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HEXACHLOROBENZENE-INDUCED PORPHYRIA IN JAPANESE QUAIL

EFFECT OF PRETREATMENT WITH PHENOBARBITAL OR β-NAPHTHOFLAVONE

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Abstract—In an effort to determine the role that metabolism by the cytochrome P-450 system plays in the development of hexachlorobenzene (HCB)-induced porphyria, Japanese quail were pretreated with either β-naphthoflavone (BNF) or phenobarbital (PB) and then treated with HCB. PB or BNF pretreatment appeared to have no effect on the response of quail hepatic enzymes to HCB. There were no differences between the two groups in either the content of cytochrome P-450 or the activities of NADPH–cytochrome *c* reductase, glutathione transferase (microsomal or cytosolic), ethoxycoumarin-*O*-deethylase or ethoxyresorufin-*O*-deethylase following HCB treament. These pretreatments did, however, markedly influence the development of porphyria in quail. BNF-treated birds had higher δ-aminolevulinic acid-synthetase (ALA-S) activities and developed porphyria much more rapidly than birds treated with HCB alone. Birds pretreated with PB did not exhibit porphyria even following 10 days of HCB. Although the ALA-S activities in this group were elevated slightly following HCB, they were about one-half of those seen in the BNF-pretreated HCB-treated group. These results may reflect a difference between the PB and BNF groups in the production of a porphyrogenic metabolite of HCB.

The biochemical mechanism for the induction of porphyria cutanea tarda (PCT) by polyhalogenated aromatic compounds (PHAs) such as hexachlorobenzene (HCB) has remained elusive [1, 2]. However, recent data have indicated that the metabolism of HCB by the hepatic cytochrome P-450 system is somehow involved in its ability to generate PCT [3–6]. One of the major reasons why the mechanism has not yet been determined is the lack of an adequate model system. Although rats have been the most extensively used experimental species, they respond very slowly to the porphyrogenic actions of PHAs, like other mammals, making difficult a correlation between early changes in the hepatic drug-metabolizing system and later changes in the heme synthetic pathway [7].

Studies which demonstrated that Japanese quail develop porphyria very rapidly in response to PHAs suggested the potential usefulness of this species as a model system for the study of PHA-induced PCT [8, ‖]. HCB treatment of quail induced changes in the hepatic drug-metabolizing system which were similar to, but distinct from, those produced by the cytochrome P-448 inducer β-naphthoflavone (BNF).‖ In addition, several of these changes, including increases in the specific content of cytochrome P-450 and the activity of glutathione transferase (GSH-t), correlated with (i.e. occurred simultaneously to) the onset of porphyria.‖

Since both phenobarbital (PB) [4] and BNF [5, 6] have been reported to exacerbate porphyria in rats and chick embryo liver cells, respectively, it was thought that treatment of Japanese quail with these compounds prior to the administration of HCB would shed light on the correlation (if any) between HCB-induced changes in hepatic drug-metabolizing enzymes and the appearance of porphyria.

MATERIALS AND METHODS

Chemicals. HCB was obtained from the Aldrich Chemical Co. and recrystallized repeatedly in toluene until pure (as assessed by gas chromatography-mass spectrometry). Also purchased from Aldrich were β-naphthoflavone, 7-hydroxycoumarin and 7-ethoxycoumarin. 1-Chloro-2,4-dinitrobenzene was obtained from the Eastman Chemical Co. and 7-hydroxyresorufin and dimethylaminobenzaldehyde were from Matheson Coleman & Bell, Inc. δ-Aminolevulinic acid, cytochrome *c*, coproporphyrin I, NADPH and glutathione were obtained from the Sigma Chemical Co.

Animals. Sexually mature female Japanese quail (*Corturnix coturnix japonica*) were obtained from an

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‖ Additional experiments have been presented in abstract form and have been submitted elsewhere for publication. They are as follows: H. M. Carpenter, D. E. Williams and D. R. Buhler, *Fedn Proc.* **41**, 1637 (1982); H. M. Carpenter, D. E. Williams, M. C. Henderson, R. C. Bender and D. R. Buhler, *Toxicologist* **3**, 115 (1983); and H. M. Carpenter, D. E. Williams and D. R. Buhler, *J. Toxic. environ. Hlth*, three manuscripts submitted (1984).

inbred colony maintained by the Department of Poultry Science at Oregon State University. Birds were group housed (sexes combined) in layer cages until approximately 3 days before use when they were randomly selected, weighed, banded and, for ease of handling, placed in individual cages. All birds were maintained on a 16–8 hr light–dark schedule and received standard layer feed and water *ad lib*. Birds were pretreated with i.p. injections of either phenobarbital (PB) (150 mg/kg/day for 5 days) in saline solution or β-naphthoflavone (BNF) 150 mg/kg/day for 4 days) in corn oil. Following pretreatment, HCB was administered orally for 1, 2, 5 or 10 days (500 mg/kg/day) via gelatin capsule using lactose as filler (Table 1). The dosages chosen for each of these chemicals have been shown previously to be effective in causing changes in quail microsomal enzyme systems.* Following HCB treatment, birds were killed and weighed. Livers were removed, weighed and placed in beakers containing ice-cold homogenization buffer.

Preparation of microsomes. Livers were weighed, placed in ice-cold buffer (0.1 M Tris–acetate, pH 7.4; 0.1 M KCl; 1 mM EDTA; 20 μM butylated hydroxytoluene; and 0.1 mM phenylmethylsulfonylfluoride), minced, rinsed, and homogenized in 4 vol. of the same buffer. A microsomal pellet was prepared by centrifuging the homogenate at 10,000 *g* for 30 min (Sorvall RC-2B) and then centrifuging the resulting supernatant fraction for 90 min at 100,000 *g* (Sorvall OTD-2 ultracentrifuge). The microsomal pellet was resuspended to a concentration of 2–10 mg microsomal protein/ml buffer (0.1 M phosphate buffer, pH 7.25; 20% glycerol; and 1 mM EDTA). Microsomes were used immediately or frozen (−20°) until use. The supernatant fraction from the high speed spin was retained for the assay of cytosolic glutathione *S*-transferase.

Estimation of cytochrome P-450. The specific concentration of microsomal cytochrome P-450 was calculated from the difference spectra of CO versus CO-sodium dithionite reduced microsomes [9] obtained on a Cary 219 recording spectrophotometer. An extinction coefficient of 100 mM^{-1} cm^{-1} was used.

Enzyme assays. Dealkylation of 7-ethoxycoumarin and 7-ethoxyresorufin was measured fluorometrically using a Perkin–Elmer 650-10S recording spectrofluorometer [10]. The 7-ethoxycoumarin *O*-deethylase (ECOD) assay, run at 35°, contained a standard incubation mixture of 0.85 ml of 0.1 Tris–HCl buffer, pH 7.6; 0.1 ml of ethoxycoumarin, 0.1 mM in 0.1 mM Tris–HCl, pH 7.6; and 0.05 ml (0.1 to 0.5 mg protein) of microsomes in a fluorescence cuvette. The reaction was initiated by the addition of 10 μl of a 10 mM NADPH solution. Activity was measured as a change in fluorescence (360 nm excitation; 460 nm emission) and quantitated with 7-OH coumarin as the standard. The 7-ethoxyresorufin *O*-deethylase (EROD) assay, also run at 35°, contained: 0.9 ml of 0.1 M Tris–HCl buffer, pH 7.8; 0.05 ml ethoxyresorufin (10 μM, in 0.1 M Tris–HCl buffer, pH 7.8, containing 1 mM dimethyl sulfoxide); and 0.05 ml (0.2 to 0.5 mg protein) of microsomes. Activity was measured as a change in fluorescence (530 nm excitation; 585 nm emission) and quantitated with 7-OH resorufin as a standard. For both assays, buffer, substrate and microsomes were placed in a fluorescence cuvette and allowed to equilibrate to the 35° assay temperature for 2 min prior to the addition of 10 μl NADPH (10 mM solution).

NADPH–cytochrome *c* reductase was assayed spectrophotometrically by the method of Imai [11]. For this assay 0.97 ml of reaction buffer (0.35 M potassium phosphate, pH 7.5, and 0.05 mM cytochrome *c*) was placed in a cuvette with 20 μl (0.04 to 0.2 mg protein) of microsomes. The reaction was then initiated by the addition of 10 μl of a 10 mM NADPH solution. Activity was measured as an increase in absorbance at 550 nm and calculated using an extinction coefficient of 21.1 mM^{-1} cm^{-1}.

Glutathione *S*-transferase (GSH-t) was assayed spectrophotometrically by the method of Habig *et al.* [12]. For this assay 0.96 ml of potassium phosphate buffer, pH 8.0, was placed in a cuvette with 10 μl of glutathione (0.1 M stock in distilled water) and 20 μl (0.04 to 0.2 mg protein) of microsomes. The reaction was initiated by the addition of 10 μl of substrate, a 0.1 M solution of chlorodinitrobenzene, and activity was measured as an increase in absorbance at 340 nm. A background rate (without protein) was routinely run and subtracted from the absorbance before calculating activity using an extinction coefficient of 9.8 mM^{-1} cm^{-1}.

δ-Aminolevulinic acid synthetase (ALA-S) was assayed using a modification of the method of Marver *et al.* [13]. Briefly, livers were homogenized (5-fold

* See ‖ footnote on first page of article.

Table 1. Treatment regimen

Pretreatment	Treatment (1, 2, 5 or 10 days)	Group
(I) None	Lactose	Untreated control
	HCB*	Unpretreated-HCB treated
(II) β-Naphthoflavone†	Lactose	BNF pretreated control
	HCB*	BNF pretreated-HCB treated
(III) Phenobarbital‡	Lactose	PB pretreated control
	HCB*	PB pretreated-HCB treated

* Dosage: 500 mg/kg/day.
† Dosage: 150 mg/kg/day for 4 days.
‡ Dosage: 150 mg/kg/day for 5 days.

in buffer containing 0.9% NaCl; 0.5 mM EDTA; and 10 mM Tris, pH 7.4), and the tissue homogenates were incubated (in a ratio of 1 ml of homogenate to 4 ml of incubation buffer which contained: 100 mM glycine; 10 mM EDTA; and 75 mM Tris, pH 7.2) for 1 hr at 37°. The reaction was stopped by the addition of 25% trichloroacetic acid (TCA) (added in a volume equal to that of the homogenate). Samples were centrifuged, and the ALA produced was removed from the supernatant fraction by ion exchange chromatography, converted to a pyrrole by reaction with acetylacetone, and quantitated colorimetrically (553 nm) following reaction with Ehrlich's reagent [19% perchloric acid in glacial acetic acid (v/v) containing 2% *p*-dimethylaminobenzaldehyde (w/v)]. Zero time samples were run, and these values were subtracted from those from the incubated samples to correct for endogenous ALA. Standard curves (using ALA) were run daily. Values from these curves were fairly consistent giving an average extinction coefficient of 65.4 mM^{-1} cm^{-1}.

Protein concentrations were determined using the method of Lowry *et al.* [14].

Determination of total hepatic porphyrins. Total hepatic porphyrin concentrations were determined using the method of Racz and Marks [15]. Samples were read on a model 111 Turner fluorometer equipped with a 405 nm band pass primary filter and a 595 nm secondary filter. Solutions of coproporphyrin I were used as standards.

All data are expressed as means ± S.E.M. Significance was determined using Student's *t*-test with the 95% confidence level used as the criterion for significance.

RESULTS

BNF or PB treatment caused increases in cytochrome P-450 specific contents, and BNF caused a shift in adsorption maximum from 450 to 449 nm of hepatic microsomes from Japanese quail (Table 2). Administration of HCB to BNF-pretreated birds resulted in additional increases in microsomal protein levels and following 10 days had increased the specific content of cytochrome P-450 by 62% over birds treated with BNF alone (Table 2). HCB treatment of PB-pretreated birds elevated microsomal protein and increased the microsomal cytochrome P-450 specific content by an average of 130%; HCB also shifted the absorption maximum for the P-450 from 450 to 449 nm in PB-pretreated birds (Table 2).

Following 5 days of HCB treatment, one of four birds was porphyric and by 10 days of treatment three of four birds were porphyric (Fig. 1). BNF pretreatment of quail greatly exacerbated HCB-induced porphyria (Table 3; Fig. 1). While BNF treatment alone had no effect on porphyrin levels, following pretreatment with BNF and a single dose of HCB, porphyria was present in two of four birds (Fig. 1) and the average porphyrin level was com-

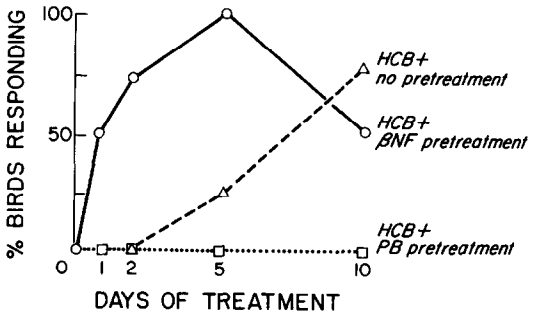

Fig. 1. Percentage of birds developing porphyria following HCB administration. N = 4 for each treatment group. Details of treatment are presented in Materials and Methods.

Table 2. Effect of phenobarbital or β-naphthoflavone pretreatment on hexachlorobenzene-induced changes in hepatic microsomal protein content and cytochrome P-450 concentration in Japanese quail*

			Cytochrome P-450	
	N	Microsomal protein (mg/g liver)	Concentration (nmoles/mg protein)	λ_{max} (nm)
Untreated controls	14	9.8 ± 0.9	0.100 ± 0.010	450 ± 0
BNF pretreatment				
Control (lactose treated)	8	13.2 ± 1.2†	0.395 ± 0.052†	449 ± 0†
HCB treatment (days)				
1	4	24.7 ± 3.2†‡	0.236 ± 0.057†	449 ± 0†
2	4	17.3 ± 3.0†	0.470 ± 0.071†	449 ± 0†
5	4	23.2 ± 3.8†‡	0.484 ± 0.112†	446 ± 1†‡
10	3	19.4 ± 4.0†‡	0.638 ± 0.028†‡	448 ± 1†
PB pretreatment				
Control (lactose treated)	8	11.5 ± 1.4	0.180 ± 0.033†	450 ± 0
HCB treatment (days)				
1	4	16.6 ± 1.8†‡	0.308 ± 0.085†‡	449 ± 0†‡
2	4	14.5 ± 0.8†	0.624 ± 0.055†‡	449 ± 0†‡
5	4	16.1 ± 3.8†	0.354 ± 0.068†‡	449 ± 0†‡
10	4	14.2 ± 3.0	0.379 ± 0.057†‡	449 ± 0†‡

* Values are expressed as mean ± S.E.M.
† $P \leq 0.05$ compared to untreated controls.
‡ $P \leq 0.05$ compared to respective control values.

Table 3. Effect of phenobarbital or β-naphthoflavone pretreatment on hexachlorobenzene-induced changes in hepatic porphyrin levels and δ-aminolevulinic acid synthetase activity in Japanese quail*

	Pretreatment		
	None	Phenobarbital	β-Naphthoflavone
	Total hepatic porphyrins† (μg/g tissue)		
Control	0.22 ± 0.03	0.13 ± 0.03‡	0.25 ± 0.03
HCB treatment (days)			
1	0.59 ± 0.06§	0.36 ± 0.10§	2.67 ± 0.90‡§
2	0.78 ± 0.23§	0.35 ± 0.04‡§	8.33 ± 3.70‡§
5	2.37 ± 1.78	0.45 ± 0.14§	8.62 ± 1.21‡§
10	6.11 ± 2.40§	0.41 ± 0.12§	27.60 ± 20.5
	δ-Aminolevulinic acid synthetase activity† (nmoles/hr/g tissue)		
Control	12.8 ± 3.1	24.7 ± 3.2‡	45.7 ± 7.4‡
HCB treatment (days)			
1	52.9‖	43.0 ± 9.6‡§	109.7 ± 36.2‡§
2		21.8 ± 4.4	103.6 ± 27.3‡§
5	28.3‖	71.9 ± 10.9‡§	162.2 ± 25.5‡§
10	30.0‖	40.5 ± 6.2‡§	92.0 ± 35.8‡¶

* Values are expressed as mean ± S.E.M.
† N = at least four for each group unless otherwise noted.
‡ $P \leq 0.05$ when compared to untreated control values.
§ $P \leq 0.05$ when compared to respective vehicle control.
‖ N = 2.
¶ N = 3.

parable to that seen following 5 days of HCB treatment when no pretreatment was given (Table 3). With BNF pretreatment and 5 days of HCB treatment all four of the treated birds were porphyric, and porphyrin levels were four times higher than in the group with no pretreatment. While only two of four birds in the 10-day group were porphyric, porphyrin levels were four times higher than in the comparable treatment period where no BNF was administered. ALA-S activities were also affected by the various treatments (Table 3). BNF pretreatment alone caused significant increases (3.5-fold) in ALA-S activity over non-treated controls and when HCB treatment was given activities increased to 8–12 times those found in non-treated controls and 2–3.5 times those found with BNF-treated controls.

In contrast, PB pretreatment greatly reduced the response of the birds to HCB-induced porphyria. Although porphyrin levels were elevated slightly, none of the PB-pretreated birds developed porphyria, regardless of the length of HCB exposure. ALA-S activities were also increased by PB pretreatment but increases were not as dramatic as those seen with BNF. PB alone resulted in a doubling of ALA-S activities compared to non-treated controls and, following HCB treatment, activities were increased still further.

NADPH–cytochrome *c* reductase and GSH-t activities were also changed (Table 4). With BNF pretreatment and 10 days of HCB treatment, reductase activities were increased. PB pretreatment caused a greater enhancement of the sensitivity of the reductase to HCB treatment as indicated by a significant increase in activity following PB dosing and only 2 days of HCB treatment.

BNF pretreatment increased both microsomal and cytosolic GSH-t activities but PB enhanced only the activity of the microsomal enzyme. Following either BNF or PB pretreatment and a single dose of HCB, microsomal GSH-t was decreased to the untreated control levels. With a second dose of HCB, microsomal GSH-t returned to levels comparable to that of the BNF or PB-treated controls. Enzyme activity in the BNF-pretreated birds remained elevated with no additional increases throughout the duration of the study. In the PB-pretreated birds, HCB treatment caused continuing increases in GSH-t activity throughout the treatment period. Cytosolic GSH-t levels were not decreased upon HCB treatment but with BNF-treated birds, 2 and 10 days of HCB treatment caused increases in GSH-t activity. In the PB pretreatment group, cytosolic GSH-t was increased compared to PB-treated controls after 2, 5 or 10 days of HCB.

BNF pretreatment of quail increased the activities of both ECOD and EROD (Table 5). Following one dose of HCB, both of the deethylases were decreased, but after 2 and 10 days of HCB treatment enzyme activities were increased and were comparable to those seen in the BNF-treated controls.

PB pretreatment increased the activity of hepatic ECOD but not of EROD (Table 5). Upon one or two treatments with HCB of the PB-pretreated quail, the activity of EROD was increased. After 10 days of HCB treatment, however, EROD activity was no longer different than the PB-treated controls. ECOD activity in PB-treated quail was unaffected by HCB exposure.

DISCUSSION

In a study examining the molecular mechanism of

Table 4. Effect of phenobarbital or β-naphthoflavone pretreatment on HCB-induced changes in the activities of NADPH–cytochrome *c* reductase and glutathione transferase in Japanese quail*

	N	NADPH–cytochrome *c* reductase (nmoles/min/mg)	Glutathione S-transferase (nmoles/min/mg)	
			Microsomal	Cytosolic
Untreated controls	14	87 ± 11	118 ± 14	699 ± 31
BNF pretreatment				
Control (lactose treated)	8	74 ± 9	214 ± 38†	1034 ± 77†
HCB treatment (days)				
1	4	82 ± 43	121 ± 13	1013 ± 203†
2	4	87 ± 6	255 ± 21†	1351 ± 104†‡
5	4	81 ± 10	208 ± 24†	1056 ± 18†
10	3	108 ± 7‡	273 ± 35†	1290 ± 43†‡
PB pretreatment				
Control (lactose treated)	8	66 ± 7	180 ± 24†	774 ± 50
HCB treatment (days)				
1	4	56 ± 9	145 ± 11	798 ± 89
2	4	109 ± 15‡	258 ± 26†	1236 ± 132†‡
5	4	93 ± 13‡	275 ± 31‡	1198 ± 125†‡
10	4	98 ± 8‡	316 ± 25‡	1015 ± 66†‡

* Values are expressed as mean ± S.E.M.
† $P \leq 0.05$ compared to untreated control.
‡ $P \leq$ compared to respective control values.

action of several PHAs, including HCB, Debets *et al.* [5, 6] used isolated chick embryo liver cells to study porphyria. They found that pre-exposure of the cells to the classic cytochrome P-450 inducers PB, 3-methylcholanthrene (3-MC) or BNF prior to the addition of HCB to the cultures markedly stimulates the porphyrinogenic action of HCB in these cells. Most significantly, stimulation by BNF pretreatment gives the greatest effect and PB only increases porphyria when administered simultaneously with HCB. Conversely, HCB-induced porphyria in chick embryo cells could be prevented by treatment with the monooxygenase inhibitor piperonyl butoxide [5, 6], but not metyrapone, a PB-type inhibitor [5]. These complicated findings indicate the importance of the metabolism of HCB by microsomal cytochrome P-450 (and cytochrome P-448 in particular) enzymes in the development of HCB-induced porphyria.

In an *in vivo* study with rats (females of the Wistar strain), it was demonstrated that PB (0.1% in drinking water) enhances the porphyrinogenic action of HCB, i.e. it increases the output of urinary porphyrins [4].

Pretreatment of Japanese quail with BNF greatly exacerbated HCB-induced porphyria in a manner apparently analogous to the *in vitro* system [5, 6]. This enhancement occurred in spite of the fact that BNF by itself caused no changes in the levels of hepatic porphyrins. PB pretreatment of the quail, on the other hand, protected against HCB-induced porphyria, a situation quite unlike that seen in the female Wistar rat [4]. While it was possible that the rats in the study of Kerklaan *et al.* [4] were never

Table 5. Effect of phenobarbital or β-naphthoflavone pretreatment on HCB-induced changes in the activities of hepatic ethoxycoumarin *O*-deethylase or ethoxyresorufin *O*-deethylase activities in Japanese quail*

	N	Ethoxycoumarin *O*-deethylase (ECOD) (nmoles/min/mg)	Ethoxyresorufin *O*-deethylase (EROD) (nmoles/min/mg)
Untreated controls	14	0.31 ± 0.06	0.08 ± 0.03
BNF pretreatment			
Control (lactose treated)	6	1.73 ± 0.35†	1.00 ± 0.24†
HCB treatment (days)			
1	4	0.38 ± 0.13‡	0.14 ± 0.03‡
2	4	0.84 ± 0.16†	0.32 ± 0.12†
10	3	1.08 ± 0.47†	0.66 ± 0.31†
PB pretreatment			
Control (lactose treated)	6	0.82 ± 0.12†	0.19 ± 0.07
HCB treatment (days)			
1	4	0.79 ± 0.15†	0.54 ± 0.10†‡
2	4	1.51 ± 0.42†	1.17 ± 0.70†‡
10	4	1.01 ± 0.17†	0.42 ± 0.07†

* Values are expressed as mean ± S.E.M.
† $P \leq 0.05$ compared to untreated controls.
‡ $P \leq 0.05$ compared to control values.

fully induced, it seemed more likely that the results reflect a basic difference in the response of the drug-metabolizing systems to PB by rats and quail.

Two possible mechanisms for the differential effects of these pretreatments can be considered. The first is an induction of alternate pathways of metabolism for HCB by BNF and PB. In this scheme PB would induce cytochromes P-450 which would produce non-porphyrinogenic metabolites of HCB while BNF pretreatment would result in the formation of porphyrinogenic HCB metabolites. The second possible explanation is a differential stimulation of detoxification enzyme systems such as GSH-t and epoxide hydrolase in the two species. Since both PB and BNF had similar effects on these enzyme systems (Table 4; *), the latter possibility seems unlikely.

The use of a PB- or BNF-pretreatment regimen would appear to offer important advantages for the study of the role of metabolism in HCB-induced porphyria. HCB treatment causes changes in drug metabolism, many of which are presumably unimportant to the generation of porphyria. Since PB pretreatment would prevent (or delay) those HCB-induced changes related to porphyria, while BNF pretreatment would cause them to occur earlier in the course of treatment (or in a different magnitude), differentiation between HCB effects related to porphyria and those which are not would be possible.

An increase in the activity of ALA-S has been reported to occur during the onset of PHA-induced porphyria in mammals [1]. The response of ALA-S to PHAs is biphasic. First, there is an initial adaptive phase where ALA-S activity is increased 2- to 3-fold over controls presumably in response to the increases in cytochrome P-450 which occur with these compounds. Second, there is a pathological phase during which ALA-S increases 10-fold or more. This latter phase is a reflection of the development of porphyria [2, 7].

In the present investigation with quail, ALA-S activities were elevated from 3 to 5 times controls by either BNF pretreatment alone or by PB pretreatment coupled with HCB treatment. No porphyria was present in any of these birds, but their cytochrome P-450 levels (specific contents) were comparable. When porphyria was discernible in the BNF-pretreated HCB-treated birds, ALA-S activities had increased from 7 to 13 times the values seen in the non-treated control birds. These results are in agreement with the previously presented idea that ALA-S, rather than playing a mechanistic role in PHA-induced porphyria, is merely responding to the decrease in heme which results from an inhibition of uroporphyrinogen decarboxylase (UROG-D) [2].

Although we have found in other experiments that increases in GSH-t correlated with HCB-induced porphyria,* this was not the case in the present investigation. Following pretreatment of quail with either PB or BNF, hepatic GSH-t activity was increased over untreated controls. Following a single dose of HCB, GSH-t activities were decreased. However, repeated HCB administration resulted in elevated GSH-t activities but no differences between PB- and BNF-pretreated quail were apparent, this despite the fact that the porphyrin levels in these groups were drastically different (*vida supra*). This appears to make the relation of GSH-t and HCB-induced porphyria (at least with CDNB as substrate) more tenuous than previously proposed.

Another effect of PB pretreatment on the drug-metabolizing system in quail was apparent with hepatic NADPH–cytochrome *c* reductase. Pretreatment of the birds with PB made the reductase more responsive to HCB. In our earlier studies on quail, no changes in reductase levels occurred until day 10 of HCB treatment.* Similar results occurred with the BNF-pretreated group in this study (Table 4). However, in the PB-pretreatment group, only 2 days of HCB treatment caused reductase activity to become elevated. These results indicate that PB pretreatment somehow increased the sensitivity of NADPH–cytochrome *c* reductase to HCB treatment.

The effect of BNF or PB pretreatment on HCB-induced changes in the monooxygenases EROD and ECOD was also examined (Table 5). It has been determined previously that these enzymes were affected by HCB treatment.* Treatment of quail with HCB for a 10-day period increased hepatic EROD levels by as much as 12 times controls.* The activity of ECOD was also increased by only two doses of HCB. In the present investigation, both BNF and PB pretreatments caused increases in ECOD levels while BNF pretreatment only elevated EROD activity. Surprisingly, in birds pretreated with BNF and given a single dose of HCB, both ECOD and EROD activities were reduced significantly at a time when 50% of the birds were porphyric (Table 5). However, following a second dose of HCB these activities increased, approaching BNF-pretreated control values. Such decreases did not occur in the PB-pretreated and HCB-dosed group. A similar situation has been reported in mammals where EROD was initially increased following HCB treatment, but then decreased with chronic feeding of HCB, coinciding with the development of porphyria [16]. The importance of these findings and how they relate to porphyria remain unclear, but a report that porphyrins are inhibitory to monooxygenases [17] suggests a possible interpretation. However, this explanation is complicated by the observation that, with HCB-induced porphyria where no pretreatment was used, the activities of these monooxygenases were not different from HCB-treated birds which were not porphyric.* It may be that the above differences were related to PB- or BNF-induced changes in the type of deethylase enzymes present.*

The toxicity of HCB, as with other PHAs, can be thought of as occurring in two steps: the first, an induction of cytochrome P-448 and related monooxygenases and the second, the metabolism of HCB by the newly synthesized cytochrome P-450 to generate reactive (toxic) metabolites. It has been suggested [18] that the rate of HCB metabolism has to be above a critical level before the heme pathway is affected and porphyrins begin to accumulate. Results presented here and in other studies* indicate that, whatever the sequence of events, it occurs more rapidly in Japanese quail than in mammals. In addition, the entire process can be further accel-

* See || footnote on first page of article.

erated by pretreating quail with BNF or prevented (or at least slowed) by PB pretreatment.

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