Notes

Biochemistry of *Neisseria gonorrhoeae* Endotoxin

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Tauber & Garson [a] recently demonstrated that the endotoxin of *Neisseria gonorrhoeae* was not a protein but a lipopolysaccharide. This lipopolysaccharide could be isolated, certain chemical characteristics could be established and its toxicity could be appraised. This discovery is believed to be of some importance since it could lead to the development of specific antigens for serological testing in gonorrhoea as well as to the possible development of diagnostic skin tests for that disease.

The method of preparation of the lipopolysaccharide has been previously described by Tauber & Russell [b] and the present note is confined to reporting specific improvements which have now been introduced in its preparation. One improvement was dispersion of the aqueous suspension of bacterial cells in the Waring blendor prior to the addition of phenol. The other improvements were the removal of the endotoxin from aqueous solutions by fractional precipitation with acetone after dialysis; ultracentrifugation; dissolving the sediment in distilled water; and drying from the frozen state. These improvements resulted in the same type of product as was described in our previous report (*op. cit.*) but the yield of lipopolysaccharide was higher. Because of the absence of protein colour reactions and because paper chromatography revealed only traces of amino-acids, we concluded in our previous report that the endotoxin was free from peptides.

We have currently examined lipopolysaccharide preparations of different Gram-negative organisms using much larger quantities of hydrolysate in our tests and have found small quantities of a few apparently very specific amino-acids and amines in all of the four preparations studied. Some amino-compounds are significantly absent from some of the endotoxin preparations. For instance, there is no α,ϵ-diaminopimelic acid or glycine in *Escherichia coli* 08 lipopolysaccharide. Compound " X ", which we found to be identical with phosphoethanolamine, is absent from *E. coli* 0111: B4 endotoxin and *Salmonella typhosa* 901 endotoxin. The trace components " Y " and " Z " have not been identified. *N. gonorrhoeae* 97 endotoxin and *E. coli* 08 endotoxin contain only traces of these amino-compounds. Qualitative data on the amino-compounds in four endotoxins are given in Table 1.

TABLE 1

QUALITATIVE PAPER CHROMATOGRAPHIC ANALYSIS OF AMINO-COMPOUNDS IN ENDOTOXINS

Amino-compound	Type of organism from which endotoxin was prepared [a]			
	N. gonorrhoeae 97	*E. coli* 08	*S. abortus equi*	*S. typhosa* 901
X	+	+	+	0
α,ϵ-Diaminopimelic acid	—	0	0	0
Aspartic acid	0	+	+	+
Glutamic acid	+	+	+	+
Y	0	—	—	+
Z	0	—	—	+
Glycine	+	0	0	+
Alanine	+	+	—	+
Glucosamine	+	+	+	+
Phenylalanine	0	+	—	+
Ethanolamine	+	+	+	+

[a] + indicates presence of compound; — indicates presence of compound in trace quantities; 0 indicates absence of compound.

The following amino-acids could not be found in any of the samples tested: β-alanine, α-amino-*n*-

[a] Tauber, H. & Garson, W. (1959) *J. biol. Chem.*, **234**, 1931.

[b] Tauber, H. & Russell, H. (1960) *J. biol. Chem.*, **235**, 961.

TABLE 2

QUANTITATIVE PAPER CHROMATOGRAPHIC ANALYSIS OF AMINO-COMPOUNDS IN ENDOTOXINS [a]

Amino-compound	R_F	*N. gonorrhoeae* 97 endotoxin	*E. coli* 08 endotoxin
X [b]	0.05	2.00	0.30
α, ε-Diamino-pimelic acid	0.08	— [c]	0 [d]
Aspartic acid	0.11	0 [d]	0.20
Glutamic acid	0.15	0.25	0.24
Y	0.24	0 [d]	— [c]
Z	0.26	0 [d]	— [c]
Glycine	0.31	0.74	0 [d]
Alanine	0.48	0.53	0.58
Glucosamine	0.55	13.80 [e]	5.90 [f]
Phenylalanine	0.64	0 [d]	— [c]
Ethanolamine	0.72	3.16	0.73

[a] All values are given in percentages.
[b] The values for X are based on the glutamic acid calibration curve.
[c] — indicates presence of compound in trace quantities.
[d] 0 indicates absence of compound.
[e] Previously reported by Tauber & Garson (*op. cit.*).
[f] Previously reported by Westphal & Luderitz (1954) *Angew. Chem.*, **66**, 407.

butyric acid, arginine, cysteine, cystine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, proline, tryptophane and valine. Nor were the amines asparagine, glutamine and necrosamine present.

Two endotoxin preparations—namely, those of *N. gonorrhoeae* 97 and *E. coli* 08—were selected for a quantitative study. Studies concerning lipopolysaccharides had previously been limited to qualitative observations only because the amino-acids were considered " impurities ". Quantitative data concerning the amino-compounds in *N. gonorrhoeae* 97 endotoxin and *E. coli* 08 endotoxin are given in Table 2. Both endotoxins were prepared by the phenol method. There are marked differences between the *N. gonorrhoeae* 97 material and the *E. coli* 08 material. The *E. coli* 08 endotoxin contains neither α,ε-diaminopimelic acid nor glycine and much less of all the other amino-compounds than the *N. gonorrhoeae* 97 preparation. *N. gonorrhoeae* lipopolysaccharide contained 3.30% nitrogen and 22.18% assayed amino-compounds containing 2.47% nitrogen. *E. coli* 08 endotoxin contained 1.00% nitrogen and 7.97% assayed amino-compounds containing 0.77% nitrogen. The amino-acids probably form a link between the several components (lipids, saccharides, amino-sugars, etc.) of the giant endotoxin molecule. D-Glucosamine, glucose and galactose were the only carbohydrates found to be present in *N. gonorrhoeae* 97 endotoxin.

The lipid moiety of *N. gonorrhoeae* endotoxin is almost all firmly bound lipid. Only a small portion could be removed by refluxing with organic solvents. Fatty acid ester groups in the lipopolysaccharide endotoxins were determined by a new method, which uses the hydroxamic acid reaction as an assay procedure.[c]

[c] Tauber, H. (1960) *Fed. Proc.*, **19**, 245.

The Status of Gonorrhoea in the USA and Current Problems in its Control*

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The gonorrhoea control programme in the United States of America following the Second World War

" The year ended 30 June 1944 may eventually come to be regarded as one of the most significant in the history of venereal disease control. Preliminary reports on large scale research in the use of penicillin for treatment of both gonorrhea and syphilis conducted during the year indicate that a therapeutic weapon may be in the process of development which if applied on a sufficiently large scale could bring the virtual eradication of venereal disease as a major public health problem."

* Note submitted to the WHO Expert Committee on Venereal Infections and Treponematoses, September 1959.