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Excretion of Bilirubin Conjugated with Glucose in Dog Bile

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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with additional acid-labile bound conjugating groups (K. P. M. Heirwegh, G. P. Van Hees, F. Compernelle & J. Fevery, unpublished work).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.

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

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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