The Immunochemistry of *Shigella flexneri* O-Antigens: a New Serologically Active Trisaccharide from Variant Y

By E. FREEDLANDER and D. A. R. SIMMONS. (*Department of Bacteriology and Immunology, University of Glasgow, Glasgow W.1, U.K.*)

The O-specific side chains of *Shigella flexneri* variant Y lipopolysaccharide are comprised of N-acetyl- α -D-glucosaminyl-(1 $\rightarrow$ 2)- α -L-rhamnosyl-(1 $\rightarrow$ 4)-L-rhamnose 'repeating units' linked together by 6-O-substitution of the N-acetylglucosamine residue in the adjacent unit (Simmons, 1969). Two trisaccharides, Y/3 and Y/4, previously isolated from this side chain by partial acid hydrolysis proved to be N-acetylglucosaminyl-(1 $\rightarrow$ 2)-rhamnosyl-(1 $\rightarrow$ 4)-rhamnose and rhamnosyl-(1 $\rightarrow$ 4)-rhamnosyl-(1 $\rightarrow$ 6)-N-acetylglucosamine respectively. The structure of a third, more recently isolated, trisaccharide (Y/3a) has now been elucidated by methods previously described (Simmons, 1969) as follows. On quantitative micro-analysis, a sample of compound Y/3a contained 218nmol of rhamnose and 96nmol of N-acetylglucosamine and on chromatography in butan-1-ol-acetic acid-water (4:1:5, by vol.) (Jermyn & Isherwood, 1949) it ran between trisaccharides Y/3 and Y/4 (R_{Glc} 0.56). These findings taken together imply that compound Y/3a is a trisaccharide containing rhamnose and N-acetylglucosamine in the molar proportions 2:1. Treatment with sodium borohydride reduced 48% of the rhamnose, which establishes this sugar as the reducing end group. After partial acid hydrolysis, α -L-rhamnosyl-(1 $\rightarrow$ 6)-N-acetylglucosamine was demonstrated among the products by comparative chromatography and high-voltage borate electrophoresis with an authentic sample of that disaccharide as a control. Trisaccharide Y/3a is therefore an α -L-rhamnosyl-(1 $\rightarrow$ 6)-N-acetylglucosaminylrhamnose. As the new saccharide gave no colour in the Morgan-Elson reaction, the reducing rhamnose is most probably 2-O-substituted. This view is consistent with the formation of 0.88mol of acetaldehyde/mol of trisaccharide after periodate oxidation. The above findings, and the structures of the oligosaccharides previously isolated from variant Y lipopolysaccharide, suggest that trisaccharide Y/3a is α -L-rhamnosyl-(1 $\rightarrow$ 6)-N-acetyl- α -D-glucosaminyl-(1 $\rightarrow$ 2)-L-rhamnose.

Complement-fixation inhibition studies in a homologous *Sh. flexneri* variant Y system showed that trisaccharides Y/3a and Y/4 were powerful inhibitors whereas trisaccharide Y/3 was virtually inactive. These findings imply that the terminal non-reducing rhamnose of trisaccharide Y/3a is the immunodominant residue and that the sequence

rhamnosyl-(1 $\rightarrow$ 6)-N-acetylglucosamine plays a major determinant role in the 3,4-group factors assigned to variant Y in the classification of Ewing & Carpenter (1966).

We thank the Medical Research Council for financial support.

Ewing, W. H. & Carpenter, K. P. (1966). *Int. J. systemat. Bact.* **16**, 145.

Jermyn, M. A. & Isherwood, F. A. (1949). *Biochem. J.* **44**, 402.

Simmons, D. A. R. (1969). *Eur. J. Biochem.* **11**, 554.

Inhibition of Growth of *Lactobacillus casei* by Tungstate and its Reversal by Molybdate

By EDWIN C. OWEN and JOHN PRINGLE. (*Department of Biochemistry, The Hannah Dairy Research Institute, Ayr, U.K.*)

In studies of metabolites of riboflavin in animals we have used *Lactobacillus casei* A.T.C.C. 7469 for assaying riboflavin (Owen & West, 1970). Tungstate has been used to demonstrate a requirement for molybdenum by species ranging from bacteria to birds and mammals, in which either growth, or the production of enzymes that contain molybdenum as an essential constituent, can be inhibited by tungstate. In many such species, additional molybdenum overcomes the tungstate-induced inhibition (for references see Owen & Proudfoot, 1968). We now report a new example of competition between molybdenum and tungsten.

Standard solutions of riboflavin, such as we routinely use for riboflavin assays, supplemented by $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ or $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ or both, were added to Difco assay medium before its inoculation with *L. casei*. With every test a normal assay with riboflavin alone was used as a control. Though growth of *L. casei* was completely inhibited by 0.2% W (calculated as the element) 0.1% Mo had no inhibitory effect at all. With 0.1% W either 0.002 or 0.01% Mo permitted only negligible growth equal to 0.15 times the standard. In contrast, 0.15% W with either 0.1% or 0.4% Mo was not inhibitory at all. In all these experiments growth was measured turbidimetrically at 600nm. With 150 ng of riboflavin/tube of 11 ml of medium $E_{1\text{cm}}^{1\%}$ 600 nm was 0.671. As a check on the method, bacteria were harvested by centrifugation after incubation and their nitrogen content was determined by using Nessler's reagent (Williams, 1964). The results showed that there was no growth in the absence of riboflavin irrespective of additions of molybdate or tungsten. With 100 ng of riboflavin/tube 138, 123, 105 and 128 μg of N/tube corresponded to no ad-