Malathion A esterase activity is not only not inhibited but is significantly increased in EPN-treated mice. Hydrolysed EPN also increases the A esterase activity but PNP does not do so. An explanation for these findings is not possible at this stage. However, the increase in the malathion A esterase activity in EPN-treated animals, may to some extent, explain one anomaly, viz. the poor over-all inhibition of malathion degrading activity in the presence of EPN *in vivo*.

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Hexachlorobenzene-induced porphyria in rats—Relationship between porphyrin excretion and induction of drug metabolizing liver enzymes

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The porphyrogenic action of the fungicide hexachlorobenzene (HCB) in man as in some types of laboratory animals has been well known for several years [1, 2].

The mechanism by which HCB induces the porphyria is not known. Repeated administration of the compound to rats causes a marked increase of the urinary porphyrin-precursors, 5-aminolaevulinic acid (ALA) and porphobilinogen (PBG) between the sixth and tenth week of treatment. The excretion of both of these precursors by humans or rabbits is not increased by HCB, but all three species excrete increased amounts of uro- and coproporphyrin in their urine. Three or four weeks after starting the experiments the neurotoxic effects of HCB including clonic convulsions, tremor, hyperexcitability, and changes in hair color become obvious. Depending on the duration of the HCB administration some of the animals die after showing signs of general weakness.

Only limited information is available about the metabolism of HCB. Parke and Williams [3] could not detect any metabolites of HCB in the urine or expired air. No glucuronidated derivatives, ethereal sulfate or mercapturic acid were excreted.

In the present experiments, a search has been made for relationships between the onset of the experimental porphyria and the changes in the activity of liver drug metabolizing enzymes. ALA and porphyrin levels were estimated in 24-hr urine specimens of 60 male rats fed with an HCB containing diet. Each week, two or three animals were sacrificed and the concentration of cytochrome P-450, microsomal protein and triglycerides and the activity of aniline hydroxylase were determined in the liver. Each week one animal of the control group was investigated in the same way as the rats of the HCB group.

MATERIALS AND METHODS

Male Wistar rats weighing approximately 200 g were fed with a standard diet containing 0·2% HCB (a control group of 15 animals was fed with the same diet containing no HCB). Twenty-four hr before being sacrificed the animals were kept in metabolic cages, during which period

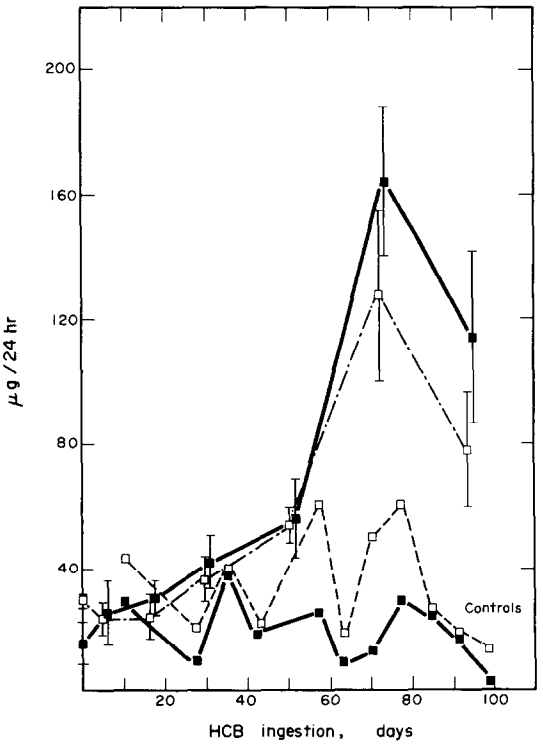

Fig. 1. Excretion pattern of urinary ALA (—□—, μg ALA/24 hr) and porphyrins (—■—, μg porphyrins/24 hr) in rats during long term exposure to HCB. Results are given as means ± S_x (vertical bars) of at least five observations.

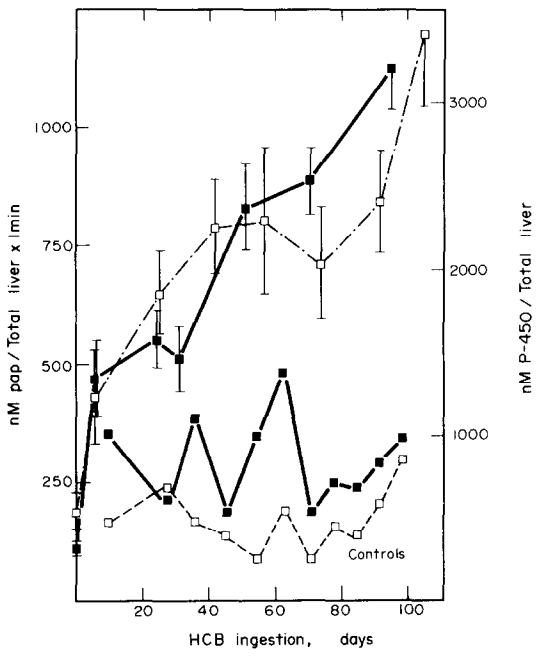

Fig. 2. Development of the contents of cytochrome P-450 (—■—, nMP-450/total liver) and of the AH activity (—·□·—, nM PAP/total liver per min) of the microsomal fraction in the livers of HCB fed rats (for details see text). Each point represents the mean of at least four observations. The standard variations are given as vertical bars.

the urine was collected for the determination of ALA and porphyrins.

The animals were killed by decapitation, the livers removed as rapidly as possible and then rinsed with an ice-cold buffer containing 0·25 M sucrose, 5·50 mM EDTA, and 0·02 M Tris–HCl pH 7·6. The microsomal suspension was prepared according to the method of Tata [4], and the protein content was determined according to Lowry *et al.* [5]. The microsomal suspension was diluted to a protein content of 6–7 mg/ml with this buffer. This suspension was used for the determination of the cytochrome P-450 content by the method of Omura and Sato [6] using a Beckman Acta C III spectrophotometer. The activity of aniline hydroxylase in this suspension was determined according to the method of Kato and Gillette [7]. The concentration of the liver glycerides was measured according to the method of Lartillot and Vogel (Haury-test) [8]. The concentration of 5-ALA in urine was determined by the method of Stich and Schmidt [9] and the concentration of total porphyrins according to Ippen and Huttenhain [10].

RESULTS

The urinary excretion of the porphyrins and ALA is shown in Fig. 1, and as can be seen, the values are clearly abnormal after the 40th day of HCB administration. On the other hand, the level of cytochrome P-450 and the activity of aniline hydroxylase in the liver were already elevated during the first week of the experiment. After an initial rise of cytochrome P-450 levels to values 3 times higher than that of the control, these values again rose steeply after the 30th day of the experiment. The aniline hydroxylase activity curve follows a similar pattern (see Fig. 2.)

DISCUSSION

The first examination of the livers from HCB fed rats showed a significant increase in the cytochrome P-450 content and the aniline hydroxylase activity. At this point the excretion of porphyrin and ALA still ranged within normal limits. The cytochrome P-450 content in the liver reached a plateau after an initial rise and remained constant until the 30th day of the experiment. Afterwards, it rose, steeply at first and then gradually until the end of the experiment. The urinary excretion of porphyrin and ALA reached pathological levels on about the 40th day and these abnormal values remained for the duration of the investigation. The initial peak of the cytochrome P-450 concentration and of the aniline hydroxylase activity is in agreement with the findings of Wada *et al.* [11], who showed a similar increase 24–48 hr after HCB administration.

Histological examinations of the liver after application of porphyrogenic compounds such as allylisopropylacetamide [12] and HCB [13] showed an increase in the number of liver cells as well as an increase in the ratio of cytoplasm to nucleus. Recently, Medline *et al.* [14] described histological changes in the liver using an almost analogous approach (male rats fed with 0·2% HCB diet were sacrificed at weekly intervals). Light and electron microscopic examination revealed a significant increase in the endoplasmic reticulum (ER) in the second week, which became more pronounced from the third to the ninth (and concluding) week of the experiment.

The initial rise of the hepatic microsomal enzyme system due to HCB exposure is certainly dependent on the chemical properties of the HCB molecule. For instance, it seems likely that, due to the lipophilicity of the compound, the HCB molecule is bound to the lipoprotein components of the ER of the liver, where it could remain unchanged for some time due to its chemical inertness. This disposition of HCB in the ER could be expected to induce the microsomal drug metabolizing enzyme system of the liver [15]. Our data supports the hypothesis that the prompt increase of liver cytochrome P-450 following HCB administration is probably caused by the unchanged compound.

The distribution of various other polyhalogenated drugs in the liver and their binding by certain cell particles [16] supports the view that the HCB molecule is partially or totally bound to the ER components thus inducing the increase of liver cytochrome P-450 to the level seen in the first three weeks of the experiments.

During the early period of HCB exposure microsomal enzyme induction could lead to the oxidation of HCB. This metabolite may be the actual porphyrogenic agent and may be a more potent inducer of the microsomal enzymes than the unchanged HCB. Such a hypothesis would explain our findings regarding the coincidence of the second rise of the hepatic cytochrome P-450 and of the increased urinary excretion of porphyrin. However, the nature of this hypothetical metabolite and its mechanism of action in porphyrin metabolism is unknown.

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Inhibition of aldehyde reductase by acidic metabolites of the biogenic amines

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Reduced NADP-dependent aldehyde reductase (EC 1.1.1.2) is an enzyme of wide specificity capable of reducing aromatic aldehydes, long chain aliphatic aldehydes and certain aldoses [1, 2]. An important function of the aldehyde reductases from brain tissue may be in the metabolism of the aldehydes derived by deamination of the neurotransmitter biogenic amines [1–4]. These "biogenic aldehydes" may either be reduced to alcohols by aldehyde reductase or oxidized to acids by aldehyde dehydrogenase (EC 1.2.1.3) and several studies have shown that the preferred route of amine metabolism appears to be mainly dependent upon the kinetic parameters of the aldehyde metabolising enzymes [5–8]. Such studies have, however, not considered the possible inhibition of aldehyde reductase by acidic or alcohol metabolites of the biogenic amines although such inhibition has recently been reported to be significant [4]. This communication examines the nature and potency of the inhibition of brain aldehyde reductase by biogenic amine metabolites and suggests that such inhibition is unlikely to be a factor that regulates amine metabolism *in vivo*.

MATERIALS AND METHODS

All chemicals were of the highest grade commercially available and unless otherwise stated were obtained from British Drug Houses Ltd. (Poole, Dorset, U.K.). Coenzymes were obtained from Boehringer (Mannheim), Germany and were stored desiccated at 4°. 3-pyridine-carboxaldehyde (PC), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), DL-4-hydroxy-3-methoxymandelic acid (vanillylmandelic acid), 5-hydroxyindole-3-acetic acid, imidazole acetic acid and 3,4-dihydroxyphenylglycol were obtained from Sigma (London) Chemical Co., U.K. Pyridine-3-methanol was from R. N. Emanuel Ltd., Wembley, U.K.

Multiple forms of reduced NADP dependent aldehyde reductase occur in brain tissue [3, 4]. The major isoenzyme in sheep brain was purified to a sp. act. of 0·6 units/mg protein by the method previously described for the isolation of this enzyme from pig brain [1]. The purified product exhibited a single band of activity following polyacrylamide gel electrophoresis [1]. Initial rate kinetic studies with this enzyme produced linear Lineweaver–Burk reciprocal plots over a 100-fold range of aldehyde concentration [3].

Routine assays of brain aldehyde reductase were performed at 30° in 200 mM sodium phosphate buffer, pH 7·2, containing 0·8 mM PC and 0·1 mM NADPH. For kinetic studies, addition of enzyme was normally used to start the reaction. The reaction was monitored continuously by following the decrease in absorbance at 340 nm in a Gilford Model 240 Spectrophotometer coupled to a Servoscribe 8-in. chart recorder. The reaction rate was linear for at least 5 min and the initial rate was proportional to enzyme concentration. A unit of enzyme activity is defined as the amount that catalyses the oxidation of 1 μmole of NADPH/min at 30°.

The concentration of PC in solution was estimated [2] assuming a molar extinction coefficient of $3{\cdot}35 \times 10^3$. Stock solutions of NADPH in glass distilled water were made freshly each day and assayed as described previously [1]. The various inhibitors tested were dissolved in the assay buffer before use and their addition to the reaction mixture at the concentrations used in these experiments did not affect the pH of the assay medium. The experimental data were initially fitted to reciprocal plots by eye to determine linearity and kinetic constants were obtained using a modification of the computer program of Cleland [9] and the University of Leeds ICL 1906A computer. Data were fitted to rate equations describing linear non-competitive, uncompetitive and competitive inhibition to determine the equation giving best fit to the data and the most valid values of kinetic parameters.

RESULTS AND DISCUSSION

The apparent Michaelis constants for NADPH and PC were estimated as $3{\cdot}5 \times 10^{-6}$ M and $9{\cdot}0 \times 10^{-4}$ M respectively which are similar in magnitude to the values previously obtained for the enzymes isolated from pig brain and kidney [1, 2]. A variety of acid metabolites of the catecholamines and 5-hydroxytryptamine were shown to cause significant inhibition of sheep brain aldehyde reductase (Table 1). In contrast imidazole acetic acid, 3,4-dihydroxyphenylglycol (an alcohol metabolite of noradrenaline) and pyridine-3-methanol (the product of PC reduction) exerted little inhibitory effect on this enzyme. Of the compounds tested, the greatest inhibition was shown by HVA, a major metabolite of dopamine.

Abbreviations: HVA: 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid); DOPAC: 3,4-dihydroxyphenylacetic acid; PC: 3-pyridine-carboxaldehyde.