NOTES

MONOSACCHARIDES ISOLATED FROM THE CAPSULE OF *PASTEURELLA MULTOCIDA*

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The type specific capsular substance of *Pasteurella multocida* has been reported to be a polysaccharide (Carter and Annau, Am. J. Vet. Research, **14**, 475, 1953). Using the method of these investigators, the capsular polysaccharide from the fluorescent variants (Hughes, J. Exptl. Med., **51**, 225, 1930) of a type 1 and a type 3 strain (Little and Lyon, Am. J. Vet. Research, **4**, 110, 1943) was removed, purified, and concentrated. The dried capsular material, just prior to hydrolysis, was serologically active as shown by mouse protection and precipitin tests, gave a positive anthrone (Morris, Science, **107**, 254, 1948) test for carbohydrates, and did not show the presence of any free sugar when subjected to paper chromatography. The material from the type 1 strain had a negligible nitrogen content (0.05 per cent) whereas that from the type 3 strain contained 2 per cent nitrogen (using the micro-Kjeldahl method).

Samples of the dried material from 3 to 5 mg were hydrolyzed at 100 C for 4 hr in $N H_2SO_4$. Following centrifugation, the remaining traces of the insoluble salt, formed when $CaCO_3$ was added to the hydrolyzate to raise the pH to approximately 6, were removed by washing the solution with dry pyridine; the salt-free hydrolyzate was dried *in vacuo*.

The ascending paper chromatographic method was used in the identification of the hydrolytic products. The dried hydrolyzate was dissolved in 50 per cent isopropyl alcohol and applied to Whatman no. 1 filter paper. The solvent, butanol:pyridine:water (45:25:40), gave the best separation of sugar and the chromatogram was processed twice. The spray used was aniline hydrogen phthalate (Partridge, Nature, **164**, 443, 1949). The unknown sugars were identified by a

comparison of R_f value and color complexes with those of known monosaccharides. Masking, a process by which the unidentified sample was reinforced with the sugar suspected, was additional proof of identity.

Qualitative examination of the hydrolytic products from the capsular polysaccharide of the 2 serological types revealed no detectable difference in monosaccharide components. Two spots appeared in each hydrolyzate; a pink-red spot indicating a pentose and a yellow-brown spot indicating a hexose. The pentose which had a higher R_f value than xylose or arabinose had the same mobility in the solvent as ribose and was tentatively identified as ribose. The hexose was identified tentatively as galactose since sufficient separation was obtained from other reference sugars such as glucose and glucosamine HCl which have similar R_f values.

Though the type specific substance of *Haemophilus influenzae* type b is a polyribophosphate (Zamenhof *et al.*, J. Biol. Chem., **203**, 695, 1953), no report in the literature of the isolation of ribose from a capsular polysaccharide has been found. Studies using the conventional Warburg respiratory technique show that intact cells of the type 1 strain of *P. multocida* actively oxidize ribose and galactose but do not metabolize D-xylose nor the stereoisomers of arabinose. This may be interpreted as additional evidence for the identification of the pentose as ribose.

Since no difference was found in the monosaccharide components of the type specific capsular material of the two strains, reported differences in virulence and serological reactions may be due to differences in the linkages or quantities of these two sugars present in the polysaccharide. The higher nitrogen content in the type 3 strain might indicate the presence of an undetected amino sugar in the capsule of that strain.

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