but it may be involved in the incorporation of sulphated tyrosine residues into proteins. Since free L-tyrosine *O*-sulphate is not incorporated into protein and in view of the specificity of this sulphotransferase for derivatives of L-tyrosine having a modified carboxyl group and a free amino group, it has been suggested that the sulphation of certain tyrosine residues may occur at the L-tyrosyl-tRNA stage of protein biosynthesis (Mattock, Barford, Basford & Jones, 1970).

When a crude preparation of sulphotransferases from rat livers is purified by chromatography on DEAE-cellulose at pH 8.5 and by isoelectric focusing, it yields three enzyme fractions with pI values 6.6, 5.8 and 5.3 and all possessing L-tyrosine methyl ester sulphotransferase activity. The last-mentioned enzyme fraction is the major component of the mixture and corresponds to enzyme B described previously (Barford & Jones, 1970). An enzyme preparation containing all three of these isoenzymes fails to catalyse the transfer of sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to L-tyrosyl-tRNA from rat livers. Again a suitably fortified enzyme preparation from rat livers failed to synthesize the *O*-sulphate ester of L-tyrosyl-tRNA under conditions that allowed the sulphation of L-tyrosine methyl ester and the synthesis of L-tyrosyl-tRNA.

Rat liver sulphotransferases catalyse an exchange of sulphate between adenosine 3'-phosphate 5'-sulphatophosphate and *p*-nitrophenyl sulphate or tyramine *O*-sulphate, but not the L-tyrosine *O*-sulphate residues of rat fibrinogen. An investigation of a large number of compounds related to L-tyrosine confirms the narrow specificity of these sulphotransferases for compounds with a free amino group and a modified carboxyl group. The best substrates were found to be L-tyrosine amide, L-tyrosine methyl ester, tyramine and 5-hydroxytryptamine (serotonin). Mixed-substrate experiments indicate that the three last-named compounds are all substrates for the same sulphotransferase.

It therefore appears unlikely that L-tyrosine methyl ester sulphotransferase is involved in the biosynthesis of proteins containing L-tyrosine *O*-sulphate residues, but it is probably important in the sulphation of physiologically active amines. These results are noteworthy in view of the demonstration (Hidaka, Nagatsu, Takeya, Matsumoto & Yagi, 1969) that the sulphation of 5-hydroxytryptamine diminishes its effect on isolated aorta and of the finding (Smith, Kellow, Mullen & Hanington, 1971) of a correlation between a diminished excretion of tyramine *O*-sulphate and susceptibility to dietary migraine in humans.

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The Fate of the Sulphate Esters of Diethylstilboestrol in the Rat

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Previous work in these laboratories on the metabolism and modes of excretion of a number of aryl sulphate esters has shown that only those aryl sulphates that are further conjugated with glucuronic acid appear in the bile to any significant extent (Hearse, Powell, Olavessen & Dodgson, 1969). These findings are consistent with current ideas on the molecular properties that render compounds suitable for biliary elimination (Millburn, 1970), for although the aryl sulphate esters satisfied the anionic and lipophilic requirements it was only after further conjugation with glucuronic acid that the molecular-weight requirement was satisfied.

Studies on the excretion of aryl sulphate esters have been extended to include investigations with diethylstilboestrol disulphate and diethylstilboestrol monosulphate, since both compounds satisfy the criteria for biliary elimination without further metabolic modification.

Mature free-ranging male and female rats received injections (either intraperitoneal or intravenous) of diethylstilboestrol disulphate (1mg/200g body wt.), and urine and faeces were collected over 48h. Of the administered radioactivity 55–80% was recovered in the urine as inorganic [³⁵S]-sulphate. Between 5 and 15% of the radioactive dose appeared in the faeces. In other experiments wholly anaesthetized rats with bile-duct and ureter cannulae received intravenous injections of diethylstilboestrol disulphate (1mg/200g body wt.). Up to 52% of the injected radioactivity was recovered in the urine in 6h as inorganic [³⁵S]sulphate, and significant amounts (12–28%) were eliminated in the bile as ester sulphate. Only traces (less than 1%) of unchanged parent ester were detected in the bile, and the major metabolite in bile was identified as diethylstilboestrol monosulphate monoglucuronide.

When diethylstilboestrol [³⁵S]monosulphate (1mg/200g body wt.) was administered to anaesthetized rats with bile-duct and ureter cannulae, between 45 and 60% of the radioactivity was recovered in the bile within 6h. The major metabolite

in bile (up to 58% of the dose) was diethylstilboestrol monosulphate monoglucuronide, and only traces (up to 2%) of parent ester were detected. Between 20 and 30% of the injected radioactivity was present in the urine after 6h as inorganic [^{35}S]sulphate.

Experiments with isolated perfused rat livers identified the liver as the major site of metabolism of both diethylstilboestrol disulphate and diethylstilboestrol monosulphate. Other experiments *in vitro* showed that both esters are desulphated by microsomal sulphohydrolase activity. Thus, in spite of the apparent suitability of diethylstilboestrol disulphate for elimination via the biliary route, this does not occur to any significant extent, perhaps owing to the activity of the desulphating system of the liver. Although diethylstilboestrol monosulphate is desulphated to some extent in the liver it would appear that the availability of a free phenolic hydroxyl group, for conjugation with glucuronic acid, allows the preferential formation of a double conjugate. The considerable biliary excretion of radioactivity after the administration of diethylstilboestrol [^{35}S]monosulphate is therefore explicable in these terms.

Although both diethylstilboestrol monosulphate and diethylstilboestrol disulphate possess physicochemical properties that, on theoretical grounds, render them suitable for biliary elimination, only traces of unchanged parent ester appear in the bile in either case.

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Mercapturic Acid Formation from Sodium Alkyl Sulphates in the Rat

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The injection of sodium benzyl sulphate and sodium 1-menaphthyl sulphate (sodium naphth-1-ylmethyl sulphate) into rats is followed by the excretion of benzylmercapturic acid and 1-menaphthylmercapturic acid respectively in the urine of the dosed animals (Clapp & Young, 1970). Similarly, sodium allyl sulphate gives rise to the excretion of allylmercapturic acid (Kaye & Young, 1970). In the present work the possibility that sodium

alkyl sulphates also give rise to formation of mercapturic acids in the rat has been investigated.

The acid sulphate esters of the alcohols in the series methanol to decan-1-ol were prepared by the action of concentrated sulphuric acid on the alcohol at -5°C (Lloyd, Tudball & Dodgson, 1961) and were separated as their sodium salts. These, and also sodium *n*-dodecyl sulphate, were injected subcutaneously into male rats, and urine extracts were examined chromatographically for the corresponding alkylmercapturic acids. No such metabolites were detected for sodium methyl sulphate and sodium ethyl sulphate, whereas sodium *n*-propyl sulphate appeared to give rise to traces of *n*-propylmercapturic acid. Significant amounts of *n*-butyl-, *n*-pentyl- and *n*-hexyl-mercapturic acid were detected as metabolites of the corresponding sulphates, but sodium *n*-heptyl sulphate gave rise to only a very small amount of *n*-heptylmercapturic acid. Very little of the corresponding alkylmercapturic acid was detected after administration of sodium *n*-octyl, *n*-nonyl, *n*-decyl and *n*-dodecyl sulphate.

After these preliminary studies, *n*-butylmercapturic acid, as the free acid and as the dicyclohexylammonium salt, was isolated from the urine of rats dosed with sodium *n*-butyl sulphate. The amount isolated corresponded to 0.8% of the administered compound. After injection of sodium *n*-pentyl sulphate and sodium *n*-hexyl sulphate into rats, *n*-pentyl- and *n*-hexyl-mercapturic acid were isolated from the urine in amounts corresponding to 1.0% and 1.1% respectively of the sulphate administered. No mercapturic acid was detected in the urine after the administration of butan-1-ol, pentan-1-ol or hexan-1-ol to rats, and it appears unlikely that these compounds are intermediates in the formation of mercapturic acids from their acid sulphate esters.

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Maternal-Foetal Relationship of Methionine and Cystine in the Pregnant Rhesus Monkey

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We have found that, in the pregnant human, cystine behaves like most plasma amino acids: the maternal plasma concentration decreased during