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Nature of Polysaccharides Obtained from Endotoxins by Hydroxylaminolysis. (26810)

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Although endotoxins have been investigated extensively, the exact relationship between chemical nature and biologic reactions is still unknown. All endotoxins contain polysaccharide, lipid, certain amino compounds(1) and phosphoric acid and are known as lipopolysaccharides (LPSP). In the present paper endotoxins from 3 different species of bacteria and their lipid-free polysaccharides are subjected to a comparative study.

Materials and methods. The preparation of endotoxin has been previously described (1). The lipid moiety of the lipopolysaccharides was removed by hydroxylaminolysis (2). The separated polysaccharides were removed from the reaction mixture by centrifugation for 10 minutes at 1.700 g. The precipitates were washed once with alcohol and once with acetone and dissolved in water. The resultant solutions were dialyzed for 24 hours. After addition of 1 mg NaCl per ml the polysaccharides were precipitated with 2 volumes of acetone. The precipitates were washed with acetone and dried over sodium hydroxide under reduced pressure. The yield of polysaccharide was 50-60% on the basis of LPSP employed. Saccharides were determined by the anthrone method(3) with galactose as the standard. Amino sugars were determined by Dische's method(4) after deacetylation. The LD_{50} values(5) of endotoxins varied from 0.25 to 0.30 mg per 18-20 g mouse. The polysaccharides were non-toxic at 5 mg. Materials used in this study were those from *Neisseria gonorrhoeae* 97, *Escherichia coli* 0117:H27 and *Salmonella abortus equi.*

Results and discussion. Analytical data concerning 3 endotoxins and their polysaccharides are summarized in Table I. The nitrogen content indicates that amino compounds have been retained in the polysaccharides and the high phosphorus content points to a high degree of phosphorylation in all materials. The polysaccharides of *E. coli* endotoxin and of *S. abortus equi* endotoxin were free of lipid and in the *N. gonorrhoeae* polysaccharide the lipid content (stearin equivalent) dropped from 23.2% to 1.0%

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TABLE I. Composition of Endotoxins and Their Polysaccharides.*

No.	Type of products	N	P	Saccharide	Hexosamine	Total saccharide	Stearin equivalent
1	*E. coli* 0117:H27 LPSP	2.2	3.5	36.6	12.5	49.1	13.2
1A	Polysaccharide of same	2.3	2.4	52.3	24.2	76.5	0.0
2	*N. gonorrhoeae* 97-LPSP	2.1	3.4	22.0	20.3	42.3	23.2
2A	Polysaccharide of same	2.2	2.9	33.1	23.1	56.2	1.0
3	*S. abortus equi* LPSP	.8	2.1	47.0	7.7	54.7	11.2
3A	Polysaccharide of same	.8	2.0	60.0	4.7	64.7	0.0

* All values are given in per cent.

after hydroxylaminolysis. As expected, there was an increase in hexosamine and total saccharide content, after removal of the lipid moiety from the lipopolysaccharide molecule.

The ultracentrifuge patterns of all 3 polysaccharides reveal the presence of a major fast moving component and a minor slow moving component. The minor component is present in lowest concentration in the *N. gonorrhoeae* 97 sample. When graphically extrapolated to zero concentration of the specimens, the sedimentation constants (expressed in Svedberg units 1 S $= 10^{-13}$ cm/sec/units field) were as follows: for *N. gonorrhoeae* 97, $S_{20} = 8.3$; *E. coli* 0117:H27, $S_{20} = 9.2$ and *S. abortus equi*, $S_{20} = 9.4$. The rotor temperature was 20°C and solvent used was 0.1 M TRIS buffer, pH 8.0. Viscosity or diffusion measurements were not performed. Molecular weights were calculated from comparison of these sedimentation constants with those found for similar products and for which particle weights have been determined. Thus, the particle weight of the major component was found to be in the order of 200,000 and that of the minor component in the order of 10,000 or a small multiple thereof.

Summary. 1. There are marked differences between endotoxins prepared from 3 different species of gram negative bacteria. 2. The differences are retained after removal of the lipid component from the endotoxins by hydroxylaminolysis. Amino compounds are an integral part of both the endotoxin and polysaccharide molecules. 3. Molecular weights of the major components of the water soluble lipid-free materials are 200,000.

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Growth Inhibition of Microorganisms by Thyroid Hormones.* (26811)

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For unknown reasons thyroactive materials increase the vit. B_{12} (cyanocobalamin) requirement in rats, mice and chicks(1,2,3). This prompted the present investigation

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