replace phenobarbital. Triton X-100, often an activator of microsomal UDP-glucuronyltransferase *in vitro*, was ineffective at 0.1% and inhibitory at 1%.

With mammalian foetal liver, phenobarbital-enriched media allowed slightly, but consistently, faster development of UDP-glucuronyltransferase activity towards both *o*-aminophenol and bilirubin. J. Fog & A. Bakken (see Bakken, 1969) suggest that bilirubin hastens development of its UDP-glucuronyltransferase activity in foetal liver; bilirubin added to media could enhance the enzyme activity of cultured foetal liver towards bilirubin (but not *o*-aminophenol), but increases so far obtained are small and poorly reproducible.

Tissues cultured with and without phenobarbital differ strikingly to the eye. Microscopically, phenobarbital delays invasion by fibroblasts and increases endoplasmic reticulum, consistent with prolonged and intensified UDP-glucuronyltransferase activity. Phenobarbital maintains protein in cultured liver, and further results indicate that induction of UDP-glucuronyltransferase in cultures requires protein synthesis. Whether barbiturates generally increase microsomal enzyme availability or produce more specific inducers of UDP-glucuronyltransferase is being investigated. The method appears convenient for such studies.

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Observations on the Biosynthesis of Thioglucuronides and Thioglucosides in Vertebrates and Molluscs

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We have previously described the biosynthesis of diethyldithiocarbamate *S*- β -glucuronide by mouse liver UDP-glucuronyltransferase and its hydrolysis by β -glucuronidase (Dutton & Illing, 1969). We now report work on another thio linkage and with further species and tissues.

The carbodithioic link in diethyldithiocarbamate *S*-glucuronide proved labile during the assay procedure. With thiophenol as substrate, an *S*-

glucuronide was formed whose thiol linkage to glucuronic acid appeared stable enough to permit more quantitative investigation.

Thiophenol was incubated with mouse liver homogenate and UDP-[¹⁴C]glucuronic acid, ethanol was added and the supernatant was chromatographed. Phenol was the substrate in parallel experiments. One rapidly moving radioactive spot was obtained from the thiophenol incubation mixtures, travelling like phenyl glucuronide in two solvent systems. The former spot, unlike phenyl glucuronide, contained organically bound bivalent or quadrivalent sulphur by the Raney nickel-lead acetate test (Feigl & Haguenaue-Castro, 1962); the spot was not due to thiophenol and appeared only when thiophenol had been incubated with UDP-glucuronyltransferase and UDP-glucuronic acid. This supposed conjugate was hydrolysed by β -glucuronidase, leaving glucuronic acid; glucarolactone prevented this hydrolysis.

Assuming negligible formation of phenol from thiophenol during 15 min incubation, comparable amounts of *S*- and *O*-glucuronides were synthesized by mouse liver homogenates. The two UDP-glucuronyltransferase activities further resemble each other in intracellular and tissue location, in pH-activity curves and in activation by UDP-*N*-acetylglucosamine. Competition studies will be reported.

S-Glucuronides are synthesized by UDP-glucuronyltransferase in vertebrate liver and kidney, but not in tissues of *Arion ater*, where the corresponding *S*-glucosides were formed from UDP-glucose; no *S*-glucosides were formed in mammalian tissues. This parallels the findings with *O*-glucosides (Dutton, 1966). After incubation of diethyldithiocarbamate and thiophenol with UDP-[¹⁴C]glucose in *Arion* gut homogenates at pH 8.5 their *S*-glucosides were identified similarly to the *S*-glucuronides. Unlike phenyl *O*-glucoside, thiophenyl *S*-glucoside resisted hydrolysis by almond emulsin β -glucosidase or by acid, confirming the results obtained by Pigman (1941); diethyldithiocarbamate *S*-glucoside was labile (see Kasander, 1966), yielding glucose on acid hydrolysis. *Arion* gut slices, but not homogenates, form *S*-glucosides from [¹⁴C]glucose.

As the *S*-glucuronides and *S*-glucosides were formed by sliced tissue the respective UDP-glycosyltransferase pathways presumably operate *in vivo*. Glucuronides were not evident with cysteine or glutathione as substrates.

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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