly positively. This suggested that the amino sugars carried the amino group at carbon position 3.

TABLE 2. Comparison of relative retention ratios of 3-amino-3,6-dideoxyhexoses and unknown compounds by amino acid analyzer chromatography

Compound	Relative retention ratio
Glucosamine ^a	100
3-Amino-3,6-dideoxy-D-galactose....	120 ± 1
3-Amino-3,6-dideoxy-D-mannose....	126 ± 1
3-Amino-3,6-dideoxy-L-glucose.....	129 ± 1
<i>Citrobacter freundii</i> 8090 unknown...	129
<i>Salmonella champaign</i> unknown.....	129
<i>S. dakar</i> unknown.....	129
<i>S. telaviv</i> unknown.....	129
<i>Arizona</i> 24 unknown.....	119
<i>S. tranoroa</i> unknown.....	119

^a Glucosamine hydrochloride was used as an arbitrary reference standard.

To determine whether the 3-amino sugars were similar to known compounds, the relative retention times on the amino acid analyzer were ascertained by comparison with reference compounds (Fig. 1) according to the procedure of Brendel et al. (*in press*). The 3-amino sugars from *C. freundii* 8090, *S. champaign*, *S. dakar*, and *S. telaviv* all exhibited relative retention times characteristic of authentic 3-amino-3,6-dideoxy-L-glucose (Table 2). The reference sugar, 3-amino-3,6-dideoxyglucose, and the bacterial samples were all eluted as a single peak when co-chromatographed in this system. The 3-amino sugars from *Arizona* 24 and *S. tranoroa* had relative retention times of 119, which is identical to that of 3-amino-3,6-dideoxygalactose.

TABLE 1. R_F values of unknown and standard reference amino sugars

Amino sugars	Antigen unknown amino sugar derived from	Free amino sugars ^a			Acetylated amino sugars ^b		
		Solvent A (PC)	Solvent B (PC)	Solvent C (TLC)	Solvent A (PC)	Solvent B (PC)	Solvent C (TLC)
Group I	28 ₁ , 28 ₂	1.5-1.6	2.0	1.7	1.2-1.3	1.3-1.5	1.25
	28 ₁ , 28 ₃	1.5-1.7	2.0	1.7	1.2-1.3	1.3-1.5	1.25
	39	1.5-1.6	2.0	1.7	1.2-1.3	1.3-1.5	1.25
	<i>Citrobacter</i> 869	1.5-1.6	2.0	1.7	1.2-1.3	1.3-1.5	1.25
Group II	55	1.2	1.6-1.8	1.4	1.1	1.3	1.16
	<i>Arizona</i> 24	1.2	1.6-1.7	1.4	1.1	1.3	1.16
3-Amino fucose		1.2	1.6-1.8	1.4	1.1	1.3-1.4	1.17
3-Amino quinovose		1.6	2.0	—	1.2	1.3	—
3-Amino rhamnose		1.8	2.2	1.7	1.4	1.55	1.3

^a The ranges in values (ratios to glucosamine) represent differences observed when several chromatograms were compared. Discrete values within the range shown were obtained on any single chromatogram.

^b Values are expressed as ratios to fucose.

^c PC = paper chromatography, TLC = thin-layer cellular chromatography.

FIG. 1. Identification of 3-amino sugars in a Technicon amino acid analyzer.

The above data do not differentiate between D and L sugar isomers. These data do demonstrate that the sugars from *C. freundii* 8090, *C. freundii* 869, and *Salmonella* groups 28 and 39 are certainly different from the galactose and mannose analogues, but they may be identical to each other and to 3-amino-3,6-dideoxyglucose; *Arizona* 24 and *Salmonella* O55 may contain the galactose analogue. Definite identification is of course not yet established.

The unknown amino sugars, as well as standard 3-amino-3,6-dideoxygalactose, -glucose, and -mannose, were subjected to the Dische-Shettles reaction used for the identification of 6-deoxyhexoses (6). The spectra obtained showed the typical absorption curves for 6-deoxyhexoses, with a maximum at 400 mμ. The color yield of the amino sugars was lower than that of the corresponding neutral 6-deoxyhexoses. The *N*-acetylated 3-amino-3,6-dideoxyhexoses yielded less color than the free 3-amino sugars.

The suggestion that the unknown sugars contain a terminal methyl group was substantiated when the sugars were oxidized with periodate (5, 11, 16). All of the unknown amino sugars produced acetaldehyde under these conditions.

Occurrence of *N*-acetylated 3-amino sugars in lipopolysaccharides. An attempt was made to establish whether the 3-amino sugars occur as *N*-acetyl compounds. When a small sample of the lipopolysaccharide of *S. typhimurium* was hydrolyzed in 1 N H₂SO₄ (4 hr, 100 C), neutralized with Ba(OH)₂, concentrated in vacuo, and submitted to paper chromatography in solvent A, staining with silver-NaOH revealed the following sugars in this sequence: galactosamine, glucosamine, galactose, glucose, the 3-amino sugar, ribose, and an unknown substance which did not stain with

ninhydrin. The unknown compound was eluted, and its analysis showed that it could not be distinguished from the *N*-acetylated derivative of the 3-amino sugar.

Distribution of lipopolysaccharides in phenol fractions. The specific lipopolysaccharide amino sugars of *S. halle* and *S. dakar* were also detected in the phenol-soluble fraction obtained by 44% aqueous phenol fractionation of these organisms, though in small yield when compared to the aqueous lipopolysaccharide fraction. The 3-amino sugar containing lipopolysaccharide of *C. freundii* 8090, however, was almost entirely confined to the phenol-soluble or interphase fractions.

Serological results. Cross-reactions of *S. dakar* 28₁, 28₃ and *C. freundii* 8090 were studied by tube agglutination and by the passive hemagglutination test, with unabsorbed and absorbed test sera. Table 3 shows that *C. freundii* 8090 cross-reacts in antiserum 28₁, 28₃ as well as in absorbed 28₃ serum. *S. dakar* in addition reacts in antiserum 28₁, 28₂.

TABLE 3. Bacterial agglutination (BA) and passive hemagglutination (PH)^a

Strain	Test	Serum			
		28 ₁ , 28 ₂	28 ₂	28 ₁ , 28 ₃	28 ₃
<i>S. dakar</i> 28 ₁ , 28 ₃	BA	640	0	2,560	640
	PH	5,120	0	10,240	2,560
<i>Citrobacter</i> 8090	BA	0	0	640	160
	PH	20	0	1,280	320

^a The supernatant fluids of boiled bacterial suspensions were used for sensitization of erythrocytes.

Similar results were obtained when the lipopolysaccharides were used in hemagglutination tests (Table 4). As expected, *S. telaviv* and *S. dakar* reacted according to their antigenic formula. *C. freundii* 869 behaves like *S. dakar* and cross-reacts with those sera containing either factor 28₁ or 28₃ antibodies. On the other hand, *C. freundii* 8090 reacts only with sera containing 28₃ antibodies.

DISCUSSION

In 1960, Kauffmann et al. (13) presented the results of sugar analyses performed on the lipopolysaccharides of *Salmonella* species. According to their sugar composition, the lipopolysaccharides were classified into chemotypes I to XVI. Heptoses, hexoses, a pentose, 6-deoxyhexoses, and 3,6-dideoxyhexoses were the neutral sugar constituents found. Galactosamine and glucosamine were the only two basic sugars then recognized and identified by paper chromatography and by their products of degradation with ninhydrin.

Since then, 2-keto-3-deoxyoctonate was found by Heath and Ghalambor (9) in *E. coli* lipopolysaccharide and was later recognized to be also present as a constituent in lipopolysaccharides of *Salmonella*.

Recently, a number of unusual amino sugars were detected in lipopolysaccharides of gram-negative bacteria, namely, *X. campestris* (2), *Citrobacter* (18), and *E. coli* (Jann et al., *in press*). We felt that a reinvestigation of *Salmonella* and related lipopolysaccharides, by methods not used in earlier investigations, was necessary. This study led to the discovery of a number of amino sugars in lipopolysaccharides of various *Salmonella* species. (In *Salmonella* groups A, B, C, D, and E, which are the most studied ones, no new amino sugars were found.) We report in this paper two amino sugars which occur in the lipopolysaccharides of *Salmonella* groups M, Q, 55, *Citro-*

bacter 869, and *Arizona* 24. According to analyses reported herein, these sugars belong to the recently discovered group of 3-amino-3,6-dideoxyhexoses. Paper chromatographic properties and retention values on column chromatography suggest that these 3-amino sugars have the glucose and the galactose configuration, respectively. Table 5 shows the list of sugars found so far in the lipopolysaccharides investigated.

Chemical and serological results described in this paper are summarized in Table 6 to reveal possible relationships between specificity and sugar composition. In Table 6, the O factors of the lipopolysaccharides as known from the Kauffmann-White scheme and as determined in this paper are opposed to those sugar constituents which are specific (i.e., not common) to the lipopolysaccharides. It can be seen that the presence of factor 28₁ is correlated with the presence of galactosamine, the presence of 28₂ is associated with ribose, and the presence of 28₃ with the presence of rhamnose; factor 39 is more remote, both serologically and chemically, from the preceding ones, and has in common with these only the presence of the amino sugars, galactosamine, and 3-amino-3,6-dideoxyglucose. *S. tranoroa* and *Arizona* 24, which do not cross-react, are also distinct with respect to some of their sugar constituents. It is unknown, however, whether these parallelisms of factors and sugars have significance. Inhibition studies with the sugars will provide more information.

As a result of the discovery of new sugar constituents in these lipopolysaccharides, they have to be classified into new chemotypes. However, the classification into new chemotypes will be done later, after the structure of other amino sugars found in various lipopolysaccharides from other species has been evaluated. This work is in progress.

It is probable that the sugars described here occur as their *N*-acetylated derivatives in the respective lipopolysaccharides. From *S. telaviv* lipopolysaccharide, a small amount of 3-*N*-acetyl-amido-3,6-dideoxyhexose has been isolated.

A frequently used method of preparing lipopolysaccharides from gram-negative bacteria has been the procedure of Westphal, Lüderitz, and Bister (24). This procedure yields an aqueous soluble fraction and a phenol-insoluble residue from whole cells. With the few exceptions noted (2, 18), only the aqueous phase had previously been examined for lipopolysaccharide content. The new amino sugars have been detected in the lipopolysaccharide of the water-soluble fraction (after phenol-water treatment of the bacteria) and could also be demonstrated in the phenol-soluble fractions. Of interest, but of unknown

TABLE 4. Hemagglutination by lipopolysaccharide-sensitized erythrocytes^a

Lipopolysaccharide coated to erythrocytes	Serum			
	28 ₁ , 28 ₂	28 ₂	28 ₁ , 28 ₃	28 ₃
<i>Salmonella telaviv</i>				
28 ₁ , 28 ₂	5,120	2,560	1,280	0
<i>S. dakar</i> 28 ₁ , 28 ₃	2,560	0	5,120	2,560
<i>Citrobacter freundii</i>				
869.....	5,120	20	5,120	2,560
<i>C. freundii</i> 8090....	40	10	640	320

^a Results are expressed as reciprocals of serum titers giving discernible agglutination.

TABLE 5. Sugar composition and chemotype classification of various enterobacterial lipopolysaccharides containing a 3-amino-3,6-dideoxyhexose^a

Species	Group	Antigen factor	3-FucN	3-QuinN	GalN	GlcN	KDO	Hep	Gal	Glc	Man	Fuc	Rha	Rib
<i>Salmonella halle</i>	M	28 ₁ ,28 ₂		+	+	+	+	+	+	+				+
<i>S. telaviv</i>	M	28 ₁ ,28 ₂		+	+	+	+	+	+	+				+
<i>S. dakar</i>	M	28 ₁ ,28 ₃		+	+	+	+	+	+	+			+	
<i>Citrobacter</i> 869		28 ₁ ,28 ₃		+	+	+	+	+	+	+			+	
<i>S. wandsworth</i>	Q	39		+	+	+	+	+	+	+	+	+		
<i>S. champaign</i>	Q	39		+	+	+	+	+	+	+	+	+		
<i>S. tranoroa</i>	55	55	+		+	+	+	+	+	+				
<i>Arizona</i> 24			+			+	+	+	+	+	+			
<i>C. freundii</i> 8090		28 ₃		+		+	+	+	+	+			+	

^a Abbreviations: 3-FucN, 3-amino-3,6-dideoxygalactose (3-amino fucose); 3-QuinN, 3-amino-3,6-dideoxyglucose (3-amino quinovose); GalN, galactosamine; GlcN, glucosamine; KDO, 2-keto-3-deoxyoctonic acid; Hep, heptose; Gal, galactose; Glc, glucose; Man, mannose; Fuc, fucose; Rha, rhamnose; Rib, ribose.

TABLE 6. Relation of antigen factors and sugar composition of the lipopolysaccharides tested

Lipopolysaccharide	Recognized antigen factor	Sugar composition ^a
<i>Salmonella telaviv</i>	28 ₁ ,28 ₂	3-QuiN, GalN, Rib, CS
<i>S. dakar</i>	28 ₁ ,28 ₃	3-QuiN, GalN, Rha, CS
<i>Citrobacter</i> 869	28 ₁ ,28 ₃	3-QuiN, GalN, Rha, CS
<i>C. freundii</i> 8090	28 ₃	3-QuiN, Rha, CS
<i>S. wandsworth</i>	39	3-QuiN, GalN, Man, Fuc, CS
<i>S. tranoroa</i>	55	3-FucN, GalN, CS
<i>Arizona</i> 24		3-FucN, Man, CS

^a The abbreviation CS refers to the sugars common to all lipopolysaccharides: glucosamine, 2-keto-3-deoxyoctonate, heptose, galactose, and glucose. The other abbreviations are defined in Table 5.

significance, is the observation that the *C. freundii* 8090 lipopolysaccharide is found almost completely in the phenol-soluble fraction, as was the case with the *X. campestris* preparation studied by Ashwell and Volk (2). We have no explanation, at present, other than that the partitioning occurs because of differential solubilities due to non-polar groups of sugars such as 3-amino-3,6-dideoxyhexose and rhamnose (10), which may alone explain why such lipopolysaccharides partition between phenol and water. Further study of composition and structure may reveal more information. Obviously, various genera and species of bacteria previously studied must be re-examined for the occurrence of carbohydrate in the phenol-soluble fractions.

The general occurrence and significance of phenol-soluble lipopolysaccharides in the *Enterobacteriaceae* is unknown at present. Much more needs to be known about composition, structure, biosynthesis, and immunological and biological properties of such materials.

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