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CONTINUED DEPRESSION OF HEPATIC UROPORPHYRINOGEN DECARBOXYLASE ACTIVITY CAUSED BY HEXACHLOROBENZENE OR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN DESPITE REGENERATION AFTER PARTIAL HEPATECTOMY

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Abstract—Hepatic uroporphyrinogen decarboxylase activity in male C57BL/10 mice was maintained in regenerated liver after recovery from two-thirds hepatectomy. In contrast, there was little increase in enzyme activity in regenerated liver from animals previously treated with hexachlorobenzene (HCB) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These chemicals initially cause depression of uroporphyrinogen decarboxylase activity over a time much longer than the period allowed for regeneration. Estimation of HCB levels showed that there was only a small amount of redistribution to the liver during regrowth. The results demonstrate that HCB and TCDD induce either formation of a toxic metabolite or some other inhibitory process and that this can be sustained for a long period which delays recovery to the normal state.

Some polyhalogenated aromatic compounds such as hexachlorobenzene (HCB), certain congeners of polyhalogenated biphenyls and, most potently of all, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), cause a hepatic porphyria in experimental animals which is characterized by a depression of uroporphyrinogen decarboxylase activity (EC 4.1.1.37) [1, 2]. TCDD and HCB have both caused porphyria in humans [1–3]. Between 1955 and 1961 several thousand people were poisoned by HCB in eastern Turkey [4, 5]. Studies of some of these patients showed that symptoms of porphyria were still present after twenty-five years even though HCB levels in milk and blood had greatly declined [6]. In an experimental model, excretion of porphyrins in the urine of rats made prophyric by HCB was still significantly elevated nine months from cessation of treatment (F. De Matteis and A. G. Smith, unpublished work). In more detailed studies, Koss *et al.* [7] reported that the time for full recovery of hepatic decarboxylase activity was surprisingly long after ending the administration of HCB. Even after 14 months depressed enzyme activity continued despite a very large drop in concentrations of HCB in the liver (>99%). Similarly, Goldstein *et al.* [8] concluded that the recovery of rats from the porphyrogenic effects of TCDD did not correlate with its biological half-life [9] and some C57BL/10 mice given one dose of TCDD still had decarboxylase activity less than 50% of controls even after 12 weeks [10] although in mice the half-life of the clearance of TCDD is apparently about 2–3 weeks [11, 12]. Thus there is both human and experimental evidence suggesting that recovery from the porphyrogenic response to these polyhalogenated compounds is slow and may not be entirely related to concentrations of these chemicals in the liver.

In the schedules that we use to produce porphyria in C57BL/10 mice with HCB (0.02% of the diet after a predose of iron) or TCDD (one 75 μg/kg dose) maximum depression of decarboxylase activity in the liver does not occur until after 5–6 weeks [10, 13]. Here we have investigated the ability of liver which is regenerating after partial hepatectomy of HCB and TCDD-treated mice to recover from "inhibition" of uroporphyrinogen decarboxylase.

MATERIALS AND METHODS

Animals and chemicals. Male C57BL/10ScSn mice were either bred in these laboratories or purchased from OLAC (1976) Ltd., Bicester, U.K. The preparation of TCDD has been described previously [14] and HCB (Organic Analytical Standard grade) was purchased from BDH Chemicals Co. Ltd. (Poole, U.K.). Safety precautions for handling TCDD were as reported elsewhere [10]. During experiments all animals were housed in negative pressure isolators. Imferon (50 mg of Fe/ml) was obtained from Fisons Ltd. (Loughborough, U.K.). Uroporphyrin III and pentacarboxyporphyrin I were purchased from Porphyrin Products (Logan, UT).

Experimental procedures. Mice were fed HCB as 0.02% of a 41BM diet which also contained 2% arachis oil after being given 0.25 ml of Imferon (12.5 mg of Fe/mouse) by subcutaneous injection. TCDD was given to mice as a single oral dose (75 μg/kg body wt) dissolved in corn oil (10 ml/kg body wt). Control animals received Imferon or oil alone as appropriate. Two-thirds hepatectomy was conducted under diethyl ether anaesthesia by removal of the left and median lobes of the liver after a single ligation. In a group of control mice this represented

67 ± 1% of the liver (± S.E., N = 8). After 2/3 hepatectomy mice were allowed to recover on control diet for 10 days.

Analyses. Excised liver samples taken at hepatectomy and after regeneration were rinsed thoroughly in cold physiological saline and frozen in liquid N_2 before storage at −70° until analysis. Tissue was homogenized in 0.25 M sucrose (1:4 w/v) and analysed for porphyrins by fluorescence spectroscopy with uroporphyrin III as a standard [15]. Uroporphyrinogen decarboxylase activity in mouse liver 40,000 *g* supernatant was estimated using uroporphyrinogen III (10 μM) at pH 6.8 or pentacarboxyporphyrinogen I (5 μM) at pH 5.4 as substrates, by reverse phase h.p.l.c. [16]. Levels of HCB in liver were determined by g.l.c. [17].

RESULTS

HCB experiment

Male C57BL/10 mice were injected with iron and fed an HCB-containing diet for 4 weeks. The median and left liver lobes were then excised and the mice allowed to recover on control diet for 10 days before they were killed and the regenerated liver removed (Fig. 1). It was estimated that the liver had regrown to more than 90% of its former size. A small increase in uroporphyrinogen decarboxylase activity was observed for control mice after recovery from hepatectomy. In contrast, in HBC-treated mice enzyme activities continued to be depressed and porphyrin levels were highly elevated and little changed from those in tissue removed at hepatectomy (Table 1). Both enzyme activities and porphyrin levels were greatly different from those estimated by assuming a dilution of original liver with newly grown tissue. HCB levels, however, had declined considerably nearly to those calculated on a proportionality basis.

Continued "inhibition" of the decarboxylase has also, been observed after hepatectomy of HCB-treated female rats (data not shown).

TCDD experiment

Porphyria in C57BL/10 mice can be produced with a single oral dose of TCDD [10]. Mice given TCDD were subjected to a one-third hepatectomy (by removal of the left lobe) after 4 weeks and allowed to recover for 8 days. Comparison of the liver samples showed no significant increase in uroporphyrinogen decarboxylase activity (12.7 ± 0.6 and 13.8 ± 0.9% of control for days 0 and 8 respectively, N = 5 with uroporphyrinogen III as substrate) but a small decrease in porphyrin levels (556 ± 55 and 398 ± 17 nmoles/g respectively). In another experiment both the median and left liver lobes were removed and 10 days allowed for recovery (Fig. 1). There was only a slight increase in uroporphyrinogen decarboxylase activity of the regenerated liver compared to the tissue removed at partial hepatectomy when estimated with pentacarboxyporphyrinogen I as a substrate and none with uroporphyrinogen III

Table 1. Uroporphyrinogen decarboxylase activity of regenerating liver from partially hepatectomised HCB-treated mice

Treatment (N)	Time from operation (days)	Uroporphyrinogen decarboxylase (pmoles copro'gen/min/mg protein)	Porphyrins (nmoles/g)	HCB (nmoles/g)
Control (4)	0	34.3 ± 1.1	0.53 ± 0.02	—
	10	36.1 ± 0.6	0.33 ± 0.02	—
HCB (5)	0	4.0 ± 0.4	229 ± 55	375 ± 20
HCB	10	6.9 ± 0.8*	247 ± 50	188 ± 9‡
HCB-estimated‡	10	25.5 ± 0.8	99 ± 18	123 ± 9

C57BL/10 mice were injected with 0.25 ml Imferon and fed a control or HCB-containing diet (0.02%) for 4 weeks. Two-thirds of the liver of each was then removed. The mice were allowed to recover on control diet for 10 days before assay of the excised and regenerated liver. Uroporphyrinogen decarboxylase was assayed with pentacarboxyporphyrinogen I as a substrate.

Significantly different from tissue removed at partial hepatectomy: *$P < 0.025$; ‡$P < 0.001$.

‡ Values estimated by assuming dilution of one part of remaining original liver with two parts of new on the assumption that regenerated liver tissue was normal tissue with control enzyme activity and porphyrin content. Results are means ± S.E.

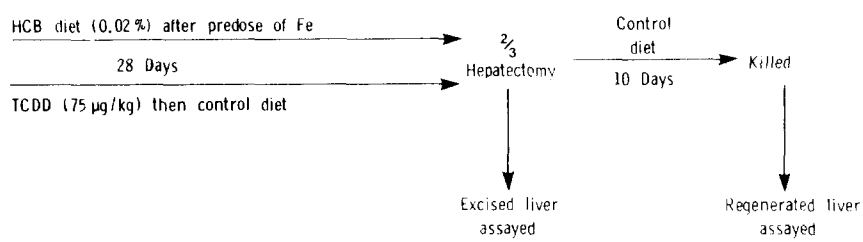

Fig. 1. Scheme illustrating experimental protocol after C57BL/10 mice were given a single dose of TCDD or fed HCB in their diet following a predose of iron.

Table 2. Uroporphyrinogen decarboxylase activity of regenerating liver from partially hepatectomised TCDD-treated mice

Treatment (N)	Time from operation (days)	Uroporphyrinogen decarboxylase (pmoles copro'gen/min/mg protein) U III	P I	Porphyrins (nmoles/g)
Control (8)	0	5.2 ± 0.6	29.6 ± 1.1	0.49 ± 0.02
Control	10	6.6 ± 0.8	32.0 ± 1.4	0.45 ± 0.03
TCDD (7)	0	0.7 ± 0.1	4.1 ± 0.3	482 ± 53
TCDD	10	0.6 ± 0.1	5.2 ± 0.5	186 ± 13*
TCDD-estimated†	10	5.0 ± 0.1	22.8 ± 0.3	159 ± 18

C57BL/10 mice were given TCDD (75 μg/kg) and left for 4 weeks. Two-thirds of the liver was then removed and the mice allowed to recover for 10 days. The excised liver lobes and regenerated liver when then analysed as described in Materials and Methods. Both uroporphyrinogen III (U III) and pentacarboxyporphyrinogen I (P I) were used as substrates.

* Significantly different from 0 day group $P < 0.001$.

† Values estimated by assuming dilution of one part of remaining original liver with 2 parts of new relative to control after hepatectomy assuming that regenerated liver was normal tissue with control enzyme activity and porphyrin content. Results are means ± S.E.

(Table 2). Both values were far less than those calculated by assuming that regenerated liver tissue had control enzyme levels. Porphyrin contents however, were lower after regeneration.

DISCUSSION

It is clear from these studies that the considerable depression of uroporphyrinogen decarboxylase activity caused by HCB and TCDD apparent at the time of hepatectomy, persists even in the newly grown liver. If the liver regenerated without any continued effects the mean enzyme activities after regrowth should be very much higher than were observed. The continued depression of hepatic uroporphyrinogen decarboxylase activity could be explained by increased mobilization of the chemical from other tissues after hepatectomy. However, in the HCB experiment not only was there continued low activity of the enzyme but hepatic concentrations of HCB had significantly fallen whereas high porphyrin levels were maintained. (Doses of HCB required to give hepatic levels of the chemical similar to that observed after regeneration are ineffective at producing porphyria within 5–6 weeks, Smith and Francis, unpublished data.) The prolonged "inhibition" of the enzyme therefore cannot be ascribed solely to the maintenance of high concentrations of HCB in the liver. In addition, only very small amounts of TCDD are required to produce similar effects to HCB [10, 18, 19] with the same slow recovery of enzyme activity after hepatectomy. Thus the activity of the enzyme was still depressed by both chemicals after the regeneration period, despite the fact that this period is considerably shorter than the time taken to produce a similar degree of "inhibition" initially [10, 13]. The decreases in porphyrin levels in the TCDD experiment were probably a consequence of the lag in their accumulation relative to lowered enzyme activity [10, 13]. Recently we have studied the recovery of male C57BL/10 mice from treatment with HCB (200 ppm) for 4 weeks (R. N. Khanna and A. G. Smith, data submitted for publication). Eight weeks after termination of treatment hepatic porphyrin levels were still approximately 400-fold greater than controls, whereas HCB levels had fallen by >90%.

There are a number of possibilities to explain these long term effects on the activity of uroporphyrinogen decarboxylase. The synthesis of the enzyme may be repressed despite regeneration or a sequence of events that is induced by HCB or TCDD, perhaps of a free radical nature which destroys the decarboxylase, may be slow to decline in regenerating tissue. Finally, an inhibitor of the enzyme may be formed initially, possibly a metabolite of HCB or TCDD [20], which is retained in sufficient quantities after regeneration to continue to cause inhibition of newly synthesized enzyme.

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