was in a metabolite, as yet unidentified, the remainder being unchanged salbutamol. The urinary-excretion findings showed that the drug was well absorbed, 80% of the dose appearing in the urine (0-72h) and 6% in the faeces. Unchanged salbutamol accounted for 40% of urinary radioactivity, 60% consisting of the same metabolite as that found in plasma.

Administration of [³H]salbutamol by aerosol resulted in a similar metabolic pattern in the plasma and urine of patients receiving the drug. The metabolite is an acid-labile compound that is not hydrolysed by β -glucuronidase or arylsulphatase. Investigations so far have shown this metabolite to be unique to man.

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Formation of Epoxides as Microsomal Metabolites of Polycyclic Hydrocarbons

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Microsomal metabolism of polycyclic hydrocarbons *in vitro* leads to the formation of reactive intermediates (Grover & Sims, 1968; Gelboin, 1969) that could be epoxides. Evidence supporting the suggestion (Boyland, 1950) that epoxides are intermediates in the microsomal hydroxylation of double bonds has been obtained with benzene and naphthalene (Jerina, Daly, Witkop, Zaltzman-Nirenberg & Udenfriend, 1968, 1970) and with phenanthrene and dibenz[*a,h*]anthracene (Pandov & Sims, 1970). Synthetic 'K-region' epoxides of polycyclic hydrocarbons are active alkylating agents that react with nucleic acid either *in vitro* (Grover & Sims, 1970) or in cells in culture (Grover, Forrester & Sims, 1971a). In view of the activity of such epoxides in transforming rodent cells in culture (Grover *et al.* 1971b), it seemed important to establish the formation of epoxides as intermediates in the metabolism of polycyclic hydrocarbons.

In the present work ³H-labelled samples of phenanthrene, benz[*a*]anthracene and dibenz[*a,h*]anthracene have been incubated with rat liver microsomal fraction fortified with an NADPH-generating system. After incubation, non-polar products were extracted into ether, the appropriate carrier 'K-region' epoxide was added, the ether was removed by evaporation and the residue was chromatographed on an alumina column. Fractions

containing the radioactive peak that was eluted with the carrier epoxide were pooled and evaporated to dryness, and the epoxide, together with a further addition of unlabelled carrier epoxide, was crystallized from cyclohexane. Radioactively labelled 'K-region' epoxides obtained in this way have been crystallized to constant specific radioactivity and the associated radioactivity has been shown to be convertible (a) by treatment with methanolic hydrochloric acid into products that possess on thin-layer chromatograms the chromatographic properties of phenols, (b) by incubation with microsomal epoxide hydrazase, in the absence of cofactors, into products that possess on thin-layer chromatograms the chromatographic properties of dihydrodiols and (c) by heating with GSH in aqueous acetone at alkaline pH into water-soluble products that possess the paper-chromatographic properties of acid-labile glutathione conjugates. In addition, the only 'non-K-region' epoxide prepared to date, benz[*a*]anthracene 8,9-epoxide, has characteristics (P. Sims, unpublished work) that readily distinguish it from the 'K-region' derivative used in these studies. The role of epoxides in the biological effects produced by polycyclic hydrocarbons, which include carcinogenesis, is currently under investigation.

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Enzymic Formation of β -D-Monoglucuronide, β -D-Monoglucoside and Mixtures of β -D-Monoxylsides and β -D-Dixylsides of Bilirubin by Microsomal Preparations from Rat Liver

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Glucosyl transfer from UDP-glucose to several steroids by microsomal preparations from rabbit liver has been demonstrated by Collins, Williamson

& Layne (1970). Demonstration of bilirubin glucoside in dog bile (Heirwegh, Van Hees, Compennolle & Fevery, 1970) stimulated attempts to synthesize *in vitro* xylose and glucose conjugates of bilirubin.

By incubating microsomal preparations from rat liver with bilirubin and UDP-glucuronic acid, UDP-xylose or UDP-glucose in the presence of Mg^{2+} transfer of the sugar moieties to bilirubin was obtained. Reaction rates were measured quantitatively by treatment of the conjugated pigments with diazotized ethyl anthranilate (Van Roy & Heirwegh, 1968). Analysis of the derived azopigments (Heirwegh, Van Hees, Leroy, Van Roy & Jansen, 1970) allowed structural elucidation of the synthesized bilirubin conjugates. The same types of conjugates were formed by using either digitonin-activated or untreated enzyme preparations.

In the presence of either UDP-xylose or UDP-glucose conjugate formation was optimum at pH 7.1. Digitonin treatment increased reaction rates by the factor 2.1. Conservation of the sugar moieties was indicated by t.l.c. with known preparations of azobilirubin β -D-xylopyranoside and of azobilirubin β -D-glucopyranoside. Enzymic hydrolysis of the conjugates with emulsin and Mylase-P together with inhibition studies with glucono-(1 $\rightarrow$ 5)-lactone demonstrated that the conjugates derived from both UDP-sugars are in the β -D-configuration.

With UDP-glucose bilirubin β -D-monoglucoside was formed under a variety of reaction conditions. With UDP-xylose mixtures of β -D-monoxylsides and β -D-dixylsides were obtained. Formation of the dixylsides was favoured by, e.g., lowering the pH of the incubation mixtures.

Previous studies of bilirubin UDP-glucuronyl-transferase with digitonin-activated (Heirwegh & Meuwissen, 1968) and untreated preparations (Van Roy & Heirwegh, 1968) were completed. Enzyme activities were increased 5–15-fold by digitonin treatment and were optimum at pH 7.9. Under all conditions bilirubin β -D-monoglucuronide was formed, as indicated by t.l.c. of derived azopigments, with azobilirubin and azobilirubin β -D-monoglucuronide as reference substances (Compennolle, Jansen & Heirwegh, 1970). The β -D-configuration of the synthesized conjugate was indicated by complete hydrolysis of the derived conjugated azopigment with β -glucuronidase and complete inhibition of the process by saccharo-(1 $\rightarrow$ 4)-lactone.

Relative values of the transferase activities found with UDP-glucuronic acid, UDP-xylose and UDP-glucose were 1:2.1:1.4 for untreated microsomal preparations and 1:0.38:0.25 after activation with digitonin.

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The Incorporation of Glycylglycine into Humic Acid

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A large proportion of the nitrogen present in soil humic acid can be released as amino acids by acid hydrolysis (Bremner, 1955). Of the remainder, it is probable that some exists as α -amino nitrogen. The association between α -amino nitrogen and aromatic units within humic acid has remained a problem, although Haider, Frederick & Flaig (1965) and Ladd & Butler (1966) have presented possible solutions.

We have investigated the reaction between humic acid and specifically radioactively labelled glycylglycine. The humic acid was extracted from the A_0 horizon of a Hiraethog podzol (Ball, 1960) with 0.1 M-sodium hydroxide. [$1^{14}C$]Glycylglycine and glycyl[$1^{14}C$]glycine were each allowed to react with humic acid in sodium borate-HCl buffer, pH 8.5. The humic acid products of each reaction were radioassayed and subjected to hydrolysis with 6M-hydrochloric acid for 24h at 110°C. The amino acids released on hydrolysis were separated by ion-exchange chromatography from the non-hydrolysable residue and non-cationic acid-soluble material.

The specific radioactivities of the humic acid products of each reaction before hydrolysis differed by 9%. The non-hydrolysable fraction of humic acid that had been allowed to react with [$1^{14}C$]glycylglycine contained 11.3% of the total specific radioactivity incorporated. The radioactivity within this non-hydrolysable fraction was eight times that in the same fraction of humic acid that had been allowed to react with glycyl[$1^{14}C$]glycine.

The results indicate that the dipeptide became bound to the humic acid and that a proportion of the radioactivity in glycylglycine was not hydrolysable. The *N*-terminal glycine residue was considerably more resistant to hydrolysis than was