

cates that there is a separate ζ -chain locus determining its structure.

Further work on the ζ -chain of the Hb-Portland 1 is currently under way on haemoglobin samples obtained in Hong Kong from Chinese stillborn infants with erythroblastosis fetalis, due to homozygous α -thalassaemia.

This work was supported by a US Atomic Energy Commission contract and a US Public Health Service research grant.

Note added in proof. Two recent papers provide similar conclusions about the uniqueness of the ζ -chain (D. J. Weatherall, J. B. Clegg and W. H. Boon (*Brit. J. Haematol.*, **18**, 357; 1970); and D. Todd, M. C. S. Lai, G. H. Beaven and E. R. Huehns (*Brit. J. Haematol.*, **19**, 27; 1970).

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Received February 19; revised March 2, 1970.

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Formate, a New Component of Bacterial Exopolysaccharides

THE occurrence of non-carbohydrate substituents in bacterial exopolysaccharides has been well documented. Acetate and pyruvate have frequently been detected either separately or together in the same polysaccharide^{1,2}. More recently, a polysaccharide secreted by *Alcaligenes faecalis* var. *myxogenes* was shown to contain succinate³. Although formate is known as a bacterial metabolite it has not previously been reported as an integral component of bacterial extracellular polysaccharides. This may be partly because of the lack of suitable methods for detection or estimation of formate and also because of the known lability of O-acetyl groups on carbohydrates.

The basic structure of the polysaccharide forming the extracellular slime or capsule of *Klebsiella aerogenes* type 54 (strain A3) was established by chemical methods⁴. A repeating unit structure was identified as β -cellobiosyl- α -glucuronosyl-1 \rightarrow 3-fucose. When the polysaccharide from this strain was hydrolysed with phage-induced depolymerase enzymes, two products were obtained⁵. Each was identified as a tetrasaccharide with the same structure as the repeating unit of the polysaccharide, but one of these oligosaccharides was acetylated while both contained an unidentified substituent.

The polysaccharide did not contain pyruvate or succinate. Unlike O-acetyl groups, the unknown component was not removed by mild alkaline treatment (0.01 M KOH at 20° C for 16 h), but was absent after mild acid hydrolysis (0.05 M H₂SO₄ at 100° C for 15 min). Hydroxamic acid derivatives were prepared from acid hydrolysates of the polysaccharide by the method of Thomson⁶ and compared with authentic compounds by descending paper chromatography in butan-1-ol-acetic

acid-water (4 : 1 : 5, upper phase) for 20 h. Spots were revealed by spraying with 10 per cent ferric chloride solution. After drying at room temperature, two spots were present with *R_F* values 0.51 and 0.43 respectively. These were exactly equidistant with acetylhydroxamic acid and with formylhydroxamic acid respectively. To confirm that formate was a substituent of the polysaccharide and of oligosaccharides derived from it, the enzyme formate dehydrogenase was prepared from *Pseudomonas oxalaticus*⁷. This enzyme is specific for the NAD-linked oxidation of formate and is unaffected by the presence of acetate. Assay of mild acid hydrolysates of the polysaccharide or the oligosaccharides obtained by depolymerase treatment gave reduction of NAD in the presence of formate dehydrogenase. By comparison with standards, the formate content of samples was calculated to be in the approximate ratio of one mole formate per mole of fucose or glucuronic acid. Calculated on a dry weight basis the polysaccharide contained 46.5 per cent glucose, 19.1 per cent fucose, 20 per cent glucuronic acid, 6.3 per cent acetate and 6.2 per cent formate. Some loss of formate could be expected because of the volatility of the free acid obtained after acid hydrolysis of the samples. A similar result was obtained for the acetylated tetrasaccharide. The non-acetylated tetrasaccharide also contained one molar equivalent of formate. It is also possible to estimate the presence of formate by reduction to formaldehyde and use of the chromotropic acid reagent⁸. Using this method, the formate content of the non-acetylated oligosaccharide was in good agreement with the value obtained by the enzymic method.

The sugar which is formylated is not known, but one residue can definitely be excluded. Treatment of either tetrasaccharide with a β -glucosidase preparation liberated one mole of glucose in each case. The other product was a trisaccharide containing either formate or formate and acetate. Further enzymic hydrolysis was not possible. Attempts to isolate a formylated sugar by graded acid hydrolysis have also proved unsuccessful.

Preliminary experiments indicate that formate is present in the extra-cellular polysaccharides of several other *K. aerogenes* strains. It is not present in all strains, however, and it remains to be seen how widespread is the occurrence of formate in polysaccharides of other bacterial species or of non-bacterial origin.

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Received August 10, 1970.

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Transduction in *Mycobacterium smegmatis*

SEVERAL people have studied lysogeny and lysogenic conversion in the advanced prokaryote, *Mycobacterium*¹⁻³, and presented evidence for reciprocal genetic exchange from the lysogenic to the non-lysogenic state. Biochemical and physiological changes induced by lysogenization of *M. phlei* with mycobacteriophage B2 have been demonstrated by Juhász⁴. We wish to report the isolation of a mycobacteriophage capable of mediating transduction in *Mycobacterium smegmatis* strain SN2, thus providing an opportunity to study the genetics of mycobacteria.

M. smegmatis strains SN2 and EB165 were provided