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THYROID FUNCTION AND THYROXINE METABOLISM IN HEXACHLOROBENZENE-INDUCED PORPHYRIA

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Abstract—The effects of hexachlorobenzene (HCB) administration on the development of porphyria and on changes in thyroid function and thyroid hormone metabolism were examined. Female Wistar rats were treated with HCB for 1 or 8 weeks. At both treatment times liver weight was notably increased with a slight change in thyroid weight at 8 weeks. Serum thyroxine (T_4) levels were depressed, whereas levels of triiodothyronine (T_3) were not depressed significantly at both treatment times. One or eight weeks of HCB treatment did not alter the incorporation and distribution of [^{125}I] into intrathyroidal aminoacids. A 50% reduction in protein bound iodine (PB[^{125}I]) was seen in both groups of animals. HCB altered [^{125}I]T_4 metabolism in rat liver slices, increasing T_4 dehalogenation. HCB administration for 1 week did not affect urinary excretion of porphyrins or their precursors, or hepatic porphyrin content. The activity of aminolaevulinate synthase was not affected, but there was a 25% and 51% inhibition in porphyrinogen carboxy-lyase (PCL) activity for the uroporphyrinogen disappearance or the coproporphyrinogen formation respectively. After 8 weeks of HCB administration the rats showed a characteristic porphyria. Our results show that HCB treatment increased hepatic thyroxine metabolism, without alterations in thyroid hormone synthesis. Serum T_4 and PCL activity behaved differently in both time- and dose-dependent studies, with serum T_4 being the more sensitive parameter which responded at earlier times and lower doses.

Hexachlorobenzene (HCB†) is a toxic polyhalogenated aromatic compound, widely distributed in the environment and a very persistent pollutant. In the past, it was used as a fungicide and is now produced as a by-product of several chlorination procedures. HCB is also a contaminant of other pesticides that are used [1]. It is a highly lipophilic compound, and it accumulates in the body fat [2].

Chronic exposure of rats and humans to HCB produces a number of effects, such as triggering of porphyria, increased synthesis of liver microsomal enzymes, and hypothyroidism [2–6]. It also has been reported that some patients who were poisoned after ingesting HCB, a fungicide added to wheat seedlings, developed porphyria (Turkish cases) and exhibited enlarged thyroids after 20–30 years [7]. Other chemicals, such as polychlorinated biphenyls (PCB) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) caused similar manifestations [8, 9] in laboratory animals.

HCB is carcinogenic in animals [10]. Hamsters fed HCB for a long period show a significant incidence of thyroid adenomas [11]. TCDD produces thyroid tumors in rats [12]. Exposure of rats to polybrominated biphenyls (PBB) results in a disruption of the normal homeostasis of the pituitary–thyroid axis and is associated with accumulation and retention of PBB in the thyroid [13].

The purpose of the present work was to investigate the effects of HCB administration on the development of porphyria and on changes in thyroid function and thyroid hormone metabolism. Since HCB porphyria appears to develop only after relatively long-term exposure, we examined the effects of chronic (8 weeks) versus short (1 week) exposure to HCB, on the induction of porphyria and on changes in thyroid status.

MATERIALS AND METHODS

Chemicals

HCB (commercial grade), with the following composition: HCB 95%, tetra-and pentachlorobenzene 5%, was a gift from Companía Química S.A. (Buenos Aires, Argentina). Uroporphyrin III was obtained from Porphyrin Products (Logan, UT) and Sephadex G-25 from Pharmacia (Uppsala, Sweden).

Porphyrinogens were prepared with sodium amalgam according to Mauzerall and Granick [14]. Propyl-2-thiouracil (PTU) and pancreatin were purchased from the Sigma Chemical Co. (St Louis, MO).

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† Abbreviations: HCB, hexachlorobenzene; ALA, δ-aminolaevulinate; PBG, porphobilinogen; ALA-S, ALA synthase; PCL, porphyrinogen carboxy-lyase; KRP, Krebs–Ringer-phosphate buffer; T_3, triiodothyronine; T_4, thyroxine or tetraiodothyronine; TSH, thyrotropin; PTU, propylthiouracil; PCB, polychlorinated biphenyls; PBB, polybrominated biphenyls; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Animals

Female Wistar rats, from the National Atomic Energy Commission, weighing 160–180 g at the start of the experiment were fed Purina 3 diet (Cabeca S.C. A., Argentina) and water *ad lib*. Environmental conditions consisted of a 12 hr light–12 hr dark cycle, 20–24° and 45–75% humidity. HCB (1 g/kg body weight) was administered daily by stomach tube for different periods of time. The drug (40 mg/mL) was suspended in water containing Tween 20 (0.5 mL/ 100 mL). Animals were killed by ether anesthesia after 1 or 8 weeks of treatment.

Intrathyroidal iodine metabolism

Animals were injected with 10 μCi (carrier free) [^{125}I] in 0.5 mL of 0.9% NaCl, 24 hr before they were killed. Thyroids were weighed, placed in 1 mL of 0.1 M Tris-buffer (pH 8.5), containing 1 mM PTU, and stored in an ice bath. PTU was dissolved by adding a few drops of NH_4OH or NaOH in a small volume of Tris-buffer. The glands were then individually homogenized and digested with pancreatin according to Inoue and Taurog [15].

Separation of the different labelled iodo compounds was achieved by ascending paper chromatography of the digests utilizing the following solvent systems: (*n*-butanol:ethanol:1.2 N ammonium hydroxide, 5:1:2 by vol.) (BEA) and (*n*-butanol:acetic acid:water, 38:5:6) (BAW), for 24 hr. Standards of iodine and of iodo aminoacids were run simultaneously to help in identification of the spots. Each strip was cut and, after counting in an automatic gamma spectrometer, the distribution of radioactivity was calculated.

The chromatography was developed with 1% ninhydrin in acetone and with 0.5% $PdCl_2$. The radioactivity in each spot was counted, and the percentage of the total radioactivity in each one was calculated.

Metabolism of [^{125}I]T_4 in liver

The animals were decapitated and the liver was washed thoroughly with cold saline. Liver slices of uniform thickness, weighing ~ 200 mg, were prepared with a Staddie–Riggs microtome (Arthur Thomas Co., U.S.A.). Liver slices were incubated at 37° in KRP (Krebs–Ringer phosphate buffer), 8 mM glucose, pH 7. 4. After 30 min, a tracer dose (usually 2 μCi) of [^{125}I]T_4 (NEN, U.S.A., sp. act. 1250 μCi/μg) was added, and the incubation was continued for another 60 min. At the end of this period, the slices were washed extensively with cold saline, blotted on filter paper, and weighed. Each slice was homogenized in 1 mL of 1 mM PTU. Homogenates were centrifuged, and aliquots of each supernatant fraction were analyzed by ascending chromatography in BEA, as already described. The labelled substrate T_4 was 90% pure as judged by paper chromatography and contained 6–8% I^- and 0.8–2% T_3.

In some experiments, some vessels were incubated at 37° but without tissues or in some cases with boiled inactivated tissue. No generation of [^{125}I]T_3 from [^{125}I]T_4 was observed in these controls, indicating the absence of artifactual conversion of T_4 to T_3 either during incubation or during chromatography. Values for the percentage generation of the several products of T_4 metabolism were always corrected for the percentage contamination by each in the substrate employed, as assessed by paper chromatography.

Iodine incorporation

Studies in thyroids. The glands were homogenized as already indicated, and 1 vol. of cold 20% trichloroacetic acid (TCA) was added. The precipitate obtained was washed twice with 10% TCA. Total and TCA precipitable radioactivities were measured, and the percentage present in the latter was calculated.

Studies in serum. One milliliter of serum was precipitated with 1 mL of cold 20% TCA, and the precipitate was treated as described before.

Uptake of [^{125}I]T_4 by liver slices

Liver slices were incubated as indicated before. The radioactivity of the slices and of an aliquot of the incubation medium was measured, at each time. The L/M ratio was calculated as follows:

$$L/M = \frac{\text{cpm/mg tissue}}{\text{cpm/mL medium}}.$$

Determination of serum thyroid hormones

Blood samples were taken and serum was stored at −20° until assayed. Total serum T_3 and T_4 concentrations were determined by radioimmunoassay (RIA), with a double antibody -PEG technique. Each sample was assayed in duplicate. Control standards prepared in serum from intoxicated rats were performed to test for possible effects of HCB in the T_4 and T_3 assays. Serum TSH was measured according to the RIA kit and the procedure for rat TSH provided by the National Pituitary Agency, U.S.A.

Tissue preparation for heme metabolism studies

Livers were weighed and divided into two portions: (1) homogenates for estimation of porphyrin content and PCL enzyme activity were made with 0.154 M KCl (1:5, w/v). They were centrifuged at 11,000 *g* for 20 min. Supernatant fraction from porphyric liver was filtered through a Sephadex G-25 column with 0.134 M potassium phosphate buffer, pH 7.0. The eluates with no, or trace, fluorescence were pooled and used as the enzyme preparation; (2) livers were homogenized in a 0.9% (w/v) NaCl, 0.5 mM EDTA, 10 mM Tris–HCl buffer (pH 7.4) mixture for determination of ALA-S activity. All procedures were carried out at 4°.

Hepatic and urinary contents of porphyrins and precursors

Urine specimens were collected in dark vessels, at room temperature.

Analyses of δ-aminolaevulinate (ALA), porphobilinogen (PBG) and porphyrins were done on 24-hr urine specimens using Dowex 1 and Dowex 50 columns and measuring ALA and PBG colorimetrically [6]. Porphyrin content in liver was determined in whole homogenate as total free porphyrins in 5% (w/v) HCl [16].

Table 1. Effect of HCB administration on body, liver and thyroid weights

Treatment	Body weight (g)	Liver weight (g)	Thyroid weight (mg)	Liver/body wt*	Thyroid/body wt†
Control	174 ± 5 (16)	7.01 ± 0.41 (16)	15.90 ± 0.46 (12)	3.99 ± 0.14 (16)	8.79 ± 0.30 (12)
HCB, 1 week	178 ± 2 (20)	10.16 ± 0.34‡ (20)	16.27 ± 0.95 (12)	5.68 ± 0.19‡ (20)	9.28 ± 0.28 (12)
Control	203 ± 9 (14)	7.19 ± 0.47 (8)	16.13 ± 0.65 (9)	3.70 ± 0.11 (8)	8.39 ± 0.58 (9)
HCB, 8 weeks	205 ± 6 (16)	9.81 ± 0.39‡ (13)	19.40 ± 1.05§ (11)	4.92 ± 0.14‡ (13)	9.06 ± 0.51 (10)

Values are means ± SE of the number of rats given in parentheses.
* Liver/body wt = liver weight (g)/body weight (g) × 100.
† Thyroid/body wt = thyroid weight (mg)/body weight (g) × 100.
‡ $P < 0.0005$ vs appropriate control.
§ $P < 0.02$ vs appropriate control.

Enzyme activities

ALA-S was assayed in whole homogenates by the method of Marver *et al.* [17] using glycine as substrate. PCL activities were assayed according to Wainstok de Calmanovici *et al.* [18].

Protein assay

Protein was determined by the method of Lowry *et al.* [19], with crystalline bovine serum albumin as standard.

Statistical evaluations

Results are means ± SE Statistical analysis was performed by Student's *t*-test. In some cases the Mann–Whitney test for independent samples was performed [20].

RESULTS

Effect of HCB administration on body, thyroid and liver weights

The effects of short- and long-term HCB administration on thyroid and liver weights, as compared to controls, are shown in Table 1. At both 1 and 8 weeks of treatment, mean liver weight and the liver/body weight ratio (liver weight (g)/body weight (g) × 100) were greater than those of the control animals.

HCB administration did not produce an increase in mean thyroid weight, following 1 week of treatment, but increased it slightly (20%) after 8 weeks of treatment. On the other hand, the thyroid/body weight ratio (thyroid weight (mg)/body weight (g) × 100) was not affected in either case.

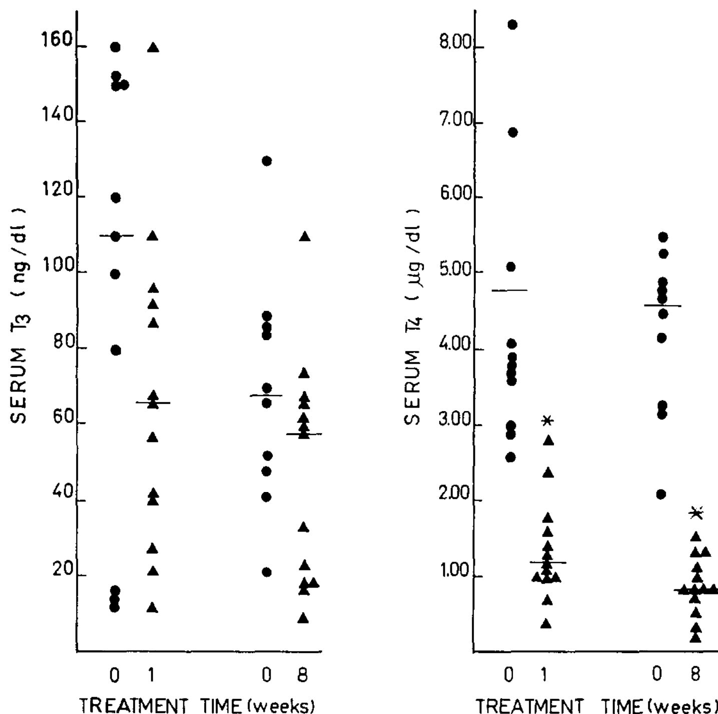

Fig. 1. Effect of HCB on serum thyroxine (T_4) and triiodothyronine (T_3) levels. Values are given for individual animals. The median value is represented by (−). The Mann–Whitney test for independent samples was performed. Key: significantly different from the control: (*) $P < 0.001$.

Table 2. [^{125}I] Incorporation by thyroid gland, and serum protein iodine (PB[^{125}I])

Treatment	Treatment time (weeks)	Thyroid gland: TCA precipitate (cpm) × 100 / Total radioactivity (cpm)	Serum PB [^{125}I]: TCA precipitate (cpm) × 100 / Total radioactivity (cpm)
Control		95.7 ± 2.3 (4)	42.3 ± 1.2 (4)
HCB	1	95.2 ± 0.6 (4)	22.4 ± 2.1* (4)
HCB	8	94.3 ± 2.4 (5)	18.2 ± 2.4* (5)

Values are means ± SE for the number of animals given in parentheses. Animals were injected with 10 μCi [^{125}I], 24 hr before they were killed.
* Significantly different from control group ($P < 0.0001$).

Effect of HCB administration on serum T_4 and T_3 levels

The effects of HCB on serum T_4 and T_3 are shown in Fig. 1. A significant decrease of the mean T_4 level in both groups of treated animals was observed. T_3 was slightly, but not significantly, depressed only after the prolonged treatment.

Effect of HCB administration on serum TSH levels

Following 8 weeks of HCB administration, serum TSH levels were elevated compared to the control levels (C = 3.06 ± 0.31 ng/mL, HCB = 5.62 ± 1.24 ng/mL, $P < 0.02$). After 1 week of HCB administration, TSH levels were not altered.

[^{125}I] *Incorporation by thyroid gland and serum PB* [^{125}I]

To explore a possible effect of HCB on iodine incorporation into thyroglobulin, [^{125}I] incorporation into the thyroid TCA precipitable protein was studied.

[^{125}I] Incorporation into the thyroid was measured after injecting [^{125}I] into rats treated for 1 or 8 weeks with HCB (Table 2). Total thyroid TCA precipitate, that is, iodine bound to thyroglobulin, did not show any difference in treated animals, at either 1 week or 8 weeks.

Serum TCA precipitable protein was reduced markedly in both cases. This fraction represents thyroid hormone bound to serum carrier proteins.

Thyroidal [^{125}I] *incorporation into the aminoacids of thyroglobulin following HCB administration*

Table 3 shows the incorporation of [^{125}I] into the aminoacids of thyroglobulin.

The relative intrathyroidal distribution of radioiodinated aminoacids was not affected by HCB (8 weeks) treatment when compared to that of the control animals. The same results were obtained for animals treated for 1 week, with both chromatographic systems (data not shown).

Table 3. Effect of HCB administration on relative intrathyroidal distribution of [^{125}I]

(a) BEA

Treatment	Treatment time (weeks)	Iodide	MIT + DIT	T_3	T_4
Control		10.95 ± 0.88	73.0 ± 1.6	1.35 ± 0.13	6.16 ± 0.51
HCB	8	9.43 ± 0.66	75.6 ± 2.5	1.31 ± 0.32	5.93 ± 0.58

(b) BAW

Treatment	Treatment time (weeks)	Iodide	MIT	DIT	$T_3 + T_4$
Control		6.21 ± 0.22	26.68 ± 1.86	40.09 ± 3.04	6.24 ± 0.56
HCB	8	8.24 ± 1.52	22.12 ± 2.05	47.05 ± 2.03	6.05 ± 0.60

Animals were injected with 10 μCi[^{125}I], 24 hr before they were killed. The glands were homogenized with 1 mM PTU and digested with pancreatin. The percent distribution of radioactivity was examined by paper chromatography. Results with the BEA (a) and BAW (b) solvent systems (see Materials and Methods) are shown. Each value is the mean ± SE from four animals. The difference in radioactivity to 100% appears in the origin (iodo proteins) and front of the chromatography (iodolipids). Abbreviations: MIT, monoiodotyrosine; and DIT, diiodotyrosine.

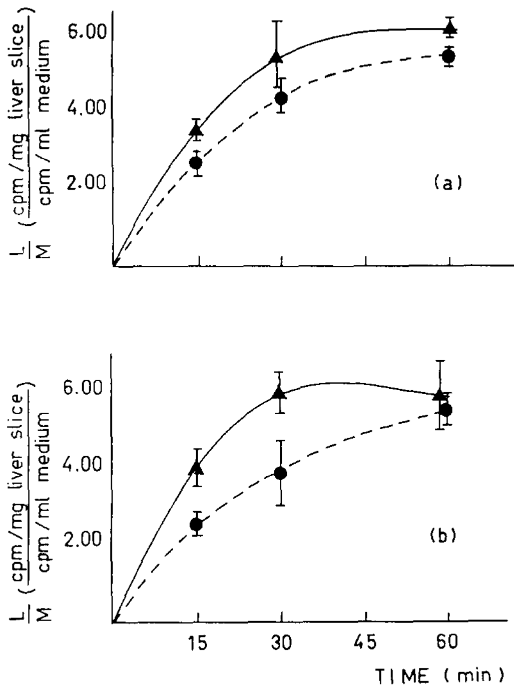

Fig. 2. Time course of the uptake of [^{125}I]T_4 by liver slices. Each value is the mean ± SE of four slices. The slices were incubated during the indicated times. L/M = liver radioactivity (cpm)/medium radioactivity (cpm). Key: (a) 1-week treatment; (b) 8-weeks treatment; (●) control; and (▲) HCB-treated rats.

Uptake of [^{125}I] T_4 by liver slices

Figure 2 shows the uptake of [^{125}I]T_4 by rat liver slices. The L/M ratio (cpm/mg liver slice)/(cpm/mL medium) for control animals increased to a value of around 4 at 30 min and reached a plateau by 60 min.

In animals treated with HCB for 8 weeks, the L/M ratio reached a value of around 6 at 30 min and stood at a plateau for up to 60 min. On the other hand, the entry of labeled T_4 was not significantly different in animals treated with HCB for 1 week when compared to that of control rats.

[^{125}I] T_4 metabolism

Results of the studies on [^{125}I]T_4 metabolism are shown in Fig. 3. Liver slices dehalogenated labeled thyroxine, generating around 30% of iodide and 5% of T_3 in control animals.

HCB treatment for 8 weeks caused a 45% increase in T_4 dehalogenation, with a significant increase in iodide generation. T_3 did not change significantly.

Urinary excretion of precursors and porphyrins

The urinary excretion of ALA, PBG and porphyrins was determined throughout the experiment to detect the onset of porphyria. As shown in Fig. 4, urinary ALA, PBG and porphyrins in HCB-treated animals started to differ significantly from normal values after 16 days of intoxication. Then at longer treatment times the precursors as well as the porphyrins underwent a marked increase. The mean values ± SE for four rats at 60 days of HCB administration were: 295 ± 63 μg/24 hr for ALA;

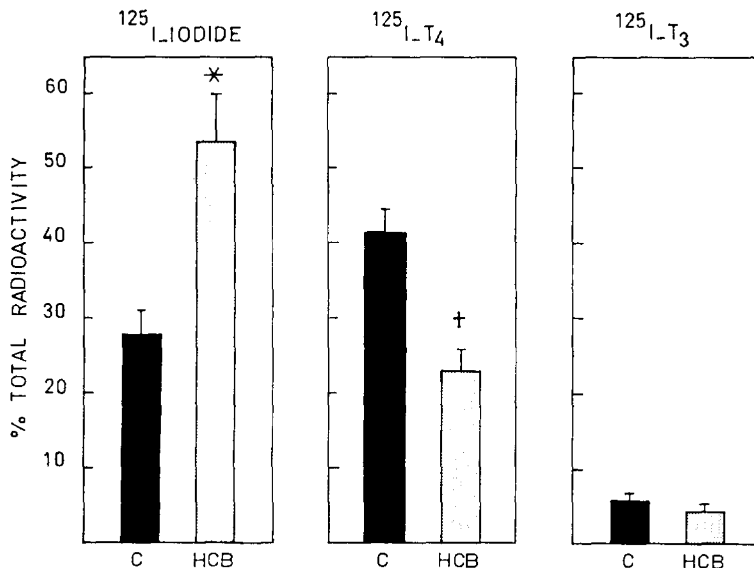

Fig. 3. [^{125}I]T_4 hepatic metabolism. Liver slices were pulse-labeled with [^{125}I]T_4 for 60 min as described in Materials and Methods. After homogenization with 1 mM PTU, the percent distribution of radioactivity was examined by paper chromatography in the BEA system. Bars and vertical brackets depict the mean and SE of values obtained for C = 11 rats and HCB = 14 rats. The absence of an indicated P value denotes lack of statistical significance between groups. Key: (*) $P < 0.005$, and (†) $P < 0.0005$.

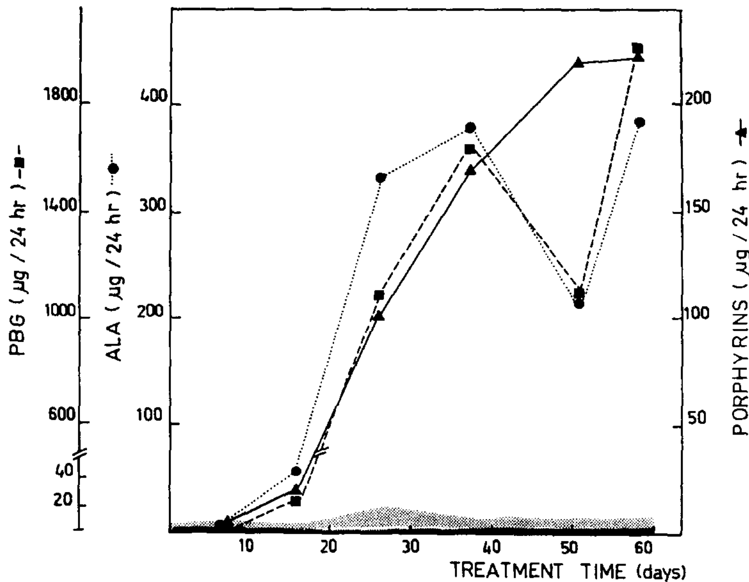

Fig. 4. Time courses of urinary excretion of precursors and porphyrins. Female rats were treated with HCB (1 g/kg daily by gastric intubation). HCB was given from week 0 and throughout the experiment. Porphyrins and precursors were estimated as described in Materials and Methods. Each point represents urinary excretion of an individual rat characteristic of the HCB-treated group. Lower shaded areas represent the range of (▩) ALA, and (▨) PBG and porphyrins obtained from ten normal rats.

1759 ± 260 µg/24 hr for PBG; and 237 ± 34 µg/24 hr for porphyrins. Control values for ten rats were: 14.82 ± 2.66 µg/24 hr for ALA; 2.16 ± 0.47 µg/24 hr for PBG; and 1.89 ± 0.51 µg/24 hr for porphyrins.

Liver porphyrin content

As can be seen in Table 4, rats treated with HCB for 1 week showed a liver porphyrin concentration no different from that in untreated rats, whereas 8 weeks of fungicide treatment sharply increased the values to 167 times that of the control.

Enzymatic activity of ALA-S

The activity of ALA-S, the rate-limiting enzyme of the heme biosynthetic pathway (Table 4), was not altered significantly after 1 week of drug treatment. At 8 weeks of HCB intoxication the specific activity was about twice the control value.

Enzymatic activity of PCL and protein concentration of liver homogenate supernatant fraction

As shown in Table 4, the activity of PCL was decreased significantly (25%) after 1 week of HCB administration. This decrease was enhanced to 81% at 8 weeks of treatment. When the activity was measured as coproporphyrinogen formation (data not shown), the same behavior was observed. In this case, the decreases observed were 51 and 95% at 1 and 8 weeks of treatment respectively.

Protein concentration was determined to cor-

Table 4. Effect of HCB intoxication on liver porphyrin content, protein concentration, ALA-S, and PCL activity

Treatment	Treatment time (weeks)	Liver porphyrins (µg/g wet wt)	ALA-S activity (nmol ALA/g liver/hr)	PCL: Protein (mg/mL)	PCL: Activity nmol porphyrin / mg protein/30 min
Control		3.1 ± 1.3	8.8 ± 1.4	15.1 ± 0.7	0.83 ± 0.03
HCB	1	6.3 ± 3.8	9.6 ± 1.7	16.5 ± 1.1	0.62 ± 0.02*
	8	233.1 ± 75.0*	17.2 ± 2.1*	14.0 ± 1.0	0.13 ± 0.03†

Treatment conditions were as described in the legend of Fig. 4. Total free porphyrin contents in 5% (w/v) HCl, proteins and ALA-S activity were measured as indicated in Materials and Methods. PCL activity: the 11,000 g supernatant fraction of control or Sephadex G-25 eluates of HCB-treated rats was used as the enzyme source. The activity was measured as the formation of 7- + 6- + 5- + 4-carboxyporphyrinogens, with uroporphyrinogen III as substrate. Data are means ± SE for eight animals.

*, † Significantly different from control: *$P < 0.005$ and †$P < 0.001$.

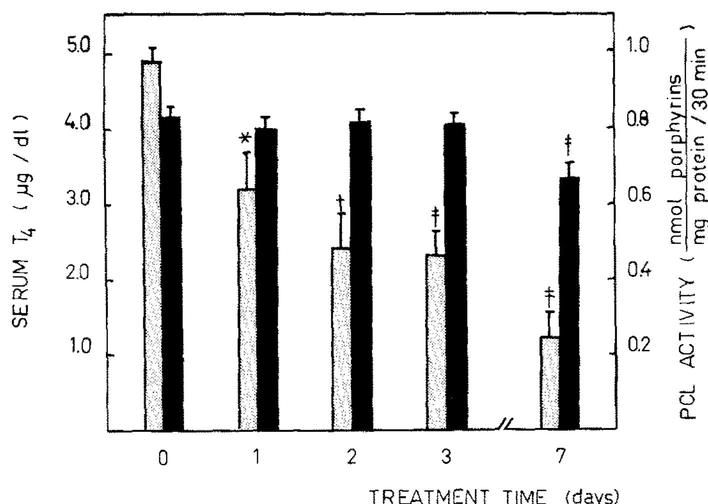

Fig. 5. Time response to HCB administration of serum T_4 levels and PCL activity. HCB (1 g/kg body weight by gastric intubation) was given for 1, 2, 3, or 7 days. T_4 serum levels and PCL activity were estimated as described in Materials and Methods. Bars and vertical brackets depict the mean and SE of values obtained for four rats. Key: significantly different from the control: (*) $P < 0.05$, (†) $P < 0.01$, and (‡) $P < 0.005$; (▩) serum T_4, and (■) PCL activity.

roborate that the slight decrease in PCL activity, observed after 1 week of HCB treatment, was not due to an increase in liver protein concentration. As shown in Table 4, protein concentration of the 11,000 *g* liver homogenate supernatant fraction was not altered by HCB treatment.

Time response to HCB administration of serum T_4 levels and PCL activity

The effects of HCB on serum T_4 and PCL activity are shown in Fig. 5. A significant decrease of T_4 levels (35%) was observed following 1 day of HCB administration, reaching to 75% at 7 days of treatment. On the other hand, PCL activity was not affected at these early times, being decreased only at 7 days of treatment.

Serum T_4 levels and PCL activity as a function of HCB dose

T_4 levels and PCL activity were determined in animals treated daily with different doses of HCB for 1 week (Fig. 6). In the present study, exposure for 1 week to 0.25, 0.5 and 1 g/kg body weight of HCB produced significant decreases in T_4 levels ranging from 40 to 65%. On the other hand, PCL activity was decreased slightly (20%) at 0.5 and 1 g/kg body weight of HCB, without any significant decrease at the lowest dose.

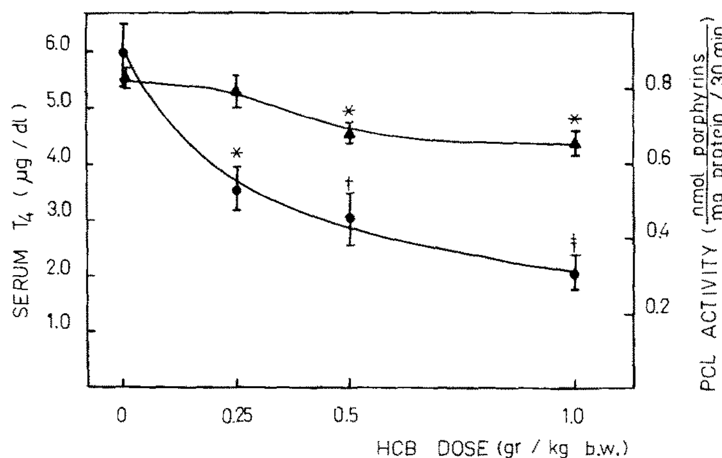

Fig. 6. Serum T_4 levels and PCL activity as a function of HCB dose. Rats were treated daily with HCB (0.25, 0.5, and 1.0 g/kg body wt) by gavage for 1 week. T_4 serum levels and PCL activity were estimated as described in Materials and Methods. Values are means ± SE for four animals. Key: significantly different from the control: (*) $P < 0.005$, (†) $P < 0.002$, and (‡) $P < 0.0002$; (●) serum T_4, and (▲) PCL activity.

DISCUSSION

Exposure of rats to HCB produces a number of effects such as porphyria and increased synthesis of microsomal enzymes, particularly in the liver [2–6]. In the present studies, HCB administration by gavage for 1 or 8 weeks resulted in a significant increase in liver weight and liver/body weight ratio as compared with control values. These results are in good agreement with those reported by other investigators [6, 21]. HCB had no effect on body or thyroid weight after 1 week, although it increased thyroid weight slightly (24%) after 8 weeks. On the other hand, the thyroid/body weight ratio was not affected in either case.

The results of this investigation and other studies in rats [5, 22] demonstrate a highly significant reduction in serum thyroxine after short- and long-term HCB administration. HCB treatment for 8 weeks lowered serum T_3, although not significantly ($0.05 < P < 0.1$). These results show, for the first time, such an early effect of the drug on serum thyroid hormone levels. Similar results were obtained by Allen-Rowlands *et al.* [13] for PBB intoxication of rats.

Previous studies showed that PCB produces ultrastructural lesions in thyroid follicular cells, and reduces serum thyroxine levels in a time-dependent manner [23].

Exposure to HCB resulted in a preferential sequestration of the fungicide by epididymal white and interscapular brown adipose tissue, and to a lesser degree in thyroid and liver [24]. These observations suggest that HCB may bind to thyroid macromolecules and may interfere with the normal synthesis and/or secretion of thyroid hormones. Results from other authors [13, 25] suggest that, in PBB- and PCB-intoxicated rats, thyroid hormone synthesis may be impaired. In the present studies, HCB did not produce any alterations in the relative distribution of [^{125}I] into the aminoacid residues of thyroglobulin. This suggests that thyroid peroxidase activity is not affected by HCB. Increased TSH levels may be an attempt to maintain normal serum levels of thyroid hormones.

Another contributing factor to reduced T_4 levels found in this study may be an enhanced hepatic dehalogenation of thyroxine. Our results show for the first time an accelerated liver deiodination of T_4 in HCB-intoxicated rats, without significant alterations in T_3, reflecting probably an increase in T_3 dehalogenation or a conversion into rT_3 (reverse triiodothyronine). This effect could possibly be due to a direct action of HCB on liver 5′-deiodinase, and the further degradation of T_3. In this respect it is interesting to take into account that most of the cellular T_3 is in kidney, muscle, and skin with a relatively small amount in the liver [26], so many agents that influence principally hepatic metabolism of hormone exert a lesser effect on T_3 than on T_4 economy. This fact could contribute to a less significant decrease in serum T_3 levels compared to T_4 depression.

Other factors tending to depress T_4 levels in HCB-treated rats may be a reduced binding of thyroid hormone to plasma proteins, an effect already reported for PCB [27], as well as reduced plasma T_4 carrier proteins caused by liver damage [25]. This and other aspects of the mechanism of action of HCB are being investigated in our laboratory.

HCB-induced alterations in hepatic function are associated with an increase in liver microsomal UDP-glucuronyltransferase activity [5, 28]. Since UDP-glucuronyltransferase is reponsible for the glucuronidation of T_4 prior to its biliary excretion, it could also contribute to the reduced thyroid hormone levels observed.

In conclusion, the data presented in these studies show that reduced serum thyroid hormone levels may be related, in part, to alterations in hepatic thyroxine metabolism, and do not result from impaired thyroid hormone synthesis.

The present results on the effect of HCB on heme metabolism show that after 1 week of HCB treatment PCL activity was decreased by 25%, whereas values for the other parameters of this pathway that were studied remained near normal. The decrease observed in PCL activity cannot be ascribed to a rise in the level of protein since its concentration in the 11,000 *g* supernatant fraction remained constant throughout the experiment (Table 4). With long-term exposure (8 weeks) the decrease in PCL activity was notable (81%), thus leading to an impairment of the heme pathway regulation. As a consequence, ALA-S activity was increased, as well as hepatic accumulation and urinary excretion of precursors and porphyrins. This is in agreement with the observation of Wainstock *et al.* [6] who reported that decreases equal or greater than 50% in PCL activity are needed to impair the heme pathway regulation, allowing the biochemical manifestation of this enzymatic failure.

What, if any, is the relationship between porphyria and alterations in thyroid function and thyroid hormone metabolism in rats caused by HCB?

Decreased serum T_4 has been shown to occur after the exposure of rats to several chlorinated aromatic hydrocarbons that are capable of inducing porphyria [8, 9, 23]. The temporal studies performed here allow one to observe that at the time of onset of increased PBG, ALA and porphyrin excretion in urine, serum T_4 was already reduced significantly. Serum thyroid hormones remained suppressed during the 8 weeks of treatment.

Serum T_4 and PCL activity behaved differently in both time-and dose-dependent studies. Our data show that serum T_4 was the more sensitive parameter which responded at earlier times and lower doses. Temporal studies show that reduced thyroid hormone levels and porphyria are not established simultaneously, although we cannot tell if they are causally related. Further studies are needed to clarify this point.

These studies are particularly relevant since there is a recent report of continued thyroid enlargement in a group of patients exposed to HCB [7]. A reduced serum T_4 level could be an early indication of HCB intoxication.

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