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DOSE–RESPONSE RELATIONSHIPS IN HEXACHLOROBENZENE-INDUCED PORPHYRIA

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Abstract—The rate of development of hexachlorobenzene (HCB)-induced porphyria in female Wistar rats was determined using HCB dosage and porphyrin analysis protocols designed to determine factors which contribute to the delay commonly observed between initial exposure to HCB and the detection of porphyria. Measurements were made of HCB and porphyrin concentrations in the livers, kidneys, and spleens of female Wistar rats exposed continuously (up to 56 days) or for 1 day to HCB (at dietary concentrations of 1000 ppm and 100 ppm). The experiments showed that when a corn oil solution of HCB was added to the diet at a concentration of 1000 ppm, HCB accumulated rapidly in all organs, and the delay in appearance of elevated liver highly carboxylated porphyrins (HCPs) was at most 4 days (approximately 8-fold elevation of HCPs on day 4). One day of exposure to this diet was sufficient to cause elevated liver HCPs, thus showing that continuous exposure to HCB was not required to cause porphyria in this species. Solid HCB added directly to the diet (1000 ppm) resulted in less rapid HCB accumulation and less rapid development of porphyria. The experiments demonstrated that the appearance of a delay in HCB-induced porphyria in the Wistar rat is caused by the rate at which HCB is absorbed, and by using total hepatic porphyrins (rather than HCPs) as the indicator of the disorder. The experiments also showed that HCB-induced liver enlargement and neurotoxicity are not necessarily associated with the severity of porphyria.

Hexachlorobenzene (HCB†), polychlorinated and polybrominated biphenyls (PCBs and PBBs), tetrachlorodibenzo-*p*-dioxin (TCDD), and other polyhalogenated aromatic hydrocarbons (PHAHs) cause a type of porphyria that is characterized by the accumulation and excretion of uroporphyrin and heptacarboxylic acid porphyrin (the highly carboxylated porphyrins, or HCPs). When severely developed, the HCPs reach levels several thousand times higher than normal, and their presence results in severe photosensitivity and skin lesions. The most widespread incident of PHAH-induced porphyria in humans was in Turkey in the 1950s. At least 4000 people developed the disorder after consuming HCB in seed grain used to prepare food during a period of grain shortage. The results of a study conducted from 1977 to 1981 showed that many of the survivors of the incident still suffered with porphyric symptoms despite the passage of 25 years [1]. Other examples of PHAH-induced porphyria in humans include workers in chemical factories [2, 3] and farmers in Michigan [4]. Two recent studies suggest that environmental contamination with PHAHs has caused porphyria in wild birds [5] and fish [6].

Despite extensive study, the mechanism of PHAH-induced porphyria is not known. Most of the proposed mechanisms regard the commonly observed phenomenon of a several week to several month delay between initial exposure to a PHAH and the onset of porphyria to be an important clue to a mechanistic explanation of the disorder [7–9]. We recently reported that two previously overlooked factors contribute to the appearance of a delay in the development of porphyria in HCB-exposed Wistar rats; (1) a relatively long time can elapse before enough HCB and/or its metabolites accumulate in the liver when it is administered as a powder in the diet, and (2) total hepatic porphyrin concentration is an insensitive indicator of the early development of porphyria [10, 11]. These results suggested that some of the earlier explanations for the delay in PHAH-induced porphyria should be re-examined. In the present paper we present further results of our studies with the Wistar rat which were designed to determine factors which contribute to the appearance of the so-called 'lag time' in HCB-induced porphyria.

MATERIALS AND METHODS

Diet preparation and administration. One control diet and three diets containing HCB (Aldrich, 97%) were prepared with powdered Purina Rat Chow. Control diet was prepared by adding corn oil to the food at a concentration of 4% (w/w). Two 1000 ppm HCB diets were prepared; 1000-CO (1000 ppm, corn oil) by mixing to homogeneity a corn oil solution of HCB with the food such that the corn oil consituted 4% (w/w), and 1000-NCO (1000 ppm, no corn oil) by mixing to homogeneity crystalline HCB with the food. One diet containing HCB at 100 ppm (100-CO) was prepared in the same manner as the 1000-CO diet. One hundred and twenty-two female Wistar

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† Abbreviations: HCB, hexachlorobenzene; HCPs, highly carboxylated porphyrins (uroporphyrin and heptacarboxylic acid porphyrin); PCBs, polychlorinated biphenyls; TCDD, tetrachlorodibenzo-*p*-dioxin; and PHAHs, polyhalogenated aromatic hydrocarbons.

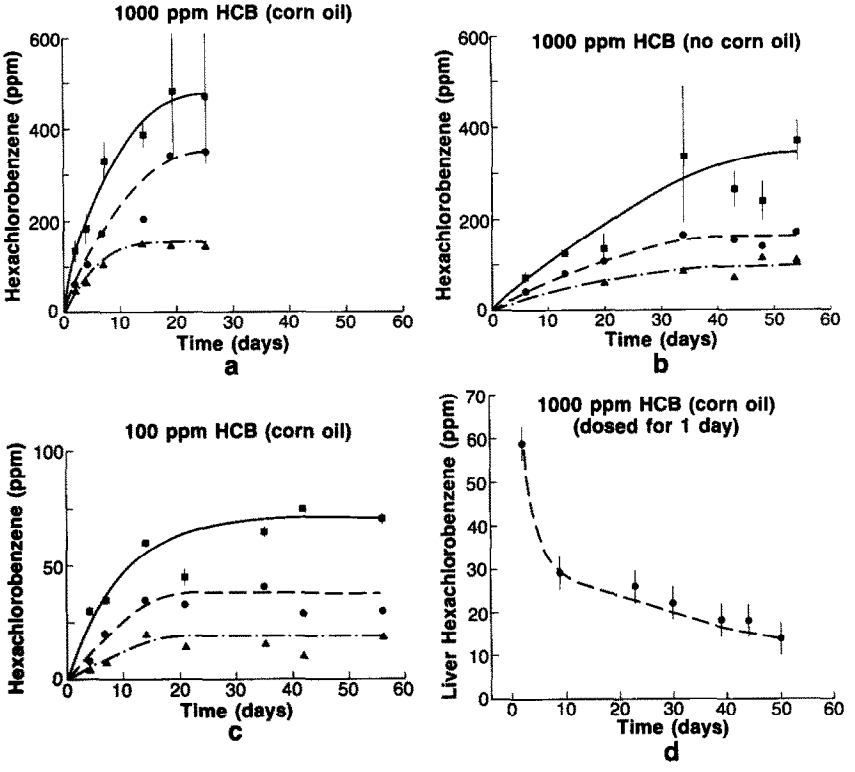

Fig. 1. Time course of hexachlorobenzene (HCB) accumulation in the livers, kidneys, and spleens of female Wistar rats. Rats were exposed to the compound in their diets at concentrations of (a) 1000 ppm (pre-dissolved in corn oil), (b) 1000 ppm (solid), (c) 100 ppm (pre-dissolved in corn oil), and (d) 1000 ppm (pre-dissolved in corn oil; exposed for 1 day only). Points shown for livers are means ± SD (N = 3–7). Points shown for kidney and spleen are those obtained from pooled samples. Key: (■) livers, (●) kidneys, and (▲) spleens.

rats (Charles River) weighing 100–150 g were assigned to five groups and provided food and water *ad lib*. Seventeen rats were fed the control diet, twenty-nine the 1000-CO diet, twenty-seven the 1000-NCO diet, and twenty-three the 100-CO diet. Twenty-six rats received the 1000-CO diet for 1 day only followed by control diet. All other rats remained on their original diets until the completion of the experiments. On the days indicated in Fig. 1, three to seven rats from each HCB-dosed group were anesthetized with ether, their left ventricles were cannulated and perfused with saline solution (0.9%, 150 mL), and their livers (separated into the three major lobes), kidneys, and spleens were removed and frozen (−20°). Similarly, organs were obtained from control rats on days 4, 14, 25, 42, and 56 (three to five rats per day).

Porphyrin analysis. Porphyrin standards were obtained from Porphyrin Products (Logan, UT). Porphyrin patterns in liver, kidney, and spleen were determined by HPLC using the method previously described [12]. Briefly, porphyrins were extracted with perchloric acid (0.9 N)/methanol, 50/50, concentrated on disposable SEP-PAK C_{18} cartridges (Waters Inc.), separated by reverse-phase HPLC (Perkin–Elmer Series 4 liquid chromatograph with a Perkin–Elmer C_{18} 3 cm long, 3 μm particle size column), and detected and quantified using a fluorescence detector (Perkin–Elmer LS-4). Recovery from liver samples spiked with uroporphyrin was 100%, and successive extractions showed that recovery of naturally occurring HCPs at concentrations ranging from 60 pmol/g wet weight (normal Wistar rat) to 400,000 pmol/g (severe HCB-induced porphyria in the Wistar rat) was at least 98%. Triplicate analyses of livers containing HCPs at concentrations ranging from 60 to 35,000 pmol/g showed variances of 3 to 8%. Using 0.5 g of tissue, the detection limits (signal-to-noise ratio of 2) for uro-, copro-, and protoporphyrin were 5, 2, and 5 pmol/g respectively.

Hexachlorobenzene analysis. Concentrations of HCB in liver, kidney, and spleen were determined by the method of Norstrom and Won [13] using a Hewlett-Packard model 5730A gas chromatograph equipped with a ^{63}Ni detector and a 5.5 ft 1% SP2100/2% SP2401 + 0.5 ft 1% SP2100/2% AN600 on a Supelcoport 100/120 mesh column (Supelco). All livers (0.5-g samples) were analyzed separately. Pooled samples of kidneys (0.3- to 0.5-g samples) and spleens (0.1- to 0.5-g samples) were analyzed.

RESULTS

Hexachlorobenzene accumulation. Panels a–c of

Table 1. Porphyrin concentrations in the livers, kidneys, and spleens of control female Wistar rats

	Porphyrin concentration (pmol/g)					
	Uroporphyrin		Coproporphyrin		Protoporphyrin	
Liver	63 ± 9*	46–76†	52 ± 8*	45–80†	(3.6 ± 2.1) × 10^2*	100–800†
Kidney	(1.2 ± 0.3) × 10^2	72–190	86 ± 27	40–130	17 ± 9	11–42
Spleen	34 ± 13	14–51	33 ± 10	21–54	98 ± 39	37–140

* Mean ± SD, N = 17 for each tissue.
† Range.

Fig. 1 show the time course of HCB accumulation in the livers, kidneys and spleens of the three groups of rats which fed continuously on diets containing the chemical (the 1000-CO, 1000-NCO, and 100-CO groups). Accumulation of HCB was more rapid in liver than in kidney, and more rapid in kidney than in spleen. Apparent steady-state* HCB concentrations were in the order liver > kidney > spleen. The compound accumulated much more rapidly in the organs of the 1000-CO rats than in the organs of the 1000-NCO rats (on day 2 the liver HCB concentration in the 1000-CO rats was 134 ± 21 ppm; it took approximately 2 weeks for a comparable concentration to be reached in the 1000-NCO rats). The apparent steady-state level was approximately 100 ppm higher in the organs of the 1000-CO rats than in the organs of the 1000-NCO rats. Both 1000 ppm HCB diets caused higher steady-state concentrations of HCB than did the 100 ppm HCB diet. In fact, kidney and spleen HCB levels in the two groups receiving the 1000 ppm diets were higher than liver HCB concentrations in the 100 ppm HCB-fed rats.

Figure 1d shows the time course of liver HCB decline in the group of rats fed the 1000 ppm HCB diet for 1 day only (HCB was not measured in the kidneys and spleens of this group). The livers of this group of rats contained 59 ± 9 ppm HCB on day 2. By day 9, the concentration was approximately 30 ppm, and by day 50 it was approximately 15 ppm.

Porphyrin accumulation. The porphyrin patterns in the livers, kidneys, and spleens of the control rats are summarized in Table 1. Uroporphyrin, coproporphyrin, and protoporphyrin were the only porphyrins detected. There were no detectable changes in the levels of each over the 56 days (data not shown). Porphyrin patterns were quite different in the three organs. In liver, protoporphyrin was the porphyrin present at the highest concentration. Uroporphyrin (uroporphyrin was the only HCP detected) and coproporphyrin levels were lower and less variable than protoporphyrin. Uroporphyrin contributed from 7 to 33% of the total hepatic porphyrin 'pool.' Spleen had slightly lower levels of uroporphyrin and coproporphyrin than liver, and much lower levels of protoporphyrin. The pattern in spleen was quite similar to that of liver (i.e. similar uroporphyrin and coproporphyrin levels, and higher protoporphyrin levels). In kidney, protoporphyrin was the porphyrin with the lowest concentration.

The time course of HCP accumulation in the livers (right lobe), kidneys, and spleens of the 1000-CO, 1000-NCO, and the 100-CO rats is shown in panels a–c of Fig. 2. Figure 2d illustrates liver HCP levels in the 1000-CO rats that were dosed for 1 day only. In all experiments, liver HCP concentrations increased soon after exposure to HCB. In contrast, delays in accumulation of HCPs were found in kidney and spleen. These delays ranged from 4 days (1000-CO) to 35 days (100-CO) in kidney and from 7 days (1000-CO) to no definite increase even at day 56 (100-CO) in spleen. Once the HCP levels were elevated, their concentrations increased exponentially in the organs of animals feeding continuously on diets containing HCB. Liver HCP concentrations appeared to reach a maximum of 300,000 ± 100,000 pmol/g by day 43 in the 1000-NCO rats, but there was no decline in the rate of increase of HCPs in kidneys and spleens of this group of rats. A decrease in the rate of HCP accumulation was not observed in liver, kidney, or spleen in the 1000-CO or 100-CO rats. Liver HCP concentration in the rats fed the 1000-CO diet for 1 day only was elevated approximately 4-fold on day 9 and did not increase further. By day 50 the HCP concentration was only marginally above normal.

Liver HCP concentration in the 1000-CO rats was elevated approximately 8-fold by day 4. It was not until day 13 that a similar elevation was observed in the 1000-NCO rats. The rates of increase from this time appeared identical in both groups. Comparison of panels a and b of Fig. 1 with panels a and b of Fig. 2 shows, however, that this similarity does not reflect similar concentrations of HCB. There was no correlation between HCP and HCB concentrations. The much higher HCB concentrations in the organs of the 1000-CO rats at all time points did not appear to cause a more rapid rate of liver HCP accumulation once the HCP levels had become elevated approximately 10-fold. However, in the 100-CO rats, the rate of HCP increase was substantially lower than it was in both of the 1000 ppm-dosed groups.

Smith *et al.* [14] found the porphyrin concentration in the posterior lobes of the livers of HCB-dosed Agus rats to be much lower than in the rest of the livers. This difference was not found in the present experiment with Wistar rats. When porphyrin concentration was high enough to result in easily visible fluorescence under long-wave UV illumination (>50,000 pmol/g), foci of fluorescence appeared to be evenly scattered throughout the liver. At lower

* We use this term to describe the situation where no further increase in HCB and/or HCPs appears to have occurred.

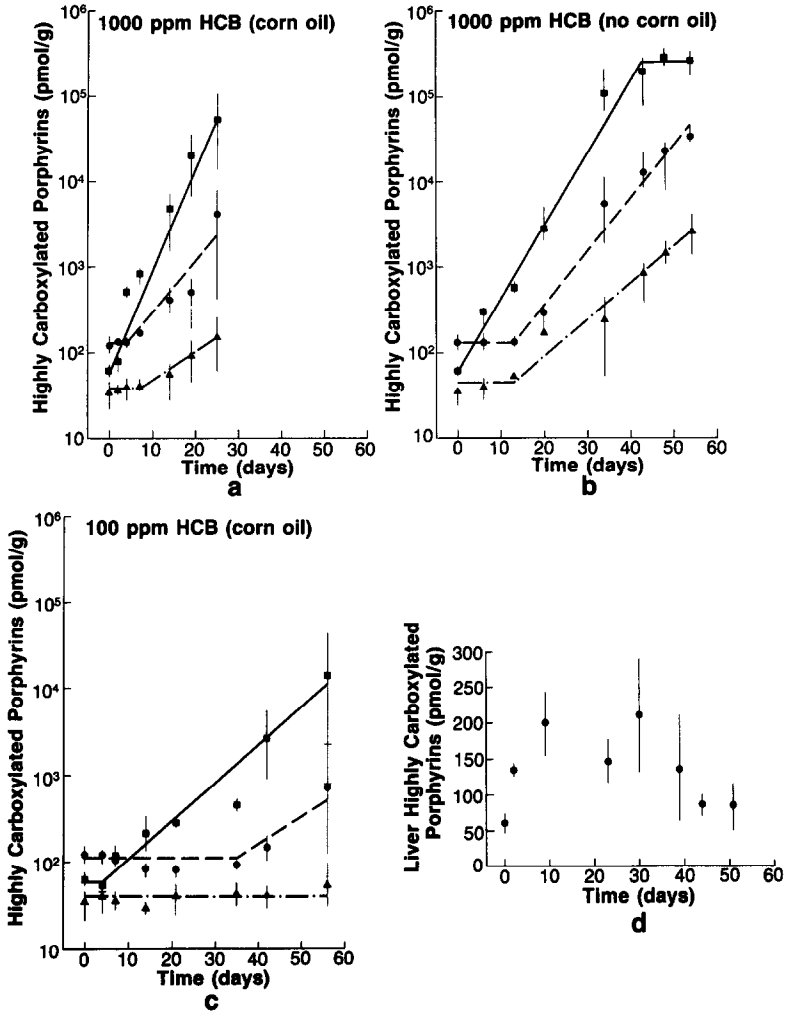

Fig. 2. Time course of highly carboxylated porphyrin (HCP) accumulation in the livers, kidneys, and spleens of female Wistar rats. Rats were exposed to hexachlorobenzene (HCB) in their diets at concentrations of (a) 1000 ppm (pre-dissolved in corn oil), (b) 1000 ppm (solid), (c) 100 ppm (pre-dissolved in corn oil), and (d) 1000 ppm (pre-dissolved in corn oil; exposed for 1 day only). The points are means (N = 3–7); the bars illustrate the range of HCP concentrations. No changes in the HCP levels in the organs of the control rats were observed over time, and therefore all control data are included on day 0 to simplify the graph. Key: (■) livers, (●) kidneys, and (▲) spleens.

porphyrin levels (≤2000 pmol/g), HPLC analysis of 0.5-g samples of livers from the same rats confirmed that significant differences did not exist in HCP concentrations between the three major lobes. Also, HPLC analysis of 0.5-g subsections of livers from individual rats which had very high HCP concentrations (10,000–85,000 pmol/g) showed less than 15% variation (data not shown).

The small variability in HCP concentration within a liver from any particular rat contrasted markedly with the variability in HCP concentrations in the livers of porphyric rats killed on the same day. This rat-to-rat variation was at least 5-fold on three of the days in the 1000-CO experiment and at least 3-fold on four of the days in the 1000-NCO rats (Fig. 2). On day 56, the HCP levels in the livers of 100-CO rats ranged from 900 to 44,000 pmol/g (N = 4), a 49-fold variation (Table 2). Rat-to-rat differences in organ concentrations of HCB were considerably smaller (Fig. 1). There was no correlation between HCP and HCB concentrations in individual rats.

Figure 3 shows the effects of HCB on the concentrations of liver HCPs, coproporphyrin, and protoporphyrin. Protoporphyrin did not become elevated in the 1000-CO rats, but appeared to be slightly higher on day 34 in the 1000-NCO rats. Coproporphyrin increased in all groups, and reached an apparent maximum of about 700 pmol/g in the two groups receiving diets containing 1000 ppm HCB.

Animal health. The rats fed the 1000-CO diet became extremely sick. By day 5 most appeared very lethargic, and their fur became rough and wet-looking in appearance. Poor grooming was evident. By day 10 most of the rats had constant tremors, and some had violent convulsions. One rat died on day 18 and another died on day 23. Weight gain and food

Table 2. Concentrations of hexachlorobenzene (HCB) and highly carboxylated porphyrins (HCPs) in the livers of female Wistar rats fed HCB (pre-dissolved in corn oil) in their diet at 100 ppm (the 100-CO group)

Day	Rat No.	HCB (ppm)	HCPs (pmol/g)
4	1	26	45
	2	23	61
	3	36	57
7	1	32	1.1×10^2
	2	33	1.2×10^2
	3	33	1.1×10^2
	4	27	1.2×10^2
14	1	61	3.3×10^2
	2	55	1.8×10^2
	3	54	1.3×10^2
21	1	29	2.9×10^2
	2	45	2.8×10^2
	3	57	2.7×10^2
35	1	71	5.4×10^2
	2	67	3.8×10^2
	3	58	4.2×10^2
42	1	66	1.6×10^3
	2	75	5.5×10^3
	3	78	8.6×10^2
56	1	70	4.4×10^4
	2	60	2.7×10^3
	3	75	9.1×10^2
	4	83	8.2×10^2

intake was considerably lower in the HCB-exposed rats then the control rats (data not shown), and the livers of the HCB-exposed rats increased greatly in size (Fig. 4). On an organ-to-body weight basis, kidney and spleen weights were increased 34 and 88%, respectively, over control weights by day 25. The 1000-CO experiment was terminated on day 25 to prevent further suffering of the animals.

The extreme toxic effects of the 1000-CO diet contrasted markedly with the toxicity of the other experimental diets. The rats on the 1000-NCO diet gained weight at approximately the same rate as the control rats (data not shown), and liver-to-body weight ratios did not differ significantly from those of control animals (Fig. 4). Similarly, kidney and spleen weights did not become elevated in the 1000-NCO and 100-CO rats (data not shown). It was not until day 34 that very slight tremors were observed in some individuals of the 1000-NCO group. These tremors continued until the end of the experiment (day 54). Most rats appeared slightly lethargic at this time, but they appeared to be in considerably better health than the 1000-CO rats were on day 5. No convulsions were observed in the 1000-NCO group.

The 100-CO rats and the rats that were fed the 1000-CO diet for 1 day only did not develop obvious health problems. They gained weight at the same rate as the control animals (data not shown), and organ-to-body weight ratios were normal. Lethargy and tremors were not observed.

DISCUSSION

Time delay. The existence of a relatively long delay between the initial exposure to PHAHs and the development of porphyria has become accepted as a fundamental characteristic of the disorder [7, 8]. In the Wistar rat, delays of at least 3–4 weeks are reported for doses of HCB as high as 3000 ppm in the diet [7] and 170 mg/rat/day as a water suspension [15]. However, as previously reported by us in short communications [10, 11], and as shown here in more detail, a long delay is not intrinsic to HCB-induced porphyria. The delay before HCPs became elevated was at most 2 days in female Wistar rats given HCB as a corn oil solution in the diet at 1000 ppm (the 1000-CO group; Fig. 2a), 6 days when in crystalline form at 1000 ppm (the 1000-NCO group; Fig. 2b), and 7 days when at 100 ppm as a corn oil solution (the 100-CO group; Fig. 2c). There were, however, considerable delays before HCPs became elevated in kidney and spleen (Fig. 2, a–c). Therefore, the extent of the delay is, in part, dependent upon where the porphyrins are measured. The fact that many researchers monitor the development of porphyria by measuring urinary porphyrins has also likely contributed to the notion of a long delay. Marks [16] stated that the accumulation of uro- and heptacarboxylic acid porphyrins occurs in the liver considerably before these porphyrins are excreted in the urine. While we believe this to be likely, we are not aware of studies which demonstrate this.

Our studies suggest that at least two factors have contributed to the widely-held notion of a delay in PHAH-induced porphyria. First, the common method of measuring total liver porphyrins (as determined by the total fluorescence of liver extracts) is insensitive to the earliest development of the disorder due to the relatively small and variable contribution of uroporphyrin to the fluorescence of such extracts (see [10] and Table 1). Second, most researchers administer HCB in a form which does not allow for rapid absorption (i.e. crystalline in the diet or as a water suspension). Our comparisons of the time courses of organ accumulation of HCB when administered at 1000 ppm in one of the traditional ways (in crystalline form—the 1000-NCO group) and as a corn oil solution in the diet (the 1000-CO group) show that HCB in corn oil is absorbed extremely rapidly, and reaches relatively high levels in liver, kidney, and spleen (Fig. 1a). Rapid absorption of HCB caused a dramatic increase in liver HCPs (Fig. 2a). The slower absorption of HCB in the 1000-NCO group caused a slower initial rate of liver HCP accumulation (compare Fig. 2a to Fig. 2b).

PHAH-induced elevation of kidney and spleen HCPs has been demonstrated by other investigators, but the origin of the HCPs is not known. Doss *et al.* [17] have suggested that, in the rat, elevated kidney porphyrins originate in the liver and that they are transported to the kidney in the serum but that spleen porphyrins are generated *in situ*. Experiments which umambiguously prove the source of kidney and spleen porphyrins, however, have not been conducted. The present study showed nothing distinctive about elevated porphyrin patterns in kidney and spleen which suggested their probable origin.

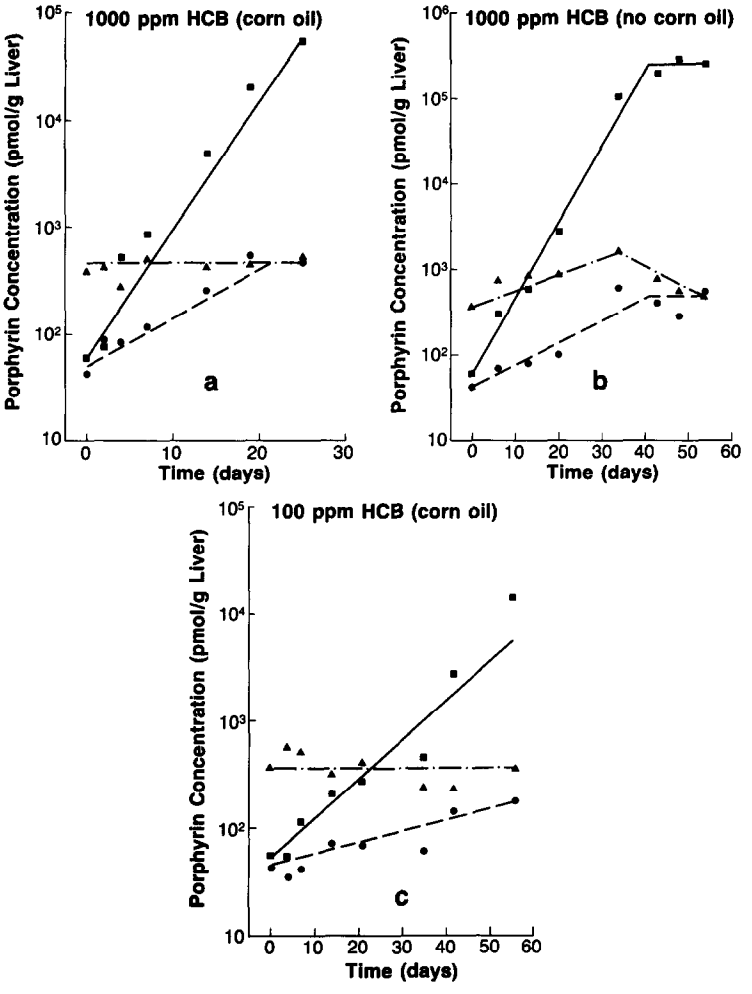

Fig. 3. Time course of liver HCP, coproporphyrin, and protoporphyrin accumulation in female Wistar rats. Rats were exposed to the compound in their diets at concentrations of (a) 1000 ppm (pre-dissolved in corn oil), (b) 1000 ppm (solid), (c) 100 ppm (pre-dissolved in corn oil). All points are means (N = 3–7). No changes in the porphyrin patterns in the control rats were observed over time, and therefore all control data are included on day 0 to simplify the graph. Key: (■) HCP, (●) coproporphyrin, and (▲) protoporphyrin.

Threshold HCB concentration. Because measurement of liver HCPs (rather than total porphyrins) and administration of HCB in corn oil (rather than as a solid) dramatically shortened the perceived "lag time" in porphyria development, we questioned (1), the commonly held belief that chronic exposure to PHAHs is required to elicit porphyria in the rat (for reviews, see [7] and [8]), and (2) whether a threshold concentration of HCB in the target organ(s) is required. The results of the 1-day dosing experiment with HCB (1000-CO) demonstrated that chronic exposure is not required to cause slight porphyria in the Wistar rat (Fig. 2d). It is worth noting that Smith and Francis [18] have reported recently that a single oral dose of a corn oil solution of HCB (100 mg/kg body weight) induces massive porphyria in male C57BL/6 mice with iron overload. Thus, continuous exposure to HCB is not required to induce porphyria in the rat or the mouse. In the present experiment with the Wistar rat, liver HCPs did not become more than 4-fold elevated, and the mean level was only 1.5 times normal on day 51. Therefore, liver HCB levels of 20–30 ppm for 5 weeks (day 9 to day 44; Fig. 1d) were sufficiently high to cause only a slight HCP elevation.

Continuous exposure to HCB at levels above 30 ppm (panels a–c of Fig. 1) caused exponential increases in HCPs (panels a–c of Fig. 2). It appears that liver exposure to HCB at a concentration above 30 ppm is required to cause porphyria. Because we were not able to discriminate between kidney and spleen porphyrins which might be generated *in situ* from those which are transported to these organs from the liver via the plasma, it is not possible to say if similar mechanistically significant thresholds exist in these organs.

Once the porphyria began, HCP concentration increased exponentially in each dosing regime where

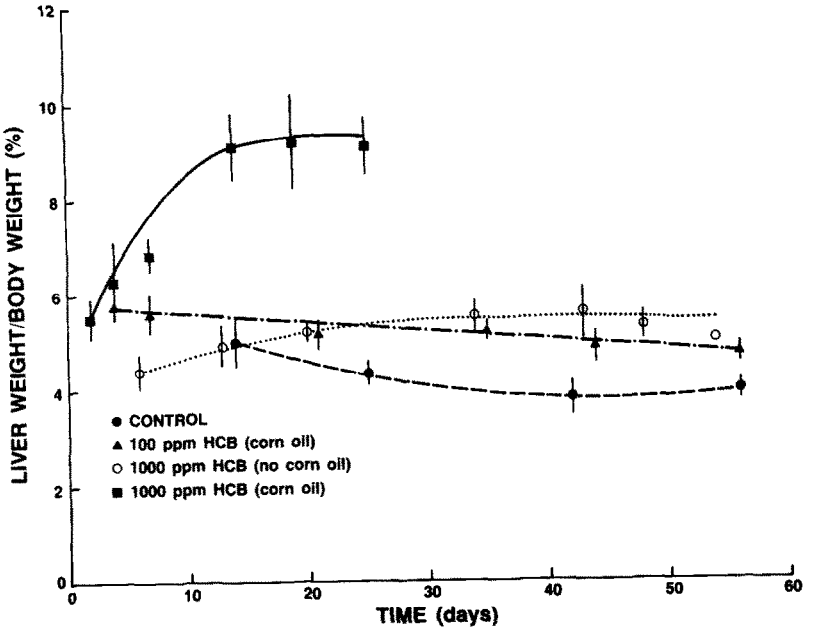

Fig. 4. Comparison of the liver-to-body weight ratios in female Wistar rats exposed to hexachlorobenzene (HCB). Rats were given HCB in their diets at concentrations of 100 ppm (pre-dissolved in corn oil) and 1000 ppm (added to diet both as a solid and as a corn oil solution). Values are means ± SD, N = 3–7.

HCB was administered continuously. Interestingly, organ-to-organ comparisons show that the rates appeared identical in the two 1000 ppm HCB groups (panels a and b of Fig. 2), yet organ concentrations of HCB in the 1000-CO group were considerably higher than those in the 1000-NCO group. From day 6 to day 20 the liver concentration of HCB in the latter group rose from approximately 75 to 135 ppm. HCB within this concentration range was, therefore, sufficiently high to cause porphyria to develop at the apparent maximal rate (compare Fig. 2a to Fig. 2b). The rate of development in the 100 ppm group (Fig. 2c) was, however, considerably slower. The maximum liver concentration of HCB in this group was 75 ppm, and it therefore appears that levels above this are required to cause porphyria to develop at the maximal rate.

HCB and HCP correlations (dose–response). There has been considerable interest in using alterations to normal porphyrin patterns as indicators of exposure to HCB and to other PHAHs [reviewed in Ref. 16 and in several articles of Ref. 19]. It would be of interest, therefore, to know if one could correlate the severity of the prophyria with liver concentrations of PHAHs. The results of the studies presented here suggest that for HCB this probably will not be possible. HCP concentrations were found to vary considerably in the livers of individual rats within particular groups and days (as much as 49-fold; Table 2). This finding is consistent with that found by others in mammals [7, 20] and birds [21, 22]. Our results show that these differences do not reflect differences in organ concentration of HCB (representative data shown in Table 2). Explanation for this phenomenon will require further research. It is possible that such large differences are the natural outcome of a response which exponential, i.e. early small differences become magnified with time. This hypothesis could be tested by monitoring individual plasma HCP concentrations in a group of identically dosed rats over the course of several weeks.

Group-to-group comparisons also show no correlation between HCB and HCP concentrations. As an example, the mean liver HCB and HCP concentrations were approximately 320 ppm and 1000 pmol/g, respectively, in the 1000-CO rats on day 9 (Figs. 1a and 2a). Similar concentrations of HCB in the livers of the 1000-NCO rats (Fig. 1b, days 34–54) caused HCPs to be at least 100,000 pmol/g.

Porphyria, liver size, and health. Because fully-developed porphyria is associated with photosensitivity, liver damage, neurotoxicity [7], and liver cancer [23, 24], there has been some interest in predicting the likelihood of developing such disorders when porphyria is detected. The presently described experiments illustrate that in the Wistar rat the degree of HCB-induced neurotoxicity and liver enlargement are not necessarily correlated with, and do not appear to be caused by, the degree of porphyria. The most dramatic illustration of this was the contrast between the extreme toxicity caused by the 1000-CO diet and the relatively mild toxicity elicited by the 1000-NCO diet. Despite the extreme toxic effects caused by the 1000-CO diet, it was the 1000-NCO diet that caused much higher HCP levels (panels a and b of Fig. 2). Therefore, as first suggested by Ockner and Schmid [25], and more recently by Rozman *et al.* [20], the mechanisms of HCB-induced mortality and porphyria appear to be different. Therefore, assessment of the health of

individuals found to have abnormal porphyrin patterns will require other measurements of hepatic, hormonal, and neurological status.

Conclusions. We conclude that:

(1) There is not a long time delay in the onset of porphyria in female Wistar rats exposed to HCB in their diets at concentrations of 100 ppm and 1000 ppm if the criterion is HCP elevation in the liver. Application of a very sensitive method of porphyrin analysis, measurement of porphyrins in the liver rather than the urine, and solubilization of the HCB in corn oil contribute to reducing the time delay.
(2) A threshold liver HCB concentration of approximately 25–30 ppm appears to be required to induce porphyria in female Wistar rats.
(3) Organ concentrations of HCB and HCPs are not necessarily correlated.
(4) Liver size and ill-health are not correlated with the severity of the porphyria.

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