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# Composition of the Lipopolysaccharide of *Neisseria gonorrhoeae*

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Analysis of glycose and fatty acid content of lipopolysaccharide extracted from 38 strains of *Neisseria gonorrhoeae* indicated that glycoses common to colonial types 1 to 5 were glucose, mannose, and galactose. *N*-acetylneuraminic acid, 2-keto-3-deoxyoctulosonic acid (KDO), glucosamine, and galactosamine were also invariably present. Virulent colonial types 1 and 2 contained no rhamnose, in contrast to avirulent types 3 to 5 and several strains of the nonpathogenic species *N. sicca* and *N. lactamica*. Fucose, characteristic of these nonpathogenic species, was not present in the gonococci. Variation in the concentration of individual glycoses in different strains was also noted. Mannose-KDO, galactose-KDO, and glucose-KDO ratios of virulent gonococci exceeded those of avirulent organisms, except that the correlation for glucose was not quite so striking. This relationship was not found in *N. sicca* and *N. lactamica* strains. Fatty acid analyses of lipid A from gonococci showed that 10-, 12-, 14-, 16-, and 18-carbon acids, as well as 3-hydroxytetradecanoic acid, were present, but differences in concentration between colonial types, although evident in some cases, appeared less significant than glycose content.

Although lipopolysaccharides (LPS) from various species of gram-negative bacteria have many properties in common, there are nevertheless major differences in their structure and chemistry. Until recently, it was not clear whether gonococcus LPS was similar to that of unrelated organisms. Tauber and Garson (17) extracted from gonococci a toxic LPS free from protein and nucleic acid. Chemically, this substance was a phosphoric acid ester, and they concluded that it had properties in common with endotoxin as defined by Westphal et al. (22). Maeland (12) found that aqueous ether-extracted endotoxin of the gonococcus contained about 90% protein and 3% each of lipid and carbohydrate. The sugars identified were glucose, galactose, glucosamine, and heptose.

Colonial types identified by Kellogg et al. (8), of importance since it was shown that type 1 and 2 gonococci are virulent for humans whereas types 3 and 4 are not, are currently being investigated in regard to immunological and chemical differences. Perry et al. (15) examined the LPS of type 1 and 4 gonococci and found that type 1 "smooth" preparations possessed characteristic sugars which were absent or different in concentration from those in "rough" type 4 LPS. Common to both were lipid A and a core fragment composed of D-glucose, D-galactose, heptose, and 2-keto-3-deoxyoctulosonic acid (KDO). Stead et al. (16), who also studied the chemical composition of gonococcus LPS, found that all preparations contained glucose, galactose, glucosamine, heptose, KDO, and phosphate. They could not detect significant differences in sugar composition of the polysaccharide component of the small number of strains tested, and they concluded that O-specific chains were absent from type 1 LPS, in contrast to Perry's observations.

Thus, information available at present is conflicting, and gonococcus colonial types other than types 1 and 4 have not been investigated. In the present study, we examined the sugar and fatty acid compositions of a number of strains representing types 1, 2, 3, 4, and 5 described by Kellogg et al. (8) and Jephcott and Reyn (5).

## MATERIALS AND METHODS

**Maintenance and preservation of strains.** Freshly isolated strains of *Neisseria gonorrhoeae* were obtained from the laboratories of the General Centre in Winnipeg, Canada. Several strains were also supplied by the Manitoba Provincial Laboratories. Strains of *N. sicca* and *N. lactamica* were supplied by C. Cates of this Department. Gonococci of types 1 to 5 were isolated from plates of GCBD (Difco) (8) medium and were selectively subcultured daily thereafter until the colonial type was stabilized. Inoculated plates were incubated for about 20 h at 37°C in 5% $CO_2$ in a Hotpack $CO_2$ incubator. The colonial types described by Kellogg et al. (8) and Jephcott and Reyn (5) were identified with a Bausch

& Lomb model 7 stereomicroscope equipped with a substage source of diffuse light. Oblique overhead illumination of colonies was suppled by a Bausch & Lomb illuminator (catalog no. 31-35-47).

Isolated colonial types in addition to being subcultured daily, were also preserved in ampoules submerged in liquid nitrogen (20). Strains to be frozen were suspended in a solution of 8% glycerol (vol/vol) and 1% proteose peptone (wt/vol) in water.

In this study, the different gonococcus colonial types were isolated in many cases from the same original patient's strain.

**Preparation of LPS.** Gonococci were grown in liquid ESP medium as described by Chan and Wiseman (2). The proportion of colonial types in the inoculum was established at the beginning and end of the growth cycle. LPS was prepared from the cells only if 95% or more of the colonies were of a single colonial type. $CO_2$ concentration above the flasks was maintained at a concentration of 16 ± 1% with the aid of twin flowmeters (Matheson & Co., Whitby, Ont., Canada; model 621 BSV).

The LPS was extracted from about 20 g (wet weight) of cells by the phenol-water method of Westphal and Jann (21). It was purified in part by centrifugation at 100,000 × *g* for 4 h. This procedure was followed by Cetavlon treatment of the centrifuged material, which further reduced the nucleic acid content, as described by these authors.

**Preparation of lipid A from LPS.** Lipid A was prepared as described by Jennings et al. (4). About 60 mg of LPS was heated at 100°C with 5 ml of 0.1 N acetic acid for 2 h. The lipid precipitate was removed by centrifugation and washed with water, dissolved in chloroform, and reprecipitated in acetone (1 part lipid to 3 parts acetone). This material was finally suspended in water, freeze-dried, and then weighed.

**Analytical methods.** Total carbohydrate was assayed as hexose by the anthrone method (18). Osborn's modification of the cysteine-sulfuric acid method (14) was used in the estimation of heptose. Aminoglycoses were quantitated by the Elson-Morgan method (7), total protein by the method of Lowry et al. (10), and total nitrogen by the method of Markham (13). The colorimetric method of Ceriotti (1) was applied to the determination of deoxyribonucleic acid, and ribonucleic acid was measured by its absorption at 260 nm in comparison with a standard. *N*-acetylneuraminic acid (NANA) was determined by the periodate-resorcinol method of Jourdian et al. (6), and phosphorus was determined as in reference 3.

Glycose analyses were performed on LPS hydrolyzed in the presence of 1 N HCl for 4 h at 105°C in a sealed ampoule. The hydrolysate was neutralized with solid $BaCO_3$ and centrifuged, and the clear solution was freeze-dried. About 50 mg of this preparation was treated with 1 ml of Sil-Prep reagent (pyridine, 0.5 ml; hexamethyldisilazane, 0.15 ml; trimethylchlorosilane, 0.1 ml), and, after standing for 60 min, the trimethylsilylated monosaccharides were analyzed in a Pye 104 gas-liquid chromatograph equipped with dual hydrogen flame ionization detectors. Separation was achieved in a stainless-steel column (0.65 by 180 cm) prepacked with 10% SE 30 on 100- to 120-mesh Gas-Chrom Q in a temperature program over the range 100 to 250°C and rising 2°C/min. The detector temperature was 250°C.

Aminoglycoses were also determined by gas-liquid chromatography (GLC) performed by the method of Laine et al. (9) in which samples were heated together with Sil-Prep reagent for 30 min at 80°C.

Qualitative assays of glycoses were also performed by descending chromatography on Whatman no. 1 paper. Samples (5-μl volumes) were applied to the paper and developed in ethyl acetate-pyridine-water (8:2:2, vol/vol) for 18 to 24 h at room temperature. The paper was dried, and glycoses were identified with silver nitrate reagent (19). Aminoglycoses were detected with ninhydrin spray (0.1% in butanol, wt/vol) after which the paper was heated for 1 min at 100°C in an oven. Known standards were included in the chromatograms.

Fatty acids were separated as methyl esters (4) in a stainless-steel column (0.65 by 180 cm) prepacked with 10% DEGA and 2% phosphoric acid on 100- to 120-mesh Gas-Chrom Q. Oven temperature was 180°C and that of the detector was held at 250°C. Known standards were used as a comparison in all GLC methods.

**Reagents.** Glycoses and Sil-Prep reagent were obtained from Applied Science Laboratories, State College, Pa. Prepacked GLC columns were purchased from the same source. Carboxylic acid methyl esters and NANA were supplied by Sigma Chemical Co., St. Louis, Mo. An authentic sample of KDO was obtained from Otto Lüderitz of the Max Planck Institute of Immunobiology, Freiburg, West Germany.

**IR specrophotometry.** Infrared (IR) spectra of the LPS preparations were obtained with a Unicam SP 200 spectrophotometer with the assistance of the Biochemistry Department. Freeze-dried LPS was ground with 100 mg of KCl and pelleted in a Research Industrial Instrument Co. 30-ton press in preparation of the disks.

## RESULTS

**IR spectra.** LPS isolated from gonococci of the KC strain (types 1 and 4) gave representa-

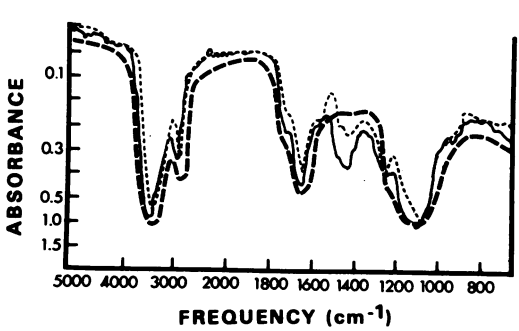


FIG. 1. *Infrared spectrum of type 1 gonococci (solid line), type 4 gonococci (dotted line), and N. sicca (dashed line).*

tive spectra which were nearly identical (Fig. 1) and are similar to that obtained for meningococcus LPS by Jennings et al. (4). The broad absorption band at 3,400 $cm^{-1}$ is due to hydroxyl radicals characteristic of carbohydrates; the bands at 1,650 and 1,500 to 1,550 $cm^{-1}$ are attributed to a monosubstituted amide such as ($-NH \cdot CO \cdot CH_3$). The ester carbonyl band at about 1,700 to 1,750 $cm^{-1}$ and absorption in the range from 1,100 to 1,250 $cm^{-1}$ characterize the material as containing carboxylic acid esters. Not all LPS preparations were IR-scanned, but material from several other *N. gonorrhoeae* and nonpathogenic *Neisseria* strains was similar to that from KC types 1 and 4. However, reference to Fig. 1 shows that the *N. sicca* scan lacks the absorption band at 1,450 $cm^{-1}$ which is characteristic of the gonococcus.

**Gross composition of LPS.** Analyses of six *N. gonorrhoeae* and several *N. sicca* strains are shown in Table 1. The preparations were essentially free from ribonucleic and deoxyribonucleic acids, although occasional traces were encountered. A small amound of protein (1 to 2%) was present which was not removed by reextraction with phenol and water. The LPS contained about 33 to 37% lipid A. Nitrogen, aminoglycose, heptose, and total carbohydrate concentrations were fairly constant in the six gonococcus samples. Carbohydrate content of *N. sicca* and *N. lactamica* strains tended to be somewhat higher, whereas nitrogen values were lower, when compared with those of the gonococcus strains.

All gonococcus LPS assayed by GLC showed evidence of NANA (see Table 2). Nineteen of these preparations were also assayed for NANA by the periodate-resorcinol method (6) and were positive. The results of several of the colorimetric assays are shown in Table 1, where it can be observed that NANA concentrations ranged from 0.7 to 2.4% (dry weight) of LPS. Assays determined colorimetrically were 25 to 50% lower than those by GLC.

**Identification of glycoses.** Glycoses identified as trimethylsilyl derivatives by GLC were mannose, galactose, glucose, and rhamnose (Table 2). In addition, aminoglycoses glucosamine and galactosamine were detected. NANA and KDO were also identified, but no xylose or fucose was found in any of the gonococcus LPS preparations. Resolution of the glycoses into D- and L-forms could not be accomplished by GLC. It is evident from Table 1 that, in contrast to types 3 to 5, type 1 and 2 gonococci contain virtually no rhamnose, with very few exceptions. There is considerable variation in concentration of the individual sugars from strain to strain. For example, glucose ranges in concentration from 0.88 to 1.92 nmol in type 1 and 2 strains. Other sugars in these strains also varied. The ratio of glucosamine to galactosamine in all strains was approximately 1.5:1 to 2:1. Data on heptose concentrations were not obtained by GLC. Gonococcus strains have been classified on the basis of the presence or absence of rhamnose and the total glycose content as determined by GLC (Table 3). It can be observed in Table 3 that most type 1 strains fall into chemotype "Ab" whereas the majority of type 4 are designated "Bc."

It is quite clear that total glycose concentration in LPS of virulent type 1 and 2 strains exceeds that in LPS from avirulent types 3 and

TABLE 1. *Chemical composition of lipopolysaccharides isolated from gonococci and nonpathogenic Neisseria strains*

| Organism | Percent (dry wt) of lipopolysaccharide | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total carbohydrate | Total hexosamine | Heptose | Phosphorus | Nitrogen | Protein | Lipid A | *N*-acetylneuraminic acid |
| Gonococci | | | | | | | | |
| 72809, type 1 | 20.6 | 9.4 | 5.7 | 2.5 | 4.7 | 1.4 | 35.2 | 2.4 |
| 48111, type 1 | 21.2 | 9.8 | 5.9 | 2.3 | 4.9 | 1.6 | 34.3 | 1.9 |
| 386023, type 2 | 21.0 | 10.1 | 5.8 | 2.0 | 4.7 | 1.3 | 36.8 | 2.2 |
| 386023, type 4 | 22.6 | 10.2 | 5.7 | 1.8 | 4.5 | 1.8 | 34.2 | —[a] |
| 72809, type 4 | 22.3 | 9.9 | 5.6 | 2.0 | 4.5 | 1.9 | 33.3 | 1.7 |
| 44269, type 5 | 21.9 | 10.3 | 5.8 | 2.7 | 4.6 | 1.7 | 35.7 | 1.7 |
| *N. sicca* | | | | | | | | |
| A | 26.4 | 11.6 | 5.1 | 1.9 | 3.6 | 1.1 | 34.8 | 0.7 |
| B | 28.2 | 11.2 | 5.3 | 2.3 | 3.1 | 1.6 | 36.1 | — |
| C | 26.9 | 11.5 | 5.3 | 2.2 | 3.4 | 1.5 | 34.2 | — |
| *N. lactamica* | 23.2 | 11.8 | 5.2 | 2.4 | 3.6 | 1.4 | 33.1 | 1.1 |

[a] Not done.

TABLE 2. *Glycose content of gonococcus lipopolysaccharide*[a]

| Strain | Type | Glycose concn (nmol per μg of lipopolysaccharide) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Glucose | Galactose | Mannose | Rhamnose | NANA[b] | KDO[c] | Glucosamine | Galactosamine | Total[d] |
| 52875 | 1 | 1.38 | 0.50 | 0.50 | 0 | 0.02 | 0.10 | 1.14 | 0.60 | 4.24 |
| KC | 1 | 1.92 | 0.40 | 0.30 | 0 | 0.08 | 0.20 | 0.84 | 0.46 | 4.20 |
| 46 | 1 | 1.66 | 1.20 | 0.44 | 0 | 0.24 | 0.26 | 1.06 | 0.74 | 5.58 |
| 9T | 1 | 1.80 | 0.40 | 0.40 | 0 | 0.24 | 0.10 | 0.82 | 0.50 | 4.66 |
| 160 | 1 | 1.44 | 0.66 | 0.52 | 0 | 0.18 | 0.12 | 1.02 | 0.58 | 4.52 |
| GC | 1 | 1.54 | 0.92 | 0.44 | 0 | 0.16 | 0.10 | 0.96 | 0.46 | 4.58 |
| 3256 | 1 | 1.37 | 0.61 | 0.37 | 0 | 0.17 | 0.15 | 0.87 | 0.48 | 4.02 |
| 4478 | 1 | 1.62 | 0.72 | 0.43 | 0 | 0.15 | 0.14 | 0.96 | 0.52 | 4.54 |
| 44269 | 1 | 1.36 | 0.77 | 0.43 | 0 | 0.16 | 0.15 | 0.95 | 0.54 | 4.36 |
| 75354 | 1 | 1.67 | 0.67 | 0.39 | 0 | 0.17 | 0.13 | 1.11 | 0.53 | 4.67 |
| 31447 | 1 | 1.72 | 0.71 | 0.36 | 0 | 0.14 | 0.16 | 1.09 | 0.59 | 4.77 |
| 75302 | 1 | 1.30 | 0.61 | 0.32 | 0 | 0.09 | 0.13 | 1.18 | 0.64 | 4.27 |
| 72809 | 1 | 1.81 | 0.76 | 0.34 | 0 | 0.13 | 0.17 | 1.04 | 0.68 | 4.93 |
| 48111 | 1 | 1.73 | 0.83 | 0.39 | 0 | 0.15 | 0.13 | 1.19 | 0.76 | 5.18 |
| 3256 | 2 | 0.88 | 0.77 | 0.61 | 0.01 | 0.40 | 0.25 | 0.85 | 0.40 | 4.17 |
| KC | 2 | 1.84 | 0.40 | 0.40 | 0.006 | 0.07 | 0.22 | 0.85 | 0.42 | 4.00 |
| GC | 2 | 1.60 | 0.38 | 0.40 | 0 | 0.16 | 0.24 | 1.02 | 0.70 | 4.50 |
| 46 | 2 | 1.20 | 0.64 | 0.24 | 0 | 0.04 | 0.16 | 0.84 | 0.62 | 3.74 |
| 386023 | 2 | 1.14 | 0.53 | 0.27 | 0 | 0.11 | 0.16 | 0.97 | 0.49 | 3.67 |
| 3256 | 3 | 0.75 | 0.58 | 0.35 | 0.15 | 0.15 | 0.11 | 0.90 | 0.41 | 3.40 |
| KC | 3 | 1.05 | 0.30 | 0.20 | 0.02 | 0.04 | 0.12 | 0.92 | 0.40 | 3.05 |
| GC | 3 | 0.96 | 0.30 | 0.04 | 0.03 | 0.04 | 0.12 | 0.84 | 0.40 | 2.73 |
| 160 | 3 | 1.00 | 0.44 | 0.08 | 0.04 | 0.06 | 0.16 | 0.96 | 0.50 | 3.24 |
| 44269 | 3 | 0.94 | 0.34 | 0.07 | 0.05 | 0.07 | 0.13 | 0.85 | 0.46 | 2.91 |
| 44871 | 3 | 0.97 | 0.38 | 0.08 | 0.04 | 0.06 | 0.12 | 0.88 | 0.44 | 2.97 |
| KC | 4 | 1.05 | 0.40 | 0.10 | 0.04 | 0.04 | 0.16 | 0.96 | 0.46 | 3.21 |
| GC | 4 | 0.80 | 0.26 | 0.02 | 0.04 | 0.05 | 0.10 | 0.88 | 0.46 | 2.61 |
| 160 | 4 | 1.06 | 0.40 | 0.06 | 0.05 | 0.07 | 0.10 | 0.96 | 0.54 | 3.24 |
| 75302 | 4 | 0.97 | 0.29 | 0.08 | 0.03 | 0.04 | 0.09 | 0.90 | 0.42 | 2.82 |
| 75354 | 4 | 1.03 | 0.36 | 0.07 | 0.05 | 0.03 | 0.11 | 0.98 | 0.45 | 3.08 |
| 72809 | 4 | 0.93 | 0.35 | 0.12 | 0.05 | 0.06 | 0.09 | 0.82 | 0.46 | 2.88 |
| 6423 | 4 | 1.26 | 0.57 | 0.19 | 0.01 | 0.05 | 0.12 | 0.91 | 0.43 | 3.54 |
| 31447 | 4 | 1.04 | 0.54 | 0.14 | 0.05 | 0.04 | 0.17 | 0.94 | 0.43 | 3.35 |
| 386023 | 4 | 1.09 | 0.64 | 0.19 | 0.07 | 0.03 | 0.14 | 0.93 | 0.44 | 3.53 |
| 3256 | 5 | 1.24 | 0.66 | 0.35 | 0.04 | 0.27 | 0.17 | 0.87 | 0.43 | 4.03 |
| 66804 | 5 | 1.19 | 0.75 | 0.22 | 0 | 0.11 | 0.13 | 0.82 | 0.47 | 3.69 |
| 44269 | 5 | 1.20 | 0.49 | 0.16 | 0.02 | 0.09 | 0.11 | 0.91 | 0.40 | 3.38 |
| 464965 | 5 | 1.14 | 0.42 | 0.09 | 0.06 | 0.09 | 0.10 | 0.87 | 0.41 | 3.27 |

[a] Determined by gas-liquid chromatography.
[b] *N*-acetylneuraminic acid.
[c] 2-Keto-3-deoxyoctulosonic acid.
[d] Total includes the hexoses, aminohexoses, NANA, and KDO.

4 (Tables 2 and 3). Total glycose in type 5 strains tended to be high and in two of four preparations was equal to that of virulent forms. It is also evident from Fig. 2A that the ratio of mannose to KDO is less than 2.0 in the majority of type 3 to 5 strains and greater than this in most type 1 and 2 strains. Similar findings are observed with galactose-KDO and glucose-KDO ratios (Fig. 2B and C), although the glucose-KDO values do not show quite the same degree of correlation.

Table 4 shows that total glycose-KDO and mannose-KDO ratios of *N. sicca* and *N. lactamica* strains are in the same range as or higher than those of type 1 gonococci. Although glucose concentrations are much lower, this is offset by the presence of rhamnose and fucose in the nonpathogenic *Neisseria* species.

The presence of glucose, galactose, mannose, rhamnose, glucosamine, and galactosamine determined by GLC was confirmed by paper chromatography.

**Identification of fatty acids.** Fatty acids present in the lipid A moiety of 20 gonococcus strains consisted of 10-, 12-, 14-, 16-, and 18-carbon acids (Table 5). Apparently there are no major differences between colonial types, but quantities vary from strain to strain. Also

found in all types was 3-hydroxytetradecanoic acid. A peak was observed to come off the GLC column between $C_{12}$ and $C_{14}$ acids in all cases, but it was not identified. Fatty acid analyses of *N. sicca* and *N. lactamica* reflect those of the gonococci.

The lipid A of two strains, KC1 and KC3, was subjected to further analysis. Glucosamine and phosphorus were the two substances identified positively. Glucosamine content in these strains was about 3.8 to 4.2 nmol per $\mu$g of lipid A, and that of phosphorus was 0.81 to 0.85 nmol. The glucosamine level in lipid A proved to be quite close to that obtained when LPS was tested.

TABLE 3. *Classification of gonococci on the basis of chemotype*

| Chemotype[a] | Colonial type | Strain |
|---|---|---|
| Aa | 1 | 46, 48111 |
| Ab | 1 | 52875, KC, 9T, 160, GC, 3256, 4478, 44269, 31447, 75354, 75302, 72809 |
| | 2 | KC, GC |
| Ac | 2 | 46, 386023 |
| | 5 | 66804 |
| Bb | 2 | 3256 |
| | 5 | 3256 |
| Bc | 3 | 3256, KC, 160 |
| | 4 | 160, KC, 75354, 6423, 31447, 386023 |
| | 5 | 44269, 464965 |
| Bd | 3 | GC, 44269, 44871 |
| | 4 | GC, 75302, 72809 |

[a] Classification method: chemotype A, rhamnose-negative lipopolysaccharide (<0.01 nmol); chemotype B, rhamnose-positive lipopolysaccharide (0.01 nmol or more); subtype a, total sugar 5.0 nmol or more; subtype b, total sugar 4.0 to 4.99 nmol; subtype c, total sugar 3.0 to 3.99 nmol; subtype d, total sugar 2.0 to 2.99 nmol.

## DISCUSSION

The present study constitutes the first report of the LPS composition of the five recognized colonial types of *N. gonorrhoeae*. Most notably, the concentrations of glucose, galactose, and mannose varied from strain to strain, but virulent types 1 and 2 generally contained a greater total glycose concentration than avirulent types 3 to 5. When mannose-KDO or galactose-KDO ratios are considered, it is clear that they tend to be higher in the virulent types, which suggests that these sugars are part of an O-specific chain, or at least that their concentration in the virulent types is greater. The glucose-KDO trend, although evident, is less striking.

Perry et al. (15) investigated a small number of type 1 and 4 strains and obtained evidence for the existence of O-specific chains in type 1 polysaccharide. In four type 1 preparations, they found considerable differences in the kinds of glycoses present. Our data differ in that we found a uniform composition of glycoses in type 1 strains together with variation in their concentration. We observed no qualitative differences between type 1 and type 4 LPS apart from the presence of rhamnose in the latter. The basis of these differences between our data and those of Perry et al. is unclear, but the culture medium was not the same and their glycose

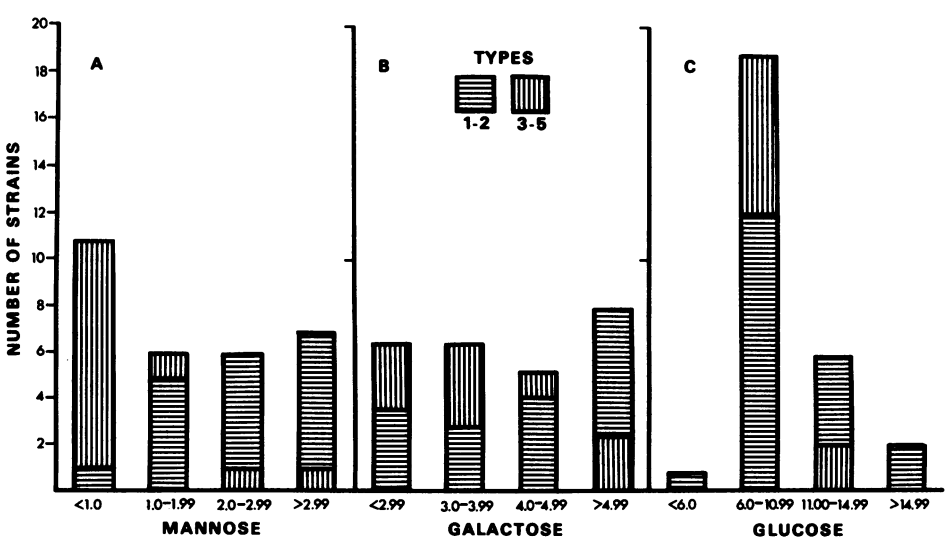


FIG. 2. *Monosaccharide to KDO ratios for lipopolysaccharide of type 1 to 5 gonococci.*

TABLE 4. *Glycose content of lipopolysaccharide from nonpathogenic Neisseria species*[a]

| Strain | Glycose concn (nmol per μg of lipopolysaccharide) | | | | | | | | | | Ratio of mannose to KDO |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose | Galactose | Mannose | Rhamnose | Fucose | NANA[b] | KDO[c] | Glucosamine | Galactosamine | Total[d] | |
| *N. sicca* A | 1.07 | 0.82 | 0.61 | 0.15 | 0.09 | 0.03 | 0.07 | 0.08 | 0.39 | 4.11 | 8.7 |
| *N. sicca* B | 1.19 | 0.80 | 0.55 | 0.20 | 0.08 | 0.05 | 0.08 | 0.93 | 0.43 | 4.31 | 6.9 |
| *N. sicca* C | 1.13 | 0.86 | 0.59 | 0.13 | 0.04 | 0.02 | 0.07 | 0.87 | 0.38 | 4.09 | 8.4 |
| *N. lactamica* | 1.24 | 1.14 | 0.30 | 0.09 | 0.11 | 0.07 | 0.11 | 0.81 | 0.46 | 4.33 | 2.7 |

[a] Determined by gas-liquid chromatography.
[b] *N*-acetylneuraminic acid.
[c] 2-Keto-3-deoxyoctulosonic acid.
[d] Total includes the hexoses, aminohexoses, NANA, and KDO.

TABLE 5. *Fatty acid content of gonococcus lipid A*

| Strain | Type | Acid chain length | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10:0 | 12:0 | 14:0 | 16:0 | 18:0 | HO-14:0 |
| *N. gonorrhoeae* | | | | | | | |
| 52875 | 1 | 0.22[a] | 0.14 | 0.12 | 0.15 | 0.22 | 0.36 |
| KC | 1 | 0.20 | 0.13 | 0.11 | 0.06 | 0.09 | 0.36 |
| 46 | 1 | 0.19 | 0.21 | 0.08 | 0.10 | 0.04 | 0.36 |
| 9T | 1 | 0.22 | 0.13 | 0.08 | 0.11 | 0.12 | 0.38 |
| 160 | 1 | 0.19 | 0.12 | 0.09 | 0.10 | 0.11 | 0.34 |
| GC | 1 | 0.21 | 0.12 | 0.11 | 0.12 | 0.13 | 0.31 |
| 3256 | 1 | 0.16 | 0.18 | 0.09 | 0.13 | 0.08 | 0.31 |
| 4478 | 1 | 0.19 | 0.13 | 0.08 | 0.11 | 0.10 | 0.32 |
| 3256 | 2 | 0.22 | 0.11 | 0.09 | 0.08 | 0.11 | 0.31 |
| KC | 2 | 0.20 | 0.13 | 0.11 | 0.06 | 0.08 | 0.30 |
| GC | 2 | 0.24 | 0.16 | 0.14 | 0.09 | 0.10 | 0.33 |
| 46 | 2 | 0.21 | 0.14 | 0.12 | 0.07 | 0.08 | 0.31 |
| 3256 | 3 | 0.31 | 0.14 | 0.14 | 0.08 | 0.06 | 0.26 |
| KC | 3 | 0.28 | 0.14 | 0.13 | 0.06 | 0.09 | 0.29 |
| GC | 3 | 0.30 | 0.16 | 0.14 | 0.07 | 0.08 | 0.27 |
| 160 | 3 | 0.27 | 0.15 | 0.08 | 0.07 | 0.08 | 0.29 |
| KC | 4 | 0.23 | 0.19 | 0.13 | 0.08 | 0.06 | 0.28 |
| GC | 4 | 0.19 | 0.13 | 0.12 | 0.07 | 0.07 | 0.33 |
| 160 | 4 | 0.23 | 0.15 | 0.11 | 0.09 | 0.07 | 0.30 |
| 3256 | 5 | 0.18 | 0.12 | 0.08 | 0.10 | 0.07 | 0.29 |
| *N. sicca* A | | 0.24 | 0.07 | 0.07 | 0.11 | 0.08 | 0.25 |
| *N. sicca* B | | 0.27 | 0.10 | 0.08 | 0.08 | 0.10 | 0.28 |
| *N. sicca* C | | 0.21 | 0.09 | 0.06 | 0.09 | 0.06 | 0.23 |
| *N. lactamica* | | 0.21 | 0.11 | 0.04 | 0.07 | 0.09 | 0.19 |

[a] Fatty acid concentration (nanomoles per microgram of lipid A), as determined by gas-liquid chromatography.

analyses were of polysaccharide material which had been separated from lipid A by acid hydrolysis.

Stead et al. (16) indicated that they were unable to detect O-specific chains in type 1 gonococci or, for that matter, differences of any kind between virulent and avirulent colonial types. However, it is uncertain whether they assessed the colonial stability of the organisms at the end of the growth cycle prior to extraction of the LPS. In our experience (2), the stability of types 1 and 2 cannot be relied upon in either liquid or solid media unless cultural conditions (particularly $CO_2$ tension) are carefully controlled throughout the period of growth. Consequently, it is possible that the inability of Stead's group to find evidence of O-specific chains may be traced to the overgrowth of type 3 or 4 gonococci in media inoculated with type 1. They also reported that deoxy, dideoxy, and *N*-acetylated sugars were not detected. Since such sugars are characteristic of O-specific chains of *Salmonella* and many other gram-negative organisms, their conclusion was that O-specific chains are not components of type 1 gonococcus LPS. Perry et al. (15) found that D-galactose, D-galactosamine, and D-glucosamine are probably present in the *N*-acetylated form

because their IR spectra show absorption bands at 1,560 and 1,650 $cm^{-1}$. Our own IR spectra support this observation. Perry et al. also detected no deoxy or dideoxy sugars, but one of their four type 1 strains contained L-rhamnose (6-deoxymannose) and fucose (6-deoxygalactose) of undetermined configuration.

The use of ratios of mannose, glucose, and galactose to KDO in assessment of the evidence of O-specific chains was based on the fact that these glycoses are frequently encountered in O-repeat units of *Salmonella* LPS. Although rhamnose is often present in the O-repeat unit as well, it is associated in the present study with avirulent "rough" gonococci rather than with virulent colonial forms. In the two strains in which aminoglycose content of lipid A was estimated, its concentration was approximately the same as that for unhydrolyzed LPS, which indicates that the core contained no glucosamine.

With the exception of *N. lactamica*, mannose-KDO ratios and total glycose concentrations of the nonpathogenic *Neisseria* species were much higher than corresponding values for the gonococci. It is not clear at present how this is to be interpreted, but it may not be valid to compare *N. sicca* and *N. lactamica* with the gonococcus in this way.

Luderitz et al. (11) and others have classified LPS from various gram-negative bacteria on the basis of sugar composition. Type 1 and 2 gonococci in our study are similar to those organisms which they designated chemotype IV, but it is not possible to place type 3 to 5 gonococci, *N. sicca*, and *N. lactamica* in their scheme at present unless a new chemotype is created.

Fatty acid analysis of gonococcus lipid A has revealed that the identified acids are similar to those found by Stead et al. (16), although there are some differences. They found HO-10:0, HO-12:0, 16:1, and 18:1 acids which we did not detect. Differences in strains, growth conditions, and analytical techniques are likely responsible for the variations.

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## LITERATURE CITED

1. **Ceriotti, G.** 1952. A microchemical determination of desoxyribonucleic acid. J. Biol. Chem. 198:297–303.
2. **Chan, K., and G. M. Wiseman.** 1975. Cultivation of type 1 *N. gonorrhoeae* in liquid media. Br. J. Vener. Dis. 51:382–386.
3. **Fiske, C. H., and Y. Subbarow.** 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375–400.
4. **Jennings, H. J., G. B. Hawes, G. A. Adams, and C. P. Kenny.** 1973. The chemical composition and serological reactions of lipopolysaccharides from subgroups A, B, X and Y of *Neisseria meningitidis*. Can. J. Biochem. 51:1347–1354.
5. **Jephcott, A. E., and A. Reyn.** 1971. *Neisseria gonorrhoeae*. Colony variation. I. Acta Pathol. Microbiol. Scand. Sect. B 79:609–614.
6. **Jourdian, G. W., L. Dean, and S. Roseman.** 1971. XI. A periodate-resorcinol method for the quantitative estimation of free sialic acids and their glycosides. J. Biol. Chem. 246:430–435.
7. **Kabat, E. A., and M. M. Mayer.** 1961. Experimental immunochemistry, 2nd ed., p. 504–507. Charles C Thomas, Publisher, Springfield, Ill.
8. **Kellogg, D. S., W. L. Peacock, W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to colonial variation. J. Bacteriol. 85:1274–1279.
9. **Laine, R. A., W. J. Esselman, and C. C. Sweeley.** 1972. Gas-liquid chromatography of carbohydrates, p. 159–167. *In* V. L. Ginsbury (ed.), Methods in enzymology, vol. 28. Academic Press Inc., New York.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
11. **Luderitz, O., O. Westphal, A. M. Staub, and H. Nikaido.** 1971. Isolation and chemical and immunological characterization of bacterial lipopolysaccharides, p. 145–233. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
12. **Maeland, J. A.** 1969. Antigenic determinants of aqueous ether extracted endotoxin from *Neisseria gonorrhoeae*. Acta Pathol. Microbiol. Scand. 76:475–483.
13. **Markham, R.** 1942. A steam distillation apparatus suitable for micro-Kjeldahl analysis. Biochem. J. 36:790–791.
14. **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.A. 50:499–506.
15. **Perry, M. B., V. Daoust, B. B. Diena, F. E. Ashton, and R. Wallace.** 1975. The lipopolysaccharides of *Neisseria gonorrhoeae* colony types 1 and 4. Can. J. Biochem. 53:623–629.
16. **Stead, A., J. S. Main, M. E. Ward, and P. J. Watt.** 1975. Studies on lipopolysaccharides isolated from strains of *Neisseria gonorrhoeae*. J. Gen. Microbiol. 88:123–131.
17. **Tauber, J., and W. Garson.** 1959. Isolation of lipopolysaccharide endotoxin. J. Biol. Chem. 234:1391–1393.
18. **Trevelyan, W. E., and J. S. Harrison.** 1951. Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. 50:298–303.
19. **Trevelyan, W. E., D. P. Procter, and J. S. Harrison.** 1950. Detection of sugars on paper chromatograms. Nature (London) 166:444–445.
20. **Ward, M. E., and P. J. Watt.** 1971. The preservation of gonococci in liquid nitrogen. J. Clin. Pathol. 24:122–123.
21. **Westphal, P., and K. Jann.** 1965. Bacterial lipopolysaccharides, p. 83–89. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press Inc., New York.
22. **Westphal, O., O. Luderitz, and F. Bister.** 1952. Uber die Extraktion von Bakterien mit Phenol/Wasser. Z. Naturforsch. 76:148.