of the enzyme–substrate complex (Jeuniaux, 1959). Cherkasov, Kravchenko & Kaverzneva (1966) showed that lysozyme was adsorbed on chitin by buffers of high ionic strength and desorbed by buffers of low ionic strength.

Convenient sources of glycoside hydrolases such as chitinase and β-(1→3)-glucan hydrolase have been reported to be present in *Streptomyces albidoflavus* (Sturgeon, 1964) and in the digestive juice of *Helix pomatia* (Takeda, Strasdine, Whitaker & Roy, 1966). The present work involved studies on the adsorption of these enzymes on chitin and the β-(1→3)-glucan pachyman (Warsi & Whelan, 1957). Chromatography of the crude culture filtrate from *S. albidoflavus* was carried out on columns of chitin equilibrated with 0·1M-phosphate buffer, pH5·2. One protein peak was obtained, fraction I, exhibiting activity towards laminarin, *p*-nitrophenyl *N*-acetyl-β-glucosaminide and chitin. Development with 0·1M-borate buffer, pH8·2, yielded a second enzyme fraction that exhibited only chitinase and *N*-acetyl-β-glucosaminidase activity. That the two chitinase fractions are different has been shown by their different pH optima and thermal stabilities, indicating a similarity to the chitinase of *Streptomyces griseus* (Berger & Reynolds, 1958).

Chromatography of the β-(1→3)-glucan hydrolases of *H. pomatia* was carried out on columns of pachyman with 0·1M-citrate buffer, pH4·8, followed by 0·1M-borate buffer, pH8·2. The protein fraction (*A*) eluted with the former buffer showed activity towards cellulose, chitin, pachyman, laminarin and *p*-nitrophenyl β-D-glucoside. The fraction (*B*) eluted at pH8·2 was able to hydrolyse pachyman and laminarin. However, the mode of action of the two fractions on β-(1→3)-glucans was shown to be different, since glucose was the only product obtained on incubation with fraction *A* and a homologous series of β-(1→3)-linked oligosaccharides was obtained from digests with fraction *B*.

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## The Effect of Isoniazid on the Alkali-Extractable Polysaccharides of *Mycobacterium tuberculosis*

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When growing *Mycobacterium tuberculosis* BCG is exposed to isoniazid there is an increase in the amount of carbohydrate in the soluble pool and a decrease in the amount of carbohydrate extractable with alkali from the remainder of the cells (Winder, 1964). The changes in the soluble carbohydrates have been studied in some detail (Winder, Brennan & McDonnell, 1967), but recent investigations have shown that somewhat similar changes can be produced by other growth-inhibitory drugs and by adjustments in growth conditions (S. A. Rooney & F. G. Winder, unpublished work). The decrease in alkali-extractable carbohydrate is more specific to isoniazid and hence this fraction has been further investigated.

*M. tuberculosis* BCG was grown, exposed to 10μg. of isoniazid/ml. for 6–12 hr., harvested and washed, and the soluble pool and lipids were extracted, as described by Winder, Brennan & Flynn (1967). The alkali-extractable polysaccharides were obtained by treating the residue with N-KOH for 18 hr. at 25°, making the mixture acid with $HClO_4$ and removing insoluble material by centrifugation.

Hydrolysis with acid, followed by paper chromatography, showed that mannose, glucose, arabinose, galactose, ribose and 6-*O*-methylglucose, in amounts diminishing in that order, were present in this material. Quantitative assays, by a combination of chromatography on Dowex 50 columns and paper chromatography, with measurement of carbohydrate in the eluates by the phenol method, showed that the effect of isoniazid was mainly on the amounts of glucose and 6-*O*-methylglucose, and that the effect on the other sugars was smaller. Measurement of glucose by the glucose oxidase method confirmed that its amount was decreased by isoniazid.

The alkali-soluble polysaccharides were completely excluded from Sephadex G-25. They were separated on Sephadex G-100 into three clearly resolved fractions, two of which showed evidence of heterogeneity. All the peaks gave several sugars on hydrolysis. The first peak had a high content of arabinose, and the second and third peaks had a high content of mannose. 6-*O*-Methylglucose was restricted to the slower shoulder of the second peak, and ribose to the third peak, where presumably it was present in ribonucleotides derived from RNA. The amount of carbohydrate in each of the three fractions was decreased by isoniazid.

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 (AI 07254) 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