The work was supported by a grant from Roche Products Ltd.

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The Enzymic Deacetylation of *p*-Acetamidobenzoic Acid

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Earlier work (Bridges & Williams, 1963) suggested that the enzymic deacetylation of *N*⁴-acetyl-sulphanilamide varied with tissue and species. The deacetylation of *p*-acetamidobenzoic acid (AABA) *in vitro* shows similar variations since it has been found in homogenates of rat, guinea-pig and mouse kidney and guinea-pig and mouse liver, but only slight activity was found in rat and rabbit liver and rabbit kidney. The activity when found was similar in both sexes and occurred in the cell-sap fraction (105 000g supernatant).

The AABA deacetylase activity in the rat was found only in the kidney. The activity was considerably increased (150%) by Mn²⁺, but not by other metal ions (Mg²⁺, Ca²⁺, Fe²⁺, Na⁺, K⁺) or a wide range of common cofactors. The effect of Mn²⁺ on the mouse and guinea-pig kidney enzyme was less marked (50 and 30% respectively). The optimum Mn²⁺ concentration was 7.5 mM for the rat kidney enzyme with an optimum pH range of 7.5–8.0. This enzyme preparation did not deacetylate *N*⁴-acetylsulphanilamide, but acetanilide was deacetylated to some extent, although the main acetanilide-deacetylating activity was associated with the microsomes.

The rat kidney enzyme showed an Mn²⁺-dependent and an Mn²⁺-independent activity. Storage of the enzyme preparation (10000g supernatant) at 4° for 4–5 days (alone or in the presence of nicotinamide, glutathione or added Mn²⁺) destroyed the Mn²⁺-dependent activity, but the Mn²⁺-independent activity remained constant. *p*-Chloromercuribenzoate also destroyed the Mn²⁺-dependent activity, but did not affect the activity of the preparation that had been stored at 4° for 5 days. CoA, thioglycollate, Hg²⁺, Cu²⁺ and Zn²⁺ inhibited both the Mn²⁺-dependent and Mn²⁺-independent activity.

These and other observations suggest that the rat kidney AABA deacetylase may possess either

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two active sites, one requiring Mn²⁺ for its activity, or a Mn²⁺-binding site that causes allosteric activation of the active site. The Mn²⁺ binding or activated site is much more susceptible to inactivation than the original active site.

This work was supported by a grant from the Ministry of Defence.

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Observations on the Metabolism of Allyl Compounds in the Rat

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1-Menaphthylmercapturic acid has been isolated from the urine of rats after the injection of various 1-menaphthyl compounds including 1-menaphthyl acetate and 1-menaphthyl benzoate (Hyde & Young, 1968), and these appear to be the first examples of mercapturic acid formation arising from the administration of carboxylic acid esters to animals. It is generally accepted that biosynthesis of mercapturic acids takes place by the mechanism proposed by Barnes, James & Wood (1959) and Bray, Franklin & James (1959a,b), whereby the administered compound or an active derivative reacts with glutathione to give an *S*-substituted glutathione, which is subsequently metabolized to the mercapturic acid. As it is known that the esters of allylic alcohols are able to transfer an allylic group to a strongly nucleophilic anion such as a thiol (Albert, 1965) it became of interest to determine whether an ester such as allyl acetate gives rise to a mercapturic acid *in vivo*.

The urine of rats that had been dosed with allyl acetate by subcutaneous injection was shown by paper chromatography and thin-layer chromatography to contain a hydroxypropylmercapturic acid. This compound was identified as 3-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine, by the gas-liquid-chromatographic examination of its methyl ester by the procedure of James, Waring & White (1966). There was no evidence for the presence of allylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine, in the urine of the dosed animals. Similar results were obtained after the administration of allyl alcohol to rats.

Some allyl compounds were found to give rise to allylmercapturic acid in the organism, however, for this compound was isolated from the urine of rats that had been injected subcutaneously with allyl chloride, and by chromatographic examination was

shown to be present in the urine of rats injected with *S*-allylglutathione and *S*-allyl-L-cysteine. The animals dosed with allyl chloride also excreted 3-hydroxypropylmercapturic acid.

The reactions of a number of foreign compounds with glutathione are known to be catalysed by glutathione *S*-transferases. These include glutathione *S*-alkyltransferases (Johnson, 1966) and glutathione *S*-alkenyltransferases (Boyland & Chasseaud, 1967, 1968), which catalyse the reaction of glutathione with alkyl compounds and with $\alpha\beta$ -unsaturated compounds respectively. The observations made in the present work on the metabolism of allyl compounds were considered in the light of knowledge concerning the glutathione *S*-transferases.

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The Effect of Phenobarbitone on the Incorporation of δ -Aminolaevulinic Acid into Rat Liver Microsomes

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Phenobarbitone given in the drinking water causes an increase in the microsomal drug-metabolizing enzyme system including cytochrome *P*-450. Values of cytochrome *P*-450 content rise from 20 ± 3 nmoles/g. in controls to 135 ± 3 nmoles/g. in treated rats. This high value is maintained as long as phenobarbitone is given (Marshall & McLean, 1969). The increased content of cytochrome *P*-450 could be due to increased synthesis (Conney & Gilman, 1963), decreased breakdown (Holtzmann & Gillette, 1968), or a combination of both. In order to study the synthesis and breakdown of microsomal haem, we have given ^{14}C -labelled δ -aminolaevulinic acid (δ -ALA) and measured radioactivity in various liver components as a percentage of the dose given.

Two groups of 150–200g. male rats, one group

having been pretreated with oral phenobarbitone (1mg./ml.) for 20 days, were given $2\mu\text{C}$ of [^{14}C] δ -ALA (specific radioactivity 19mc/m-mole)/100g. by intraperitoneal injection. Rats were killed 4, 8, 24 and 48hr. after injection, and liver microsomes were prepared. Haem was extracted from the microsomal suspension with acid-acetone and its specific radioactivity found.

Of the administered dose of δ -ALA, 16% was incorporated into the livers of both groups by 4hr. After 48hr. the activity had fallen to 6% and 8% for control and phenobarbitone-pretreated groups respectively. In the phenobarbitone-pretreated group, essentially all the activity was found to be in the microsomal haem. In the control group, however, the radioactivity in the microsomes after 4hr. was 10%, and only half of this was ^{14}C -labelled haem.

Regression analysis of the fall in radioactivity of microsomal haem from 8 to 48hr. gave a half-life of microsomal haem in control animals of 42hr., compared with 45hr. in phenobarbitone-pretreated rats. The contribution to the total microsomal haem radioactivity by cytochrome *b*₅ was not significant. There does not appear to be any gross difference in the rate of breakdown of microsomal haem between control and phenobarbitone-pretreated groups. This suggests that the inductive effect of phenobarbitone is not mediated by a decrease in the rate of breakdown.

This work has been supported by Grants from the British Empire Cancer Campaign and the U.S. Public Health Service.

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Destruction of Reduced Nicotinamide-Adenine Dinucleotide Phosphate by Bromotrichloromethane and by Carbon Tetrachloride *in vitro* and *in vivo*

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The administration of CCl_4 to rats results in severe centrilobular necrosis and fatty degeneration of the liver. Numerous biochemical changes occur during the first 1hr. of poisoning (for references see Slater, 1966), and some of these are related to the development of necrosis (Slater & Sawyer, 1969). One such early change is the decrease in total liver