Following these observations an examination of the effect of digitonin on the activity of glucuronyl-transferase with bilirubin as acceptor (Van Roy & Heirwegh, 1968) was made. Microsomal preparations (final protein concn. 1.2-1.6 mg./ml.) from rat liver were treated at 0° for 30 min. with various digitonin concentrations between 0 and 8.2 mg./ml., and were then assayed for glucuronyltransferase activity. By increasing the digitonin concentration it was possible to raise the enzyme activity to a maximal value which was 5 to 7 times that found with untreated microsomes. The preparations were fully activated at detergent-protein ratios between 2.2 and 6.4.

After standing for 30 min. at 0° the microsome-digitonin mixtures were separated into the corresponding sediment and supernatant fractions by centrifuging at 4° for 72 min. at 80000g_{av.}. The transferase activities of fully activated microsomes and of the derived sedimentable and non-sedimentable fractions were stable for at least 24 hr. at 4°. The sum of the activities of the latter two fractions amounted to 65-80% of the value found for the corresponding microsome-detergent mixtures. However, the initial activity could be restored by re-suspending the sediments in the corresponding supernatant solutions. In the solubilized fraction 20-30% of the activity was recovered.

Chromatographic analysis of the azopigments derived from conjugated bilirubin produced *in vitro* by enzymic incubation of microsomal preparations showed that azopigments A and G were formed in equal amounts (Van Roy & Heirwegh, 1968). Identical results were obtained with digitonin-treated microsomes and with the derived fractions.

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The Fate of 4,5- and 5,6-Dichloro-2-trifluoromethylbenzimidazole in the Rat and Rabbit

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Various derivatives of 2-trifluoromethylbenzimidazole possess antibacterial, antiviral, herbicidal or insecticidal activity (Bishop, Chelton & Jones, 1964; Fara & Cochran, 1963; Burton *et al.* 1965) and some are potent uncouplers of oxidative phosphorylation (Jones & Watson, 1967).

We have studied the fate of orally administered 4,5- and 5,6-dichloro-2-trifluoro[¹⁴C]methylbenzimidazole in the rat and rabbit. Both compounds were more toxic to rabbits than to rats. In the rat (dose, 50 mg./kg.), 82%, and in the rabbit (dose, 6 mg./kg.), 79%, of the ¹⁴C of the 4,5-isomer was excreted in the urine in 3 days. None of the compound was excreted unchanged. The urine of the first day was examined for metabolites by paper, thin-layer and gas chromatography and by comparison with synthetic reference compounds. The urines contained after acid hydrolysis, 6-hydroxy- (25, 11%), 7-hydroxy- (27, 8%) and 6,7-dioxo-4,5-dichloro-2-trifluoromethylbenzimidazole (28, 20%), the figures in parentheses being the % of the dose excreted as these metabolites by the rat and the rabbit respectively. These metabolites were excreted mainly as glucuronic acid or sulphate conjugates or both, and it appears that the 6,7-quinone is formed, during the hydrolysis, from the corresponding 6,7-diol.

The excretion pattern of the 5,6-isomer was different from that of the 4,5-isomer. In the rat (dose 33 mg./kg.), 33% of the ¹⁴C appeared in the urine and 28% in the faeces in 3 days, but in the rabbit (dose, 3.3 mg./kg.) in the same time only 10% appeared in the urine and none in the faeces. In 5 days after the dosing only 24% of the dose of ¹⁴C had been excreted by the rabbit and the remainder was found distributed evenly in the tissues. Only traces of the original compound were excreted by both species. The urine of the first 24 hr. after the dosing in the rat and of the first 5 days in the rabbit was examined for metabolites. There were identified as 5,6-dichloro-2-trifluoromethylbenzimidazole-1-glucuronide (6, 9%) and the glucuronic acid or sulphate conjugates (or both) of the 4-hydroxy (9, 2%) and 4,7-dihydroxy (4, 15%) derivatives of the 5,6-isomer (the figures in parentheses are the % of dose in the rat and the rabbit respectively). The 4,7-dihydroxy derivative was detected after acid hydrolysis as the corresponding 4,7-quinone.

Although both compounds are metabolized mainly by aromatic hydroxylation at the two unsubstituted carbon atoms there is a marked

difference in the rate of elimination of the metabolites. These findings will be discussed.

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The Conversion of *S-n*-Propyl-L-Cysteine into its Sulphoxide by Microsomal Preparations from Rat Liver

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The oxidation of thioethers to sulphoxides has been observed in biological systems (e.g. Salzman & Brodie, 1956; Burns *et al.* 1957). The sulphoxidation of 4,4'-diaminodiphenyl sulphide and chlorpromazine in the presence of guinea-pig liver microsomes was found to require NADPH and oxygen (Gillette & Kamm, 1960).

In the present work, microsomes were prepared from the livers of male Wistar albino rats (approx. 250g.) and were suspended in freshly oxygenated 0.25M-sucrose at a concentration of approx. 3mg. of N/ml. Incubations were performed in open flasks at 37° for 30min. with *S-n*-propyl-L-[³⁵S]-cysteine as substrate, EDTA (3mM) and either an NADPH-generating system (NADP⁺, glucose 6-phosphate and glucose 6-phosphate dehydrogenase) or NADH and CN⁻ (7.5mM). Samples of the reaction mixtures were pipetted directly on to Whatman no. 3 paper and were subjected to high-voltage electrophoresis at 100v/cm. in a formic acid-acetic acid mixture, pH 2.0. The electrophoretograms were scanned for radioactivity and the sulphoxide present was determined as a percentage of the total radioactivity measured on the scan.

The extent of conversion into sulphoxide was influenced by the concentrations of substrate and microsomal protein, but under comparable conditions, the following yields were obtained: with an NADPH-generating system, 160μmoles of sulphoxide/mg. of N/30min; with NADH-CN⁻, 340μmoles of sulphoxide/mg. of N/30min. The inclusion of CN⁻ (7.5mM) in the reaction mixture containing the NADPH-generating system gave a 50% increase in the yield of sulphoxide.

Sulphoxidation was not significantly inhibited by SKF 525A (0.4mM), sodium azide (3.0mM), 2,4-dichlorophenol (0.85mM) or *p*-chloromercuribenzoate (0.25mM), but 100% inhibition was observed with dimethyl-*p*-phenylenediamine (2.0mM) and 90% inhibition with CO.

Paper chromatography of the *S-n*-propyl-L-[³⁵S]-cysteine sulphoxide in butan-1-ol-water-acetic acid, (12:3:5, by vol.) showed that *S-n*-propyl-L-[³⁵S]cysteine (+)sulphoxide was the predominant diastereoisomer formed. This is consistent with the isolation of *S*-ethyl-L-cysteine (+)sulphoxide from the urine of rats dosed with bromoethane (Barnsley, Thomson & Young, 1964).

Evidence so far indicates that the present system that converts *S-n*-propyl-L-cysteine into its sulphoxide differs from the sulphoxidation system reported by Gillette & Kamm (1960). The possible roles of EDTA and the inhibitory effect of CO will be discussed.

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Genetic Variation of Cod and Coalfish Lactate Dehydrogenase

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Genetic variations (polymorphism) can be a useful tool in the study of the sub-unit composition of enzymes. This is particularly true when, as in the lactate dehydrogenase (LDH) of many fish species, a complex mixture of isoenzymes is present.

In cod (*Gadus morrhua*) a polymorphism of one LDH polypeptide can be demonstrated by starch-gel electrophoresis of tissue extracts from fish caught off the Atlantic coast of Canada (Odense, Leung & Allen, 1966) and also in fish caught within 40 miles of Aberdeen. The polymorphic polypeptide, which is abundant in the heart, is referred to as the H polypeptide and the corresponding genetic locus as *LDH_H*. The frequency of the more common allele in 184 of the Aberdeen cod was found to be 0.61. There was a slight but significant excess of homozygotes in this sample.

In coalfish (*Gadus virens*) obtained from fishing grounds off the west coast of Scotland, and also from Icelandic and Faroese waters, we have found a polymorphism of a different polypeptide, M, which occurs mainly in the skeletal muscle. The frequency