- Kaslander, J. (1966). Ph.D. Thesis: University of Utrecht.
 Pigman, W. W. (1941). *J. Res. natn. Bur. Stand.* **26**, 197.

Excretion of Bilirubin Conjugated with Glucose in Dog Bile

By K. P. M. HEIRWEGH, G. P. VAN HEES, F. COMPERNOLLE and J. FEVERY. (*Laboratory for Liver Physiopathology, Academic Clinic of St Rafael, Minderbroedersstraat 10, B-3000 Leuven, Belgium*)

In mammals, birds, amphibia and fish conjugation with glucuronic acid constitutes a major pathway in the end metabolism of endogenous compounds (Dutton, 1966) and drugs (Smith & Williams, 1966). Recent studies have demonstrated that a considerable fraction of bilirubin in human bile is conjugated with uronic acid (not necessarily identical with glucuronic acid) and with additional acid-labile bound conjugating groups (K. P. M. Heirwegh, G. P. Van Hees, F. Compernelle & J. Fevery, unpublished work).

It has now been shown that about 30% of total azopigment derived from dog bile by diazotization with the diazonium salt of ethyl anthranilate (Van Roy & Heirwegh, 1968) consists of azobilirubin monoglucoside. The aglycone and the sugar moiety are bound in ester linkage involving the carboxylic acid group of azobilirubin and the 1-hydroxyl group of D-glucose, probably in the β -D-configuration. The pigment in question has been called α_3 -azopigment.

The structural assignment is based on the following evidence. After application of purified α_3 -azopigment to t.l.c. plates treatment with ammonia vapour leads to the formation of (1) a mixture of azobilirubin and of its carboxylic acid amide and (2) D-glucose. The derived azopigments were identified by mass spectrometry. The nature of the sugar moiety was established as follows. The isolated sugar reacted promptly with glucose oxidase test paper, and moved as D-glucose on (1) silica-gel plates with various sugar solvents (Lewis & Smith, 1967) and (2) anion-exchange columns in boric acid, pH 7 (Kesler, 1967). Control experiments indicated that the ammonia treatment used did not affect the chromatographic properties of D-glucose. Treatment of the α_3 -azopigment with β -glucosidase preparations led to the formation of azobilirubin, and the process was inhibited with glucono-(1 $\rightarrow$ 5)-lactone.

The isolation from urine of man (Arcos & Lieberman, 1967) and of rabbit (Layne, Sheth & Kirdani, 1964; Layne, 1965; Collins, Williams & Layne, 1967) of steroids conjugated with N-acetylglucosamine, together with the observations men-

tioned above, suggest that conjugation with sugars different from glucuronic acid may have a more important role in animals than hitherto suspected.

We are indebted to the 'Fonds voor Wetenschappelijk Geneeskundig Onderzoek' of Belgium for financial support.

- Arcos, M. & Lieberman, S. (1967). *Biochemistry, Easton*, **6**, 2032.
 Collins, D. C., Williams, K. I. H. & Layne, D. S. (1967). *Archs Biochem. Biophys.* **121**, 609.
 Dutton, G. J. (1966). In *Glucuronic Acid, Free and Combined*, p. 185. Ed. by Dutton, G. J. New York and London: Academic Press.
 Kesler, R. B. (1967). *Analyt. Chem.* **39**, 1416.
 Layne, D. S. (1965). *Endocrinology*, **76**, 600.
 Layne, D. S., Sheth, N. A. & Kirdani, R. Y. (1964). *J. biol. Chem.* **239**, 3221.
 Lewis, B. A. & Smith, F. (1967). In *Dünnschichtchromatographie*, 2nd ed., p. 769. Ed. by Stahl, E. Berlin, Heidelberg and New York: Springer-Verlag.
 Smith, R. L. & Williams, R. T. (1966). In *Glucuronic Acid, Free and Combined*, p. 457. Ed. by Dutton, G. J. New York and London: Academic Press.
 Van Roy, F. P. & Heirwegh, K. P. M. (1968). *Biochem. J.* **107**, 507.

Studies on the Reverse Action of Amylo-1,6-Glucosidase

By J. R. STARK and V. THAMBYRAJAH. (*Department of Brewing and Biochemistry, Heriot-Watt University, Edinburgh EH1 1HX, U.K.*)

The reverse action of amylo-1,6-glucosidase, in which radioactive glucose is incorporated into glycogen, has been widely used as an assay procedure (Hers, 1964). This reaction was further studied by Manners & Stark (1967), with human muscle and liver homogenates and maltosaccharides in place of glycogen as the acceptor substrate, and by Nelson & Larnar (1970), who showed that the reaction proceeds in both the synthetic and degradative directions with retention of anomeric configuration.

These studies have now been extended by using a purified rabbit muscle amylo-1,6-glucosidase preparation. The muscle homogenate was fractionated by acid treatment, ammonium sulphate precipitation and column chromatography on Bio-Gel A1.5m, giving a 39-fold purification. The preparation moved as a single zone on gel electrophoresis, but when incubated with radioactive glucose material corresponding to glucose phosphates on paper chromatography was produced, indicating the probable presence of traces of hexokinase. When the enzyme was incubated with radioactive glucose and maltosaccharides, radioactive branched oligosaccharides were formed. Maltotetraose was

the smallest acceptor to give an incorporation product. The linkage of the incorporated radioactive glucose to the maltosaccharide was shown to be (1→6) by partial acid hydrolysis and methylation studies on a branched heptasaccharide formed from maltohexaose and radioactive glucose. The incorporation products were resistant to pullulanase, indicating that the radioactive glucose residue is present as a single stub. The action of β -amylase on the branched oligosaccharides gave further information on their structures.

The variation of the extent of incorporation with pH was studied with different acceptor substrates. With glycogen or Schardinger dextrans as acceptor substrates optimum incorporation occurred at pH 7.0 and 5.6, whereas with glycogen β -limit dextrin as acceptor a single optimum value at pH 7.0 was produced. With maltosaccharides of degree of polymerization 7 and 13 maximal incorporation occurred at pH 5.6. These results indicate that two related types of incorporation reaction that differ in pH optima are taking place.

We thank Professor D. J. Manners for his advice and encouragement during the course of this work.

Hers, H. G. (1964). *Adv. metab. Disorders*, 1, 2.

Manners, D. J. & Stark, J. R. (1967). *Abstr. FEBS 4th Meet., Oslo*, p. 14.

Nelson, T. E. & Larner, J. (1970). *Biochim. biophys. Acta*, 198, 538.

Properties of an α -Mannosidase from *Streptomyces* WL 6

By J. K. BAIRD and W. L. CUNNINGHAM. (*Department of Brewing and Biochemistry, Heriot-Watt University, Edinburgh EH1 1HX, U.K.*)

The purification and properties of α -mannosidases from several sources have been described recently. The enzyme from jack-bean meal (Li, 1967; Snaith & Levvy, 1968) hydrolyses α -mannosides containing aromatic aglycones, whereas the enzyme from an *Arthobacter* species shows little activity against such compounds but will degrade yeast mannan extensively (Jones & Ballou, 1969), a property that is not always associated with the other α -mannosidases.

Tanaka & Phaff (1965) observed that glucose and mannose were liberated from yeast cell walls by *Streptomyces* WL 6, an organism isolated by them from soil. They concluded that the streptomycete produced both glucanase and mannanase. During a study on the production of protoplasts from yeast cells by using the culture filtrate of this organism, we observed that an active α -mannosidase was also present. This α -mannosidase could be induced, in

quantity, by substituting yeast mannan as the carbon source, as found with *Streptomyces griseus* (Hockenhull, 1960).

Streptomyces WL 6 was grown for 6 days at 25°C in medium of pH 6.5, containing yeast mannan (0.5%), yeast extract (0.05%), tryptone (0.05%) KH_2PO_4 (1.36%) and salts. Ammonium sulphate was added to the culture fluid after removal of the cells. The fraction precipitated by 10–70% saturation was dialysed against 10 mM-phosphate buffer, pH 6.5, containing 0.1 mM- Ca^{2+} . Non-diffusible material was further purified by chromatography on DEAE-Sephadex.

A fraction was obtained that showed a 36-fold increase in purification of α -mannosidase activity measured with *p*-nitrophenyl α -mannoside as substrate. This preparation also hydrolysed manno-biose, mannotriose and mannotetraose derived from yeast mannan, methyl α -mannoside to a limited extent, and *p*-nitrophenyl α -glucoside, methyl α -glucoside, isomaltose, maltose and laminaribiose not at all. K_m values obtained were: for *p*-nitrophenyl α -mannoside, 3.3×10^{-3} M; for manno-biose, 2.0×10^{-3} M; for mannotriose, 7.5×10^{-3} M. Yeast mannan was slowly hydrolysed to mannose to a limited extent, indicating that the enzyme has exomannanase activity. The activity of the enzyme against *p*-nitrophenyl α -mannoside was stimulated by Ca^{2+} but inhibited by Cu^{2+} , Mg^{2+} and Zn^{2+} . There are therefore some differences between this enzyme and the α -phenylmannosidase present in *Streptomyces griseus* (Hockenhull, Ashton, Fantes & Whitehead, 1954).

We are indebted to Dr H. J. Phaff for a culture of *Streptomyces* WL 6. We thank the Science Research Council for a research studentship to J. K. B.

Hockenhull, D. J. D. (1960). *Prog. ind. Microbiol.* 2, 133.
Hockenhull, D. J. D., Ashton, G. C., Fantes, K. H. & Whitehead, B. K. (1954). *Biochem. J.* 47, 93.

Jones, G. H. & Ballou, C. E. (1969). *J. biol. Chem.* 244, 1043.

Li, Y.-T. (1967). *J. biol. Chem.* 242, 5474.

Snaith, S. M. & Levvy, G. A. (1968). *Biochem. J.* 110, 663.

Tanaka, H. & Phaff, H. J. (1965). *J. Bact.* 89, 1570.

An Effect of Amino Acid Analogues on Extension Growth in Pea Root Segments under Aseptic Conditions

By D. VAUGHAN and EVELYN CUSENS. (*Department of Biochemistry, The Macaulay Institute for Soil Research, Aberdeen AB9 2QJ, U.K.*)

Segments cut 2–4 mm from the root tip of 2-day-old seedlings of *Pisum sativum* (var. Meteor) elongate by 30% when cultured aseptically in water for 18 h. Elongation is enhanced by the