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LOBES OF RAT LIVER RESPOND AT DIFFERENT RATES TO CHALLENGE BY DIETARY HEXACHLOROBENZENE

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Abstract—The lobes of livers from rats fed hexachlorobenzene (HCB) develop porphyria at different rates. The caudate lobe reacts significantly slower than the median, although eventually all lobes become equally porphyric. Uroporphyrinogen decarboxylase and 5-aminolaevulinate synthetase activities are less depressed and less elevated respectively in the caudate lobe than the remainder of the liver. The variations in porphyrin levels do not appear to be due to differences in clearances of the porphyrins from the tissues. HCB levels in the lobes, although similar at the later stages of porphyria, are significantly different in the initial course of treatment. The slower reacting caudate lobe contains the highest concentrations of HCB as shown by both tissue analysis and [U-^{14}C]HCB experiments. The differences in rate of porphyria development between median and caudate lobes cannot therefore be accounted for by differences in uptake of HCB but must reflect variations in metabolism of HCB and/or in the porphyrogenic response. Some of the possibilities have been investigated.

Hexachlorobenzene (HCB) and some other polychlorinated aromatic compounds cause a chronic hepatic porphyria when fed to rats and other mammals [1]. Related conditions were reported in people in Turkey after accidental poisoning by HCB through the consumption of contaminated wheat [2]. Another similar human syndrome called porphyria cutanea tarda is sometimes seen in patients with liver damage caused by alcohol, and in some instances where oestrogens have been given therapeutically [3]. Experimentally, the livers of poisoned rats are seen to fluoresce brightly red under ultra-violet light owing to high amounts of accumulated porphyrins (mostly uroporphyrin). This accumulation is caused by the inhibition of uroporphyrinogen decarboxylase [EC 4.1.1.37] which occurs in this type of porphyria [4].

During previous studies of HCB-induced porphyria in rats we observed that the various lobes of rat liver seemed to respond at different rates [5]. For most biochemical, pharmacological and toxicological studies the liver is commonly regarded as a homogenous organ. We therefore deemed it important to examine this phenomenon in more detail and found that the caudate lobe of rat liver develops porphyria and the characteristic depression of uroporphyrinogen decarboxylase much more slowly than the median lobe. Evidence suggests that this difference is not due to differential accumulation of HCB in the liver lobes but may represent variations in the response. A slower reaction rate of the caudate lobe has also been observed with 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and cobaltous chloride.

MATERIALS AND METHODS

Materials. HCB (Organic Analytical Grade) was purchased from B.D.H. Chemicals Co. Ltd. Poole, Dorset, U.K. [2,3-^{14}C]Succinic acid (68.1 mCi/mmole) was obtained from New England Nuclear, Postfach 401240, 6072 Dreieich, F.R.G. and [U-^{14}C]HCB (12 mCi/mmole) from California Bionuclear Corp. Sun Valley, Ca, U.S.A.

Animals and treatments. Female Agus rats (35–45 days old) were fed diets of powdered 41B (Labsure Animal Foods, Poole, Dorset, U.K.) containing 2% arachis oil and 0.01 or 0.02% HCB. The rats given [U-^{14}C]HCB were 70 days old (162–172 g) and over 24 hr each was allowed to eat 10 g of diet containing 4.25 μCi of labelled compound (0.0014% of the diet).

Animals were killed by decapitation and the livers rinsed, blotted and weighed. They were usually homogenized in either 0.25 M sucrose or in 0.1 M Na_2HPO_4–NaH_2PO_4 buffer (pH 6.8) containing 0.1 mM EDTA (1:4 w/v) and analysed as described below.

Porphyrins. These were determined by fluorescence spectroscopy as described previously [5, 6] and calculated in terms of uroporphyrin.

HCB. Portions of homogenates (0.2 ml) were mixed with water (5 ml), acetonitrile (5 ml) and hexane (5 ml) and extracted by shaking for 3 hr [7]. The HCB levels in the hexane phases were then determined by electron capture (^{63}Ni detector) gas chromatography using a 1.5 m 3% Dexsil column at 185° with N_2 as the carrier gas (30 ml/min). Homogenates of livers from rats fed [U-^{14}C]HCB were diluted with water and digested with NaOH (80 mM final concentration). After neutralization with 0.5 M HCl, portions were mixed with Instagel (10 ml) (Packard Instrument Co. Inc.) and the radioactivity assayed in a Phillips P4510 liquid scintillation analyser. Liver samples spiked with [^{14}C]HCB gave 100 per cent recovery of radioactivity.

Glutathione. The method was based on that of Beutler *et al.* [8, 9].

Cytochrome P-450. Measurements were made on the homogenate as described by McLean and Day [10] and related to protein concentration [11].

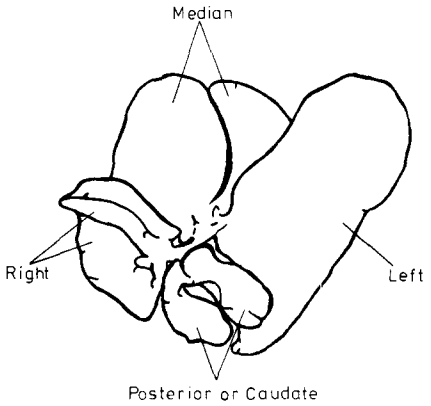

Fig. 1. Nomenclature used for lobes of rat liver. The liver is viewed from a ventral–posterior position.

5-Aminolaevulinate synthetase. Activity was estimated essentially as described by Condie and Tephly [12], using [2,3-^{14}C]succinate at a final concentration of 0.37 mM.

Haem oxygenase. Assayed on a post-mitochondrial supernatant by the method of Schacter *et al.* [13] modified as reported previously [14].

Uroporphyrinogen decarboxylase. The enzyme levels in the homogenates were assayed by the method of Smith and Francis [15] using uroporphyrin (mainly isomer III) isolated from the livers of rats made porphyric with HCB. Activity is expressed as nmoles of coproporphyrinogen formed/hr/g wet tissue.

RESULTS AND DISCUSSION

The variation in porphyria between rat liver lobes was studied in a group of animals fed HCB (0.01% of the diet) for 98 days. The livers were divided into four main lobes, median, left, right and caudate (Fig. 1). A significant difference in accumulated porphyrins between the median and caudate lobes was visually apparent by the red fluorescence when viewed under u.v. light. Quantitative analysis of the porphyrin levels confirmed the greater porphyria in the median lobes with concomitant decreases in uroporphyrinogen decarboxylase activities (Table 1). However, no significant differences in the HCB content could be detected. In another experiment the liver lobes were analysed for porphyrins and decarboxylase activity during the course of the development of porphyria. Both accumulation of porphyrins (Fig. 2a) and inhibition of uroporphyrinogen decarboxylase (Fig. 2b) appeared more rapidly in the median lobe than in the caudate lobe, with the left and right lobes having intermediate responses. When porphyria was very advanced all of the lobes were equally affected.

The partial block in haem biosynthesis due to loss of activity of uroporphyrinogen decarboxylase would be expected to result in depletion of the haem pool and in a secondary stimulation of 5-aminolaevulinate synthetase activity [EC 2.3.2.37]. The levels of this latter enzyme were therefore measured in rats fed HCB (0.02%) for 70 days and found to differ in the various lobes and to be more elevated in those exhibiting a marked porphyria. This was consistent for each liver but because of the wide variations between rats often seen in the earlier stages of porphyria development, statistical tests could not be applied. Values seen for porphyrin concentrations in the median lobes of the livers from three different animals were as follows, with the values observed in the caudate lobes of the same liver in parentheses: 5.8 (0.57); 179.8 (1.73); 15.1 (0.78) nmoles/g of tissue. Corresponding values estimated for 5-aminolaevulinate synthetase were 0.55 (0.39); 1.72 (0.42); 0.78 (0.35) nmoles/min/g. The right and left lobes gave intermediate responses. Control animals showed no variations between lobes, i.e. porphyrins, median 0.41 ± 0.04; caudate 0.33 ± 0.3 nmoles/g (± S.E.M. N = 4); 5-aminolaevulinate synthetase, median 0.34 ± 0.01; caudate 0.34 ± 0.01 nmoles/min/g (± S.E.M. N = 4). These results confirmed that the haem biosynthetic pathway had been more severely affected in the median lobe and also showed that the different porphyrin accumulations could not be merely ascribed to varying rates of clearance from the various lobes.

Table 1. Porphyrins, uroporphyrinogen decarboxylase activity and hexachlorobenzene in different lobes of rat liver*

Liver lobe	Porphyrins (nmoles/g)	Uroporphyrinogen decarboxylase (nmoles/hr/g)	Hexachlorobenzene (nmoles/g)
Median	900 ± 110	4.0 ± 0.7	270 ± 15
Left	867 ± 57	4.1 ± 0.7	273 ± 15
Right	578 ± 142	4.2 ± 0.6	280 ± 11
Caudate	209 ± 61†	7.6 ± 0.7‡	289 ± 12

* Female AGUS rats were fed a powdered diet containing HCB (0.01%) for 98 days. The livers were then analysed as described in Materials and Methods. Results given are means ± S.E.M. (N = 4) per gram of tissue.

† Significantly different from median lobe $P < 0.005$.

‡ $P < 0.01$ as assessed by Student's *t*-test.

Control animals (N = 4) showed no difference in either porphyrin levels (median, 0.27 ± 0.01; left 0.24 ± 0.01; right 0.26 ± 0.02; caudate 0.26 ± 0.02 nmoles/g tissue) or in uroporphyrinogen decarboxylase activity (22.8 ± 0.5; 23.9 ± 0.5; 24.2 ± 0.7; 22.8 ± 0.9 nmoles of coproporphyrinogen/hr/g, respectively).

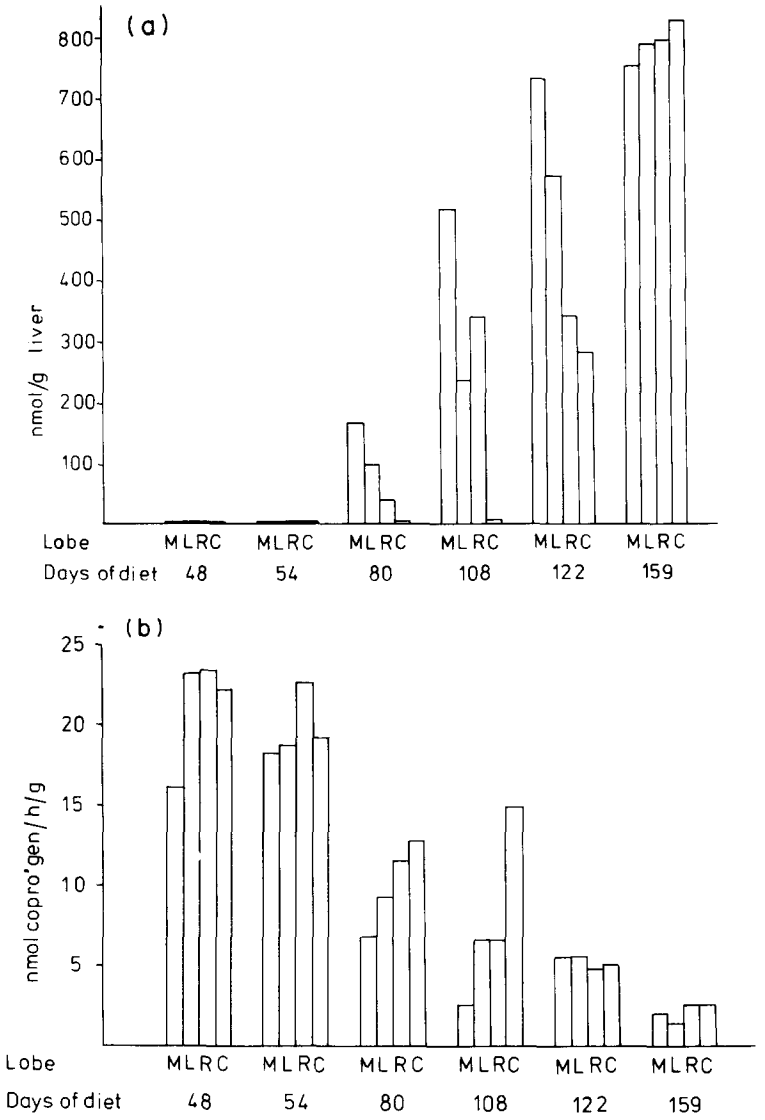

Fig. 2. Comparison of porphyrins and uroporphyrinogen decarboxylase activity in the liver lobes of female rats during feeding of HCB (0.01%) in the 41B diet. Single animals were killed at intervals from 48 days. M = median, L = left, R = right, C = caudate. (a) Porphyrins (estimated as uroporphyrin) per gram of wet tissue. (b) Uroporphyrinogen decarboxylase; coproporphyrinogen formed from uroporphyrinogen III per hr per gram of wet tissue.

Although no significant differences in HCB content could be detected between the lobes in the experiment described in Table 1 and in previous work [5], consistent variations were observed in the livers of animals during early stages of porphyria. The caudate lobes appeared to contain more HCB per wet weight of tissue than those lobes that were affected more rapidly. When the lobes were compared during the first few weeks of diet it was indeed found that the caudate lobe accumulated more HCB than the median (Fig. 3). This was confirmed by using [U-^{14}C]HCB fed to rats overnight, in a similar manner to chronic feeding experiments. Estimations of the radioactivity in the four lobes showed that the median had the lowest radioactivity per gram of wet tissue (Table 2). Therefore a reduced HCB uptake is not likely to be the reason why the caudate lobe reacts more slowly.

2-Allyl-2-isopropylacetamide (male rats injected s.c. with 300 mg/kg and left for 5 hr) and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (males dosed orally with 100 mg/kg and left 7 hr) also appeared to cause a lower accumulation of porphyrins in the caudate lobe compared to the median (results not shown). Cobaltous chloride (female rats injected subcutaneously with 60 mg/kg, left 16 hr) stimulated haem oxygenase activity to a lesser extent in the caudate lobes (0.385 ± 0.27 nmoles/min/mg protein ± S.E.M. N = 4) than the remainder (0.481 ± 0.024; $P < 0.05$). No differences in total cytochrome

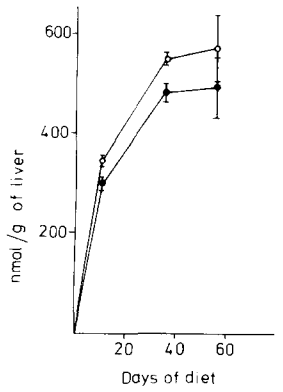

Fig. 3. Comparison of the HCB concentrations in the median and caudate lobes of livers of female rats during the earlier stages of feeding HCB (0.02%) in the diet. Values are means of three estimations ± S.D. ●——● Median lobe; ○——○ caudate lobe.

P-450 content between lobes of the livers of young female rats could be detected (median 0.14 ± 0.02; left 0.13 ±0.02; right 0.12 ± 0.01; caudate 0.13 ± 0.01 nmoles/mg protein ± S.E.M. N = 4).

As an uneven distribution of GSH in the hepatocytes of liver lobules has been suggested as partially explaining the greater susceptibility of centrilobular cells to electrophilic attack by toxic metabolites of inert xenobiotics [16], the GSH levels of the liver lobes were examined. No significant differences between lobes were observed in control animals (median 6.8 ± 0.3; left 6.6 ± 0.1; right 6.6 ± 0.1; caudate 6.2 ± 0.2 μmoles/g wet tissue ± S.E.M. N = 4). Rats fed HCB (0.02% of the diet) for 58 days showed no significant increases as previously reported [17, 18], but the caudate lobe contained slightly lower levels than the median (median 6.9 ± 0.2; left 6.6 ± 0.3; right 6.2 ± 0.4; caudate 6.0 ± 0.3* μoles/g ± S.E.M. N = 5: (* significantly different from the median $P < 0.05$).

It would thus appear that the caudate lobes of rat liver respond less markedly to HCB and probably to other chemicals. There are several possible explanations for these findings. Firstly, the rate of uptake of the toxic agent may be different in the various parts of the liver because the lobes differ instrinsically in their ability to take up and retain certain chemical compounds from the blood. Incorporation of [^{3}H]leucine into proteins has been found to be identical in all lobes when the isotope was injected into the external jugular vein but differences were observed after administering the isotope via the spleen or lumen of the jejunum, probably due to streamlining of blood flow [19]. In addition, in perfused rat liver variable synthesis of cholesterol and fatty acids by different parts of the liver has been reported [20] and differential removal and metabolism of chylomicrons and chylomicron remnants [21]. No evidence was obtained in this present study for a preferential uptake of HCB by the fast reacting lobes; in fact the opposite was found to be the case during the first few weeks of treatment.

Table 2. Incorporation of radioactivity from dietary hexachloro-[U-^{14}C]benzene into rat liver lobes*

Liver lobe	Incorporation of radioactivity (d.p.m./g tissue)	(d.p.m./lobe)
Median	52,255 ± 1881	89,150 ± 3207
Left	55,243 ± 1678	101,346 ± 4616
Right	54,136 ± 576	71,742 ± 5378
Caudate	61,464 ± 721†	35,141 ± 2845

* Female rats were each fed 10 g of 41B diet containing 4.24 μCi [^{14}C]HCB (14 p.p.m.) over 24 hr. The livers were then analysed for radioactivity as described in Materials and Methods. Values are averages ± S.E.M. (N = 4).

† Significantly different from median lobe ($P < 0.005$) as assessed by Student's *t*-test.

Another possible explanation is a difference in drug metabolism between different lobes. If metabolic activation of HCB is required for the subsequent inhibition of uroporphyrinogen decarboxylase as has been suggested [22–24], a faster rate of metabolism in the median lobe (as compared with the caudate lobe) may explain the difference in response and similar differences in response to 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine. Although *in vivo* metabolites of HCB have been extensively studied [25, 26], *in vitro* experiments have not been very successful [27] and further progress is probably required before any possible variations in metabolism of HCB can be explored. Carbon tetrachloride and dimethylnitrosamine have been shown to affect preferentially different parts of the rat liver, in both cases those lobes with greater activating enzymes being more affected [28]. This explanation would be difficult to reconcile however, with differences observed with cobalt, as no activating enzymic step has been postulated in the mechanism of action of this metal.

In conclusion, the present results illustrate that livers cannot always be regarded as homogenous organs for toxicological studies and that the caudate lobe of rat liver reacts less markedly to HCB and to other chemicals. However, we are unable to provide a satisfactory explanation for the differences.

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