Experiments are under way to determine whether isoniazid inhibits the synthesis of these polysaccharides, stimulates their breakdown, or causes their release from the cells.

We thank Miss Alice Harrison for technical assistance. This work was supported by Grants from the U.S. Public Health Service (AI07254) and from Shell International Petroleum Co. Ltd.

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Free Trehalose in *Corynebacterium xerosis*

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Similarities between the cell-wall constituents of mycobacteria and corynebacteria (Cummins & Harris, 1958) and the presence of dimannophosphoinositides in both genera (Brennan & Ballou, 1967; Brennan, 1968) indicate a close relationship between them. The soluble carbohydrates of *Corynebacterium xerosis* were investigated and compared with those of mycobacteria (Winder, Brennan & McDonnell, 1967) in a further examination of this relationship.

The growth and harvesting of *C. xerosis* were as described by Brennan (1968). Washed organisms were extracted several times with 50% (v/v) ethanol at 0°. Ethanol was removed from the extracts and they were fractionated with barium (LePage, 1957). The 'barium-insoluble' fraction contained about 50% of the total cell phosphate and about 10% of the total carbohydrate. Most of the phosphate in this fraction was inorganic, but some of the organic phosphate was apparently in the form of a phosphorylated arabinose. The 'barium-soluble ethanol-insoluble' fraction contained about 40% of the total phosphate and 20% of the total carbohydrate. Hydrolysis and paper chromatography showed the presence of glucose and mannose. Glucose 6-phosphate was identified. The 'barium-soluble ethanol-soluble' fraction contained 75% of the total carbohydrate. This material was further fractionated on a column of charcoal-celite (Whistler & Durso, 1950). Most of the carbohydrate was eluted with water and was identified as glucose by several chromatographic systems. Elution of the column with 10% (v/v) ethanol removed at least three oligosaccharides. These were further purified by paper chromatography in ethyl acetate-pyridine-water (10:4:3, by vol.). The major oligosaccharide contained only glucose and had the chromatographic properties of $\alpha\alpha'$ -trehalose in four solvent systems (Winder *et al.* 1967). The other oligosaccharides are now being examined.

There are several similarities between the above pattern and that obtained previously for mycobacteria (Winder *et al.* 1967): mycobacteria contain fairly large amounts of trehalose and appreciable amounts of glucose 6-phosphate, and evidence for phosphorylated arabinose has also been found in them.

A glycolipid containing $\alpha\alpha'$ -trehalose has been found in *Corynebacterium diphtheriae*, but there has been no previous report on the presence of the free sugar in corynebacteria. Substantial amounts of free trehalose are thus present in corynebacteria, mycobacteria, streptomyces (Elbein, 1967) and propionibacteria (Stjernholm, 1958). Other similarities between these genera will be discussed.

I thank Professor F. G. A. Winder for advice. This work was supported by a Grant (AI07254) from the United States Public Health Service.

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Phosphoglycopeptides from Yeast Cell Walls

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The major components of the yeast cell wall are mannan, glucan, and protein, which are present in the form of macromolecular complexes. Lampen (1968) suggested a structure for the cell wall in which the outer layer is made up of large mannan molecules held together by phosphodiester bonds. About 20% of the total mannan is present in an adjacent layer that also contains glucan and protein, and this layer appears to be covalently linked to the glucan network that gives the cell rigidity and its characteristic shape. The present report describes studies on the chemical composition of phosphoglycopeptides that are released from the outer layers of yeast cell walls by the action of Pronase.

Yeast cells (*Saccharomyces cerevisiae*, Guinness strain 1406) were suspended in water and disintegrated in the cold by shaking with glass beads for 1–2 min. Cell-wall fragments were isolated by

differential centrifugation as described by Falcone & Nickerson (1956). Defatted cell wall gave a typical analysis of carbohydrate, 85%; N, 1.3%; P, 0.6%; mannose:glucose ratio, 1.05:1. Pronase digestion of this material solubilized 54% of the total carbohydrate and 38% of the total phosphorus. The mannose:glucose ratio of the residue was 1:2. The soluble fraction from the Pronase digest was fractionated on DEAE-cellulose to give phosphoglycopeptide material (250 mg. from 1g. defatted cell wall; carbohydrate, 94%; mannose:glucose ratio, 4.6:1; N, 0.8%; P, 0.9%).

The nature of the phosphodiester linkages in the phosphoglycopeptide was investigated by measuring the elimination of inorganic phosphate from its periodate-oxidation product. The native phosphoglycopeptide yielded no inorganic phosphate when treated with 1N-KOH at room temperature for 12 hr., but mild alkali treatment (pH 10.5) of the periodate-oxidation product resulted in the elimination of 59% of the phosphorus as inorganic phosphate. Almost complete conversion into inorganic phosphate was achieved only by adding the periodate-oxidation product to 1N-KOH. Our results suggest that the phosphodiester linkages in the glycopeptide are similar to those reported for the extracellular phosphomannan from *H. holstii* (Jeanes & Watson, 1962) in which the phosphodiester groups are linked between C-6 of one mannose unit and C-1 of another, which is in turn glycosidically linked at C-2 by another mannose residue.

T. N. C. acknowledges the receipt of a Guinness Research Scholarship.

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Partial Purification of Deoxyribonucleic Acid Polymerase from *Mycobacterium smegmatis*

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A nucleoside triphosphate-dependent DNA-breakdown system has been found in *Mycobacterium smegmatis* (Winder & Coughlan, 1967a) and in bacteria from two other genera (Tsuda & Strauss, 1964; Anai, 1967). It has been suggested that this system might represent the reversed action of a DNA polymerase (deoxynucleoside triphosphate-DNA deoxynucleotidyltransferase, EC 2.7.7.7) able to use ATP in place of pyrophosphate, with a consequent shift of equilibrium towards depolymerization (Winder & Coughlan, 1967c). Hence,

the purification of DNA polymerase from *M. smegmatis* was undertaken as a preliminary to testing this hypothesis.

The bacteria were grown in iron-limited medium and harvested after 72 hr., and extracts were prepared as described by Winder & Coughlan (1967a,b). DNA polymerase was determined by using [³H]TTP in the assay mixture of Lehman (1963) but by employing the procedure of Patel, Howk & Wang (1967).

The crude extracts incorporated about 0.2 mμmoles of thymidine into DNA/30 min./mg. of protein. Treatment with 0.5% streptomycin gave a precipitate that showed a 50-fold increase in specific activity over the crude extract, with a recovery of 65%. The supernatant contained a similar total activity, the total recovery in this step being about 130%. Dialysis of this supernatant, addition of 5 mg. of DNA/ml. and addition of 2% streptomycin gave a second precipitate with a similar specific activity and recovery to the first. The dialysed solutions of the two precipitates were treated with deoxyribonuclease and then with C₆ alumina gel, and eluted as described by Lehman (1963). The total activity was substantially increased by the deoxyribonuclease treatment, but there was difficulty in eluting the activity from the gel. However, a good recovery was achieved with the material from the second precipitate, and a 430-fold increase in specific activity over the crude extract was obtained.

Determination of ATP-dependent DNA-breakdown activity was carried out during the purification. A marked variation in the ratio of this activity to DNA polymerase activity was observed. This suggests that the two activities are not due to one enzyme, but further investigations will be required to show this conclusively.

This work was supported by grants from the Medical Research Council of Ireland and the Irish Cancer Society.

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