formation of 2- and 3-hydroxypentylmercapturic acid and two additional compounds, which could be the 4- and 5-hydroxy derivatives. One of these was detected in the urine extract but no peak corresponding to the second was observed. *N*-Acetyl-*S*-(4-carboxybutyl)cysteine, described in the preceding communication (James & Needham, 1970), was, however, detected as a metabolite of 1-bromopentane and may arise by further oxidation of a 5-hydroxy-substituted intermediate.

The preceding communication (James & Needham, 1970) shows that oxidation of the pentyl chain of *S*-pentylcysteine occurs, but it is possible that hydroxylation of 1-bromopentane takes place before the conjugation with glutathione and the subsequent formation of hydroxylated derivatives of pentylmercapturic acid and the further oxidized 4-carboxybutylmercapturic acid. The suitability of 1-bromopentane as a substrate for the liver drug-metabolizing enzymes was examined. 1-Bromopentane reacted with cytochrome *P*-450 prepared from the microsomal fraction of rat liver by the method of Schenkman, Remmer & Estabrook (1967), giving a difference spectrum corresponding to that of a Type 1 substrate. It stimulated the oxidation of NADPH as measured by a decrease in fluorescence *in vitro* in the system described by Das, Orrenius & Ernster (1968). This effect was inhibited by carbon monoxide. The K_m value for pentyl bromide in this system was 2mM, which is of the same order as that for other known substrates. These results suggest that 1-bromopentane may be hydroxylated by the cytochrome *P*-450 system of rat liver.

Pretreatment of rats with phenobarbitone resulted in an increase of at least one-third in the proportion of hydroxymmercapturic acids excreted on dosing with 1-bromopentane.

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The Metabolism of 2-Aminobiphenyl by Rats *in vivo*

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2-Aminobiphenyl, in contrast with 4-aminobiphenyl, lacks carcinogenic activity (Weisburger

& Weisburger, 1966). It was therefore decided to compare the metabolism of these structurally similar compounds to determine if a specific metabolic route could be implicated in carcinogenesis.

Adult male albino rats received daily 2-aminobiphenyl (50mg) intraperitoneally until 5g had been administered. Urine was collected and metabolites were concentrated by adsorption on to charcoal followed by elution with methanolic NH_3 . The concentrate was chromatographed by using a cellulose column (65cm $\times$ 6.5cm) eluted with butan-1-ol-cyclohexane-2M- NH_3 (9:2:1, by vol.). The polarity of the eluent was increased during the chromatography. Paper chromatography was used to monitor the elution of the metabolites from the column. Diazotization followed by coupling with alkaline 2-naphthol was used to locate the unchanged amine and conjugated aminophenols.

Fractions with similar chromatographic characteristics were bulked for isolation experiments. In addition to a small amount of unchanged 2-aminobiphenyl, the following metabolites were isolated from the 5g of amine administered: 2-amino-3-biphenyl sulphate (300mg), 2-amino-5-biphenyl sulphate (1600mg) and 2-amino-5-biphenyl glucosiduronate (75mg). The metabolites were characterized, by having chromatographic properties identical with the metabolites obtained after the corresponding aminophenols were administered to rats, or by chemical analysis or specific tests.

Examination by g.l.c. or t.l.c. of an ethereal extract of urine from rats that had received 2-aminobiphenyl failed to provide evidence for the formation of 2-hydroxylaminobiphenyl or 2-nitrosobiphenyl as metabolites. Consistent with this was the lack of methaemoglobinaemia, during the period when the rats were receiving 2-aminobiphenyl, which would have indicated the presence of *N*-hydroxylated metabolites in the blood. No metabolite was detected in which the amino group was conjugated with either acetic acid, sulphuric acid or glucosiduronic acid. No mercapturic acids, glutathione conjugates or their precursors were detected as metabolites.

4-Aminobiphenyl is metabolized by hydroxylation at position 3 or 4' on the nucleus (Bradshaw & Clayson, 1955) or on the nitrogen atom. All three metabolites have been shown to be carcinogenic (Gorrod, Carter & Roe, 1968). The present results, taken with those of Radomski & Brill (1970) on the comparison of the metabolism of 1- and 2-aminonaphthalene, indicate that the absence of *N*-hydroxylation of 2-aminobiphenyl by rats could account for the lack of carcinogenicity of this compound in rats. However, the failure to detect 2-amino-3-biphenyl glucosiduronate as a metabolite

may implicate this type of metabolite as suggested by Clayson, Jull & Bonser (1958) and Boyland (1963).

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Mercapturic Acid Formation from Allyl Compounds in the Rat

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In an earlier investigation (Clapp, Kaye & Young, 1969) the urine of rats that had been injected subcutaneously with allyl chloride was shown to contain allylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine, together with 3-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine. Examination of the urines of rats that had been dosed with allyl alcohol and with allyl acetate revealed that they contained 3-hydroxypropylmercapturic acid, but no evidence was found for the presence of allylmercapturic acid.

In the present work allylmercapturic acid was isolated from the urine of rats dosed with *S*-allyl-L-cysteine in an amount corresponding to 16.3% of the injected compound and was also shown to be excreted by rats after the injection of allyl bromide and allyl iodide. It was also possible to isolate crotylmercapturic acid from the urine of rats injected with crotyl chloride.

A further study of the metabolism of allyl alcohol led to the isolation of 3-hydroxypropylmercapturic acid as its dicyclohexylammonium salt from the urine of the dosed rats in an amount that represented 6.3% of the administered alcohol. No evidence was obtained for the excretion of allylmercapturic acid by these animals. As had been reported earlier for allyl acetate, other carboxylic acid esters of allyl alcohol, namely allyl propionate, allyl stearate and allyl benzoate, were found in the present work to give rise to formation of 3-hydroxypropylmercapturic acid, but not to allylmercapturic acid.

Some esters of allyl alcohol do give rise to the biosynthesis of allylmercapturic acid, however, for

it was found that from the urine excreted in the 24 h period after injection of triallyl phosphate into rats it was possible to isolate allylmercapturic acid in an amount corresponding to 3.9% of the administered compound. In a similar experiment in which sodium allyl sulphate was injected into rats allylmercapturic acid corresponding to 4.0% of the administered compound was isolated from the urine. It is noteworthy that two other sulphates, namely sodium benzyl sulphate and sodium 1-menaphthyl sulphate, have recently been shown to give rise to formation of benzylmercapturic acid and 1-menaphthylmercapturic acid in rats (Gillham, Clapp, Morrison & Young, 1970).

These various observations were discussed in the light of current views concerning the mechanism for the biosynthesis of mercapturic acids.

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The Induction of Hepatic Microsomal Enzymes by Safrole

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It is well known that administration of a number of drugs and other nutrient chemicals to animals may result in hypertrophy of the liver and an increase in the activities of various hepatic enzymes (Conney, 1967). This has also been shown to occur with certain natural nutrients, e.g. β -ionone, which, as other terpenoids, gives rise to induction of the hepatic microsomal drug-metabolizing enzymes (Parke & Rahman, 1969). Certain methylenedioxyaryl pesticides, inhibitors of the hepatic microsomal oxygenases, stimulate the rat hepatic microsomal enzymes that catalyse the 2-hydroxylation of biphenyl but inhibit those that catalyse the 4-hydroxylation of this substrate (Jaffe, Fujii, Guerin, Sengupta & Epstein, 1969).

Safrole (4-allyl-1,2-methylenedioxybenzene), an nutrient that occurs naturally in many essential oils, e.g. sassafras, and is present in the flavourings of root beer and sarsaparilla, has now been shown to produce in the rat liver hypertrophy accompanied by marked increases in a number of the hepatic microsomal enzymes including biphenyl hydroxylase, nitro reductase, glucuronyltransferase and cytochrome *P*-450. The addition of safrole to rat liver microsomal preparation *in vitro* did not result in any similar increase in enzyme activities. The increase in activity of biphenyl 4-hydroxylase,