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.

Albert, A. (1965). *Selective Toxicity*, 3rd ed., p. 287. London: Methuen and Co.

Barnes, M. M., James, S. P. & Wood, P. B. (1959). *Biochem. J.* **71**, 680.

Boyland, E. & Chasseaud, L. F. (1967). *Biochem. J.* **104**, 95.

Boyland, E. & Chasseaud, L. F. (1968). *Biochem. J.* **109**, 651.

Bray, H. G., Franklin, T. J. & James, S. P. (1959a). *Biochem. J.* **71**, 690.

Bray, H. G., Franklin, T. J. & James, S. P. (1959b). *Biochem. J.* **73**, 465.

Hyde, C. W. & Young, L. (1968). *Biochem. J.* **107**, 519.

James, S. P., Waring, R. H. & White, D. A. (1967). *J. Chromat.* **26**, 255.

Johnson, M. K. (1966). *Biochem. J.* **98**, 44.

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.

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Conney, A. H. & Gilman, A. G. (1963). *J. biol. Chem.* **238**, 3682.

Holtzmann, J. L. & Gillette, J. R. (1968). *J. biol. Chem.* **243**, 3020.

Marshall, W. J. & McLean, A. E. M. (1969). *Biochem. Pharmacol.* **18**, 153.

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

NADP + NADPH₂ (Slater, Sträuli & Sawyer, 1964); the mechanism underlying this decrease has been studied in this investigation.

At 1–2 hr. after dosing with CCl₄ there is no change in the total content of ATP in the liver or in total liver NAD + NADH₂, which are substrates for the NAD kinase reaction; NAD kinase is slightly increased in activity at this period of poisoning. These results suggest that there is no failure in the synthesis of NADP during the first 2 hr. of poisoning with CCl₄, but this interpretation is complicated by intracellular shifts of ATP and the nicotinamide-adenine dinucleotides during this period.

The decrease in total liver NADP + NADPH₂ produced by CCl₄ *in vivo* is diminished by the concomitant administration of various free-radical scavengers with the CCl₄ (Slater, Sawyer & Sträuli, 1966). This indicates that the breakdown of the nucleotide is homolytic in character. This is further suggested by experiments *in vitro* that show that NADPH₂ is rapidly destroyed by u.v. irradiation in the presence of CBrCl₃ and, to a lesser extent, CCl₄. This rapid degradative reaction can be followed by fluorimetric or enzymic procedures, and is retarded by free-radical scavengers. The possibility will be considered that NADPH₂ is broken down *in vivo* as a result of the homolytic interaction of CCl₄ and the NADPH₂–cytochrome c reductase in the endoplasmic reticulum of rat liver.

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Slater, T. F. (1966). *Nature, Lond.*, **209**, 36.

Slater, T. F. & Sawyer, B. (1969). *Biochem. J.* **111**, 317.

Slater, T. F., Sawyer, B. & Sträuli, U. (1966). *Biochem. Pharmacol.* **15**, 1273.

Slater, T. F., Sträuli, U. & Sawyer, B. (1964). *Biochem. J.* **93**, 260.

The Metabolic Fate of DL-[7-³H]Isoprenaline in Man and Dog

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After intravenous injection of DL-[7-³H]isoprenaline (90 μC, 0.5 μg./kg.) in the dog, plasma radioactivity fell biphasically, the initial rapid fall coinciding with a return of the pulse rate towards normal. Free amine represented about 85% of the total radioactivity, 3-*O*-methyloisoprenaline making up the remainder. The findings were similar in man, intravenous dosage (40 μC, 0.06 μg./kg.) resulting in mostly free isoprenaline and a small amount of *O*-methylated metabolite in plasma. It is thus possible that the rapid termination of the pharmaco-

logical effect derives from a mechanism such as tissue uptake (Vane, 1969) rather than rapid *O*-methylation.

Urine samples collected in the first 24 hr. after intravenous injection in man contained 80% of the administered radioactivity (40% free amine, 35% *O*-methylated amine). A progressively increasing proportion of 3-*O*-methyloisoprenaline was excreted with time, and its sulphate conjugation also increased.

In contrast, after oral administration (70 μC, 220 μg./kg.) in man, peak concentrations of total plasma radioactivity (95 min.) and maximum pharmacological effect (50 min.) did not correspond. The major plasma metabolite was isoprenaline sulphate, with a small amount of *O*-methylated amine; 89% of the dose was excreted in the urine in 24 hr., largely as isoprenaline sulphate conjugate, although 10% was the conjugated *O*-methylated derivative. A little free isoprenaline was also present in early urine samples. Intraduodenal administration in the dog (240 μC, 600 μg./kg.) gave a similar pattern; conjugated amine was largely responsible for plasma radioactivity, but a little 3-*O*-methyloisoprenaline was detected. The main metabolite in the urine was isoprenaline sulphate.

Thus the pattern of metabolic degradation of isoprenaline, some aspects of which have previously been studied in the rat (Hertting, 1964) and the dog (Conway, Minatoya, Lands & Shekosky, 1968), varies with the route of administration.

Conway, W. D., Minatoya, H., Lands, A. M. & Shekosky, L. M. (1968). *J. pharm. Sci.* **57** 1135.

Hertting, G. (1964). *Biochem. Pharmacol.* **13**, 119.

Vane, J. R. (1969). *Brit. J. Pharmacol.* **35**, 209.

Regulation of Cytidine Aminohydrolase

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A possible regulatory role in nucleic acid synthesis for cytidine aminohydrolase (EC 3.5.4.5) has been suggested (Wisdom & Orsi, 1967) that implicates it in base conservation after nucleic acid breakdown. Further work on this enzyme has confirmed these conclusions and seems to indicate some rather novel characteristics for cytidine aminohydrolase from sheep liver.

The enzyme has been purified by the method of Wisdom & Orsi (1969) with slight modifications. The most important of these is the substitution of dithiothreitol (1 mM) for 2-mercaptoethanol throughout the purification: this greatly increases the yield and stabilizes the enzyme enough to permit it to be freeze-dried or frozen for storage.