Enhancement of the UDP Glucuronyltransferase Activity and Biliary Excretion Rate of 17β-Estradiol in the Female Rat Fed Hexachlorobenzene

Celso E. Mendoza* and Hisako Watanabe

Virgin female Wistar rats (190–200 g) were fed ad libitum either control or 80 ppm HCB diet for a period of 5 or 8 weeks. At the end of each feeding period, each rat was anesthetized, its bile duct cannulated, and 17β-estradiol-*t* injected ip. Bile was collected from 0–2 to 2–4 h. After 5 weeks of treatment with HCB, an increase in porphyrin concentration in the liver was seen. After 8 weeks, porphyrin concentration was further increased. Maximum induction of liver esterases was observed in the 5-week group. In contrast, significant enhancement of the 17β-estradiol excretion rate and uridinediphosphate glucuronyltransferase activity was seen only after 8 weeks of HCB treatment. The body, liver, or uterine weight; the metabolism of estradiol; and the bile flow rate were not affected by the HCB treatment.

Hexachlorobenzene (HCB), a persistent fungicide, is gaining some importance as an industrial and environmental contaminant (Vos et al., 1972). It has been detected as a trace contaminant in some pesticide formulations (Alam, 1977). An epidemic of human porphyria cutanea tarda (PCT) in Eastern Turkey as a result of HCB intoxication had been reported (Cam, 1960). Ockner and Schmid (1961), Cam and Nigogosyan (1963), and Patel (1963) demonstrated the relationship of HCB to induction of porphyria in experimental animals and in humans. Peters (1976) reviewed the incidence of HCB-induced porphyria in Turkey with a view toward evaluating the long-term effects of such an intoxication and to determine the role of chelating agents in detoxification of human and other animals exposed to HCB.

A dietary level of 2000 ppm HCB increased the urinary excretion of porphyrins and simultaneous administration of estradiol, or estradiol plus gestagen or androgen, enhanced the quantity of porphyrins in the urine (Ippen and Aust, 1972). Zimmerman et al. (1966) and Vail (1967) reported that PCT was induced by female sex hormones.

HCB has been reported to induce drug metabolizing enzymes (Stonard and Nenov, 1974; Grant et al., 1974; Mehendale et al., 1975) and carboxylesterases (Mendoza and Shields, 1976) in rat livers. Mehendale et al. showed that uridine diphosphate (UDP) glucuronyltransferase activity in the male rat was also induced by this compound. Other organochlorine compounds found to induce UDP glucuronyltransferase were 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Lucier et al., 1973), and aldrin and dieldrin (Vainio and Parkki, 1976).

Since estrogens are metabolized by monooxygenases and are conjugated with glucuronic acid, the effect of HCB on UDP glucuronyltransferase activity and the excretion rate of estrogen was investigated. The effects on some carboxylesterases and liver porphyrin concentration were also determined.

MATERIALS AND METHODS

Four groups of virgin female Wistar rats (190–200 g) were fed ad libitum for 5 and 8 weeks with either HCB diet [powdered rat diet containing 80 ppm HCB and 4% corn oil (Mazola)] or control diet containing 4% corn oil. At the end of the feeding periods, each rat was weighed, given 0.4 mL of urethane (1 g/mL) ip and its bile duct was cannulated. A 0.2-mL saline solution containing 10% ethanol, 0.1 μg of 17β-estradiol, and 1 μCi 17β-estradiol-6,7-*t* (sp act. 40 Ci/mmol) (New England Nuclear Corp., Boston, Mass.) was given subcutaneously. The bile was immediately and quantitatively collected for two successive 2-h periods at 37 °C milieu. The liver and uterus were then excised and weighed. Duplicate samples of these organs and 25-μL bile aliquots were taken for radioactive determination using the oxygen combustion technique.

Bile aliquots from controls, or treated, animals were pooled and 5 mL of 0.1 M acetate buffer (pH 5) was added to the pooled aliquots. The sample was then extracted with three times 2-mL chloroform to remove the unconjugated materials, which were about 2% of the total radioactivity. The radioactivity of a portion of the aqueous phase was counted. Carrier 2-hydroxyestrone (0.1 mg) and 0.5 mL of Ketodase solution (Warner-Lambert Co., Morris Plains, N.J.) were added to the remaining aqueous solution, which was then incubated overnight at 37 °C. The sample was again extracted with chloroform and the radioactivity was counted. Aliquots were analyzed for estrogen metabolites by thin-layer chromatography (TLC) using a solvent system consisting of chloroform–cyclohexane–acetic acid (2:2:1).

Rat liver microsomes were prepared according to the procedure of Matthews et al. (1971) using 0.05 M Tris–HCl buffer, pH 7.4. UDP glucuronyltransferase activity was determined according to the method of Lucier et al. (1971) using 1-naphthol-*1*-^{14}C (Amersham/Searle Corp., Ill.) as substrate. UDP glucuronic acid (ammonium salt) was purchased from Sigma Chemical Co., St. Louis, Mo.

The liver esterases were extracted by homogenizing the tissue (20 parts in weight) with cold distilled, deionized water (80 parts in volume) in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 20000*g* (~4 °C) for 20 min (Mendoza et al., 1976). The liver esterase activities in the supernatant were determined using as substrates thiophenyl acetate (TPA) (Polysciences, Inc., Warrington, Pa.); *p*-nitrophenyl acetate (PNPA) (Sigma Chemical Co., St. Louis, Mo.); and indophenyl acetate (IPA) (Eastman Kodak Co., Rochester, N.Y.). The esterase activity toward TPA was measured according to Ellman's method (Ellman et al., 1961; Augustinsson et al., 1972) and that toward PNPA and IPA was measured according to the method of Mendoza et al. (1976). The rate of substrate hydrolysis was determined spectrophotometrically (Unicam SP 800).

Protein was determined by the biuret method (Gornal et al., 1948) after solubilization with 0.8% sodium desoxycholate. Crystalline albumin from bovine serum

Health Protection Branch, National Health and Welfare, Ottawa, Ontario, Canada, K1A 0L2.

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Table I. Esterase Activity in Livers of Female Rats Fed 80 ppm HCB Diet for 5 or 8 Weeks

Treatment	n	Esterase activity (μmol min^{-1} g^{-1} of protein) ± SE[a] TPA	PNPA	IPA
Five weeks				
Control	10	283 ± 9	544 ± 23	77 ± 2
80 ppm HCB	10	351 ± 20[c]	853 ± 40[c]	94 ± 2[c]
Eight weeks				
Control	10	244 ± 19	451 ± 38	65 ± 2
80 ppm HCB	16	315 ± 28[b]	652 ± 44[c]	91 ± 6[c]

[a] SE = standard error of the mean; n = number of analyses, two analyses per sample; TPA = thiophenyl acetate; PNPA = p-nitrophenyl acetate; IPA = indophenyl acetate. [b] Significantly higher than the control, $p < 0.05$ (Student's t test). [c] Significantly higher than the control, $p < 0.01$.

Table II. Porphyrin Concentrations in Livers of Control and HCB-Fed Female Rats[a]

No. of weeks on the diet	Porphyrin (nanomol/g of tissue ± SE) n	Control	n	80 ppm HCB
5	5	0.409 ± 0.044	5	0.850 ± 0.124[c]
8	5	0.487 ± 0.162	3	58.4 ± 35.8[c]
			5	0.856 ± 0.210[b]

[a] n = number of rats examined; SE ± standard error of the mean. [b] Not significantly different from the controls, $p < 0.05$. [c] Significantly higher than the control, $p < 0.01$ (Student's t test).

(General Biochemicals, Grand Island, N.Y.) was used as a protein standard.

HCB residues were extracted from the tissues and determined by gas–liquid chromatography using the previously published method (Mendoza et al., 1975). Porphyrin concentrates were determined according to the method of Abbretti and De Matteis (1971).

RESULTS

At each of the sacrificial times, no significant difference was found between control and treated rats in terms of body, liver, or uterine weights. At the end of the 5- and 8-week periods, body weights were approximately 242 g and 287 g; liver weights, 8 g and 10 g; and uterine weights, 0.4 g and 0.6 g, respectively.

Table I shows that liver esterase activities toward TPA, PNPA, or IPA were significantly increased with the HCB treatment. A maximum increase in esterase activity was observed within 5 weeks.

Table II shows that porphyrin concentrations were ca. twofold higher in the HCB-fed rats than in the control rats after 5 weeks on the diet. By 8 weeks, a dramatic increase of liver porphyrin concentrations was observed in three out of eight females fed HCB. The porphyrin concentrations in the other five rats were also elevated but were not significantly different from the controls.

Table III shows that rats fed a diet containing HCB for 8 weeks had significantly higher HCB residues in the liver, uterus, and bile than those fed for 5 weeks. However, the mean concentration of HCB excreted in the bile at 0 to 2 h was not significantly different from that at 2 to 4 h.

There was no significant change in bile flow rates with HCB treatment. The proportion of the radioactive estrogen excreted was unaltered in the group treated for 5 weeks as compared with the corresponding control group (data not shown). However, after 8 weeks on the HCB diet (Table IV), there was a significant increase in the estrogen excretion rate. A proportionately larger quantity of the radioactive dose was found to be excreted within the first 2 h.

TLC of the chloroform extracts showed that there was no difference in the nature or proportion of the aglycon metabolites excreted in the bile of the control and HCB-fed rats (Table V). Furthermore, the mean radioactivity per organ of the HCB-fed rats (liver: 52366 dpm; uterus: 2273 dpm) was not statistically different ($p > 0.05$) from that

Table III. HCB Residues ± SE (ppm) in Liver, Uterus, and Bile[a]

Treatment	n	Liver	Uterus	Bile 0–2 h	Bile 2–4 h
Five weeks					
Control	5	<0.01[b]	<0.01	<0.01	c
80 ppm HCB	5	47.50 ± 2.03	9.62 ± 1.01	1.01 ± 0.11	c
Eight weeks					
Control	5	1.77 ± 0.02	0.45 ± 0.08	0.05 ± 0.02	0.04 ± 0.01
80 ppm HCB	5	71.80 ± 3.18	13.59 ± 2.51	2.55 ± 0.27	2.47 ± 0.75

[a] SE = standard error of the mean; n = number of rats examined. [b] Detection limit = 0.01 ppm. [c] Not analyzed.

Table IV. Mean Bile Volume and Percent of Radioactive Dose ± SE Excreted by Female Rats Fed Either Control or 80-ppm HCB Diet for 8 Weeks[a]

Treatment	n	Bile vol, mL 0–2 h	Bile vol, mL 2–4 h	Radioactivity, % 0–2 h	Radioactivity, % 2–4 h
Control	5	1.23 ± 0.11	0.69 ± 0.03	23.4 ± 1.8	11.5 ± 0.7
80 ppm HCB	5	1.51 ± 0.16	0.79 ± 0.17	36.7 ± 3.9[b,c]	7.8 ± 8[c]

[a] SE = standard error of the mean; n = number of rats examined, one sample per rat. [b] n = four rats examined. [c] Significantly different from the control, $p < 0.01$ (Student's t test).

Table V. Mean Percentages of Five Estrogen Metabolites Examined in the Bile and Separated by TLC[a]

Treatment	n	More polar than 2-hydroxyestrone	2-hydroxyestrone	17β-estradiol	Estrone	2-methoxyestrone
Control	5	11.1 ± 1.9	45.6 ± 3.0	11.4 ± 0.3	12.8 ± 0.5	19.3 ± 2.4
80 ppm HCB	5	12.5 ± 2.7	46.5 ± 4.0	11.4 ± 0.7	11.7 ± 0.4	17.3 ± 0.7

[a] n = number of rats examined, one bile sample per rat. Student's t test indicated no significant difference between treatments, $p > 0.1$.

Table VI. UDPG Transferase Activity (μmol of Naphthyl Glucuronide Formed per 30 min ± SE) in Livers of Rats Fed Either Control or 80 ppm Diet for 8 Weeks[a]

	μmol/g of protein		μmol/g of liver	
	Control	80 ppm HCB	Control	80 ppm HCB
Microsomes	873 ± 52	1100 ± 60[b]	4.95 ± 0.32	6.18 ± 0.17[c]
Crude homogenate	45 ± 4	60 ± 5[c]		

[a] SE = standard error of the mean. [b] Significantly higher than the control, $p < 0.05$ (Student's t test). [c] Significantly higher than the control, $p < 0.01$.

of the control rats (liver: 42 295 dpm; uterus: 2138 dpm). Similar results were obtained with the control and HCB-fed rats when the radioactivity unit was expressed per milligram of tissues.

Table VI shows that the UDP glucuronyltransferase activities in liver microsomes and homogenates from rats fed HCB for 8 weeks were significantly higher than those from the controls. Likewise, the total UDP glucuronyltransferase activity in the whole liver was significantly higher in the HCB-fed rats than in the control rats.

DISCUSSION

The results show that an increase in porphyrin concentration begins to be seen at 5 weeks. In the 8-week group, about 40% of the treated females showed significantly higher porphyrin concentrations in the livers. This incidence agreed with the result reported by De Matteis et al. (1961). They observed marked disturbance of porphyrin metabolism in only 50% of the treated rabbits, although all exhibited the same final neurological symptoms. This observation indicates that the predisposition of individuals to porphyria is genetically linked. The porphyrias are usually considered hereditary abnormalities of porphyrin or porphyrin precursor metabolism (Tschudy and Bonkowsky, 1972).

HCB is known to induce microsomal enzyme activity in the liver. Mehendale et al. (1975) reported an increase in UDP glucuronyltransferase activity in the male rat on the eighth day after six daily po doses of either 10 or 25 mg/kg of HCB. This present study shows that liver UDP glucuronyltransferase was also induced in female rats fed 80 ppm dietary HCB for 8 weeks. Induction of carboxylesterase activity was observed in rats fed HCB diet for 5 weeks and no further increase in the activity was noted after 8 weeks.

The data also show an increase in biliary excretion rate of the estrogen only after feeding on 80 ppm dietary HCB for 8 weeks. It must be noted that the difference between the radioactivities in the livers, or uterus, was not statistically significant. However, there was a trend for lower radioactivity in the HCB than the control groups. Since the TLC data indicate that HCB did not change the metabolic pattern of the aglycon metabolites, it can be concluded that the increase in the excretion rate of estrogen was due to an increase in glucuronidation of the estrogen prior to biliary excretion.

In summary, it can be stated that there was an increase in carboxylesterase activity in the liver before any increase in biliary excretion of estrogen was detected. This increase in carboxylesterase activity was accompanied by an increase in the liver porphyrin concentration. The increase in the biliary excretion rate of the estrogen after 8 weeks on HCB diet was concurrent with the increase in UDP glucuronyltransferase activities in the liver. HCB, a known inducer of drug metabolizing enzymes, did not alter the estrogen metabolites excreted in the bile. The interrelation between porphyrogenesis and increased monooxygenase activity and that of UDP glucuronyltransferase and carboxylesterase after a dietary exposure to HCB should be investigated.

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