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Toxicity of PBBs with Special Reference to Porphyrinogenic Action and Spectral Interaction with Hepatic Cytochrome P-450

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Some of the polyhalogenated aromatic compounds (PHAs) which are able to produce porphyria are presently known as environmental contaminants. Chronic exposure to PHAs causes hepatic porphyria in different species. Qualitatively PBBs act comparable to PHAs. An increase in accumulation of porphyrins caused by PHAs is not simply related to an increase of δ-ALAS activity in liver. Heme cannot exert a feedback when porphyria develops. Induction of P-450 mediated drug enzymes is needed. The PHAs interact with P-450 *in vitro*. The PHAs are converted into a reactive intermediate, not a known metabolite, which depletes liver GSH and then becomes reactive to tissue structures. Mitochondria are damaged; fluorescence of porphyrins is detected in the region of central veins where degenerative change in hepatocytes is most marked. A possible pathological change in the cell membrane permeability is assumed too. In this porphyric stage uroporphyrinogen decarboxylase (urogen decarboxylase) is inhibited. The proportion of steroid hormones product by ovaries and testes compared to each other are possibly involved in sensitivity to porphyrinogenic compounds.

Introduction

Some polyhalogenated hydrocarbons, such as hexachlorobenzene (HCB), (*1*) 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, polychlorinated biphenyls (PCB) (different commercial brands), hexachlorobiphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, γ-hexachlorocyclohexane, polybrominated biphenyls (BP-6), methyl chloride, vinyl chloride (*2*), and hexabromobenzene (*3*), are able to produce porphyria in man and animals and pose health hazards because of their presence in human and animal food, in the work place, and in wildlife. PBB is a strong porphyrinogenic compound in avian systems. Compared to the porphyrinogenic action of HCB and PCB, PBB is respectively 80 and 10 times more active (*4, 5*). A feature of the porphyria evoked by these polyhalogenated aromatics is its slow onset. A long chronic exposure is needed, especially in mammals (*6*). This type of porphyria is related to liver damage (*7*).

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Materials and Methods

Separate groups of adult Japanese quail were orally dosed with gelatin capsules or fed diet containing PBB (BP-6 lot m11081). The parameter for porphyria consisted of macroscopic and microscopic porphyrin fluorescence in the liver, bile, kidneys, and blood; as well as δ-aminolevulinic acid synthase (δ-ALAS) activity in the liver and kidneys, analysis of porphyrin methyl esters by means of thin-layer chromatography, and determination of heme in liver fractions. Furthermore the serum glutamic acid dehydrogenase (GLDH) and glutamic acid pyrotartaric acid transaminase (GPT) activities were determined (*4*). To obtain P-450/PBB binding spectra, 105.000 g pellets of Japanese quail liver were used (*8*).

Results

Influence of PBB on Heme Biosynthetic Pathway

Table 1 shows the dose-effect correlation of δ-ALAS activity in liver homogenate of female Japanese quail, after administration PBB for seven

Table 1. Formation of δ-aminolevulinic acid by liver homogenate, liver weights, and tissue fluorescence in Japanese quail orally dosed with polybromobiphenyl for 7 days.

PBB, mg/kg body weight	ALA formed, nmole ALA/g liver/hr[a]	Liver, g/100 g body weight[a]	Tissue fluorescence incidence		
			Animal (macroscopic)	Liver	
				Macroscopic	Microscopic
0	88.4	2.93	0/5	0/5	0/5
25	207.6	3.65	0/5	0/5	0/5
50	259.0	3.65	0/5	0/5	0/5
100	355.0	3.69	0/5	0/5	1/5
250	271.3	5.27	2/5	2/5	3/5
500	D[b]	6.57	3/5	4/5	5/5
1000	D[b]	6.77	5/5	5/5	5/5

[a] Mean values for 5 female birds per group.
[b] Animals died.

days. A maximum δ-ALAS activity was reached with a dose of 100 mg/kg. This activity increase is two- to three-fold in value. Microscopic fluorescence in the liver was detected in three sacrificed animals dosed 250 mg/kg and one sacrificed animal dosed 100 mg/kg.

Table 2 shows that daily PBB application reduces the hepatic heme content ($p < 0.05$) prior to development of hepatic porphyria.

Table 3 shows that hemin can counteract the elevated porphyrin production of the liver which is excreted by the bile and which was induced by a single PBB dose.

According to Table 4, hemin can not suppress the porphyrin accumulation induced in the liver, bile, and kidneys by daily loading with 500 and 1000 mg/kg of PBB.

Influence of PBB on Porphyrin Biosynthesis

Table 5 shows that, starting with a daily dose of PBB of 100 mg/kg-day, porphyrin accumulation occurs in the liver and bile. Starting with a dose of 250 mg/kg-day, the animals die with porphyrin accumulation in the liver, bile, and kidneys. No porphyrins were detected in the kidneys and blood of the three sacrificed animals, although they exhibited liver and bile fluorescence. In the examined organs, porphyrin accumulation occurred only when the animals were moribund.

Macroscopic fluorescence of the whole body was observed in all dead animals and in one moribund specimen (1000, 500, and 250 mg/kg group). The livers of these animals exhibited extremely strong microscopic fluorescence. Furthermore, livers of three sacrificed animals, from the 500, 250 and 100 mg/kg groups, showed a weak microscopic fluorescence.

In a subsequent experiment, five Japanese quail were treated with a single dose of 500 mg/kg of PBB. No porphyrins were detectable in the liver and kidneys after 16 hr. The (pooled) bile contained

Table 2. Influence of PBB on the heme content of the liver.[a]

No. of animals	Substance	Dose, mg/kg	Duration of treatment, days	Heme content			Positive liver fluorescence /n animals
				μmole/g of liver	μmole/ total liver	μmole /g albumen	
5	Control	—	3	0.72 ± 0.08	1.77 ± 0.20	2.06 ± 0.14	0/5
6	PBB	1000	3	0.56 ± 0.07[b]	1.74 ± 0.12	1.73 ± 0.24	2/6
5	Control	—	5	0.56 ± 0.04	1.21 ± 0.13	1.72 ± 0.14	0/5
7	PBB	1000	5	0.41 ± 0.01[b]	1.45 ± 0.10	1.33 ± 0.09[b]	3/7

[a] Japanese quail were dosed orally with 1000 mg/kg PBB per day. The heme content in the liver homogenate was determined after 3 and 5 days.
[b] $p < 0.05$.

Table 3. Influence of hemin on the porphyrin accumulation in bile and liver with single dose of PBB.[a]

No. of animals	Substance	Duration of treatment, hr	PBB Dose, mg/kg	Positive fluorescence /n animals	
				Bile	Liver
5	Vehicle	18	—	0/5	0/5
5	Hemin	18	—	0/5	0/5
5	PBB + hemin	18	1000	2/5	1/5
5	PBB + vehicle	18	1000	5/5	0/5

[a] Japanese quail were dosed orally with PBB (1000 mg/kg). Hemin was suspended in 0.001*N* NaOH; 10 μmole hemin/100 g of animal was injected IP 1 hr before and 1 hr after dosing. Porphyrin fluorescence was determined 17 hr after dosing as a measure of the porphyrin accumulation.

Table 4. Influence of hemin on the accumulation of porphyrins in bile, liver, and kidneys caused by dosing repeatedly with PBB.

No. of animals	Substance	PBB dose mg/kg	Duration of treatment, days	Positive fluorescence/ n animals		
				Bile	Liver	Kidneys
4	Hemin	—	7	0/4	0/4	0/4
2	PBB	500	7	2/2	2/2	2/2
2	PBB	1000	7	2/2	2/2	2/2
4	PBB + hemin	500	7	4/4	4/4	4/4
4	PBB + hemin	1000	7	4/4	4/4	4/4

[a] Male Japanese quail were daily dosed orally with PBB (500 mg/kg and 1000 mg/kg) over a period of 7 days. Each day hemin was administered IP just before the dosing (12.5 mg/100 g of animal). After dosing for 7 days, the porphyrin accumulation in bile, liver, and kidneys was determined with the aid of a fluorescence microscope.

Table 5. The dose dependence of the appearance and localization of the earliest detectable porphyrin accumulation by PBB in various organs in Japanese quail.[a]

PBB dose, mg/kg	Positive fluorescence/n animals			
	Bile	Liver	Blood	Kidneys
0	0/5	0/5	0/5	0/5
25	0/5	0/5	0/5	0/5
50	0/5	0/5	0/5	0/5
100	1/5	1/5	0/5	0/5
250[b]	1/3	1/3	0/3	0/3
500[c]	1/1	1/1	0/1	0/1
1000[d]	0/0	0/0	0/0	0/0

[a] Female Japanese quail (35 total) were randomly divided into groups of 5. One group served as a control group, receiving empty capsules. The other groups were dosed daily for 7 days with different oral doses of PBB. Liver, bile, blood, and kidneys were examined for macroscopic fluorescence of porphyrins.

[b] Two animals died within the 7-day test period, with fluorescence of the liver, bile and kidneys.

[c] Four animals died in the test period, with fluorescence of liver, bile, and kidneys.

[d] Five animals died in the test period, with fluorescence of the liver, bile, and kidneys.

porphyrins with 2, 3, 4, 6, and 8 COOH groups. The experiment in Table 6 shows that PBB treatment causes the development of porphyrin accumulation in the kidneys and bile as well as in the liver.

Table 7 shows that with a daily application of 400 mg/kg of PBB for a period of 7 days, porphyrins with 5-8 COOH groups accumulate in the bile and feces, in addition to those with 2, 3, and 4 COOH groups which are also detectable in the controls. In the kidneys, no porphyrins were detectable in the controls, while those of the PBB-treated animals contained porphyrins with 4, 6, 7, and 8 COOH groups. The liver of the controls and of the PBB-treated animals contained porphyrins with 2 and with 2 and 4 COOH groups, respectively. The experiments of Table 8 indicate that porphyrins with

Table 6. The time dependence of the appearance and localization of the earliest detectable porphyrin accumulation by PBB in various organs in Japanese quail.[a]

	PBB dose, mg/kg	Duration of treatment, days	Positive fluorescence/n animals			
			Bile	Liver	Kidney	Intestine
Control	—	3	0/6	0/6	0/6	0/6
PBB	1000	3	6/6	0[b]/6	6/6	3/6
Control	—	5	0/6	0/6	0/6	0/6
PBB	1000	5	3/7	3[c]/7	7/7	3/7

[a] Japanese quail (20 total) were randomly divided into four groups. Two groups were dosed daily with 1000 mg/kg of PBB. Two groups served as control. After 3 and 5 days, one control and one treated group were examined for microscopic fluorescence of porphyrins in bile, liver, kidneys, and intestines.

[b] Two animals showed a few small spots of fluorescence.

[c] One animal showed a few small spots of fluorescence.

2, 4, and 8 COOH groups are detectable in the liver as a result of PBB loading. The kidneys of these animals which had died contained not only porphyrins with 4 and 8 but also with 6 and 7 COOH groups; porphyrins with 2, 3, and 4 COOH groups were detected in the bile.

Influence of PBB on Mitochondrial Membrane Enzymes

Whether PBB porphyria develops before, during, or after biochemically detectable liver lesions was studied on the basis of the serum enzyme activities of glutamic dehydrogenase and glutamic pyruvic transaminase (SGLDH and SGPT). GLDH is a typical mitochondrial enzyme and GPT is characteristic cytoplasmic enzyme. Liver lesions result in an elevated activity of one or both enzymes, depending on the nature of the lesion.

The experiments in Table 9 demonstrate that loading with 500 ppm PBB in the diet does not result in a difference of SGPT activity ($p > 0.05$). Starting with 10 days of PBB loading, the serum GLDH activity is elevated ($p < 0.05$). After 10, 13, and 20 days, no porphyrin accumulation was found in the liver of the PBB-loaded animals. Porphyrin accumulation in the bile was seen already after 5 days in the PBB-loaded animals.

After 5 days of PBB treatment (oral, as capsules), SGLDH is elevated and porphyrin accumulation occurs in the liver (Table 10).

Discussion

δ-Aminolevulinic Acid Synthase

First of all, the existence of a relation in quail liver between changes in ALAS activity and porphyrin accumulation in the liver was investigated.

Table 7. Qualitative differentiation of porphyrin methyl esters in PBB-loaded Japanese quail organs by means of thin-layer chromatography.[a]

	PBB dose, mg/kg	Duration of treatment, days	Number of methyl ester groups in the porphyrins[b]			
			Liver	Bile	Kidneys	Feces
Control	—	7	2	2;3;4	ND	1;2;3;4;5
PBB	400	7	2;4	2;3;4;5;6;7;8	4;6;7;8	2;3;4;5;6;7;8

[a] Five Japanese quail were treated with 400 mg/kg-day of PBB for 7 days. Five Japanese quail served as controls. No deaths occurred during the treatment period. The condition of the PBB-treated animals did deteriorate, however (weight loss). After the 7-day period, the porphyrin pattern in the liver, bile, kidneys, and feces was investigated by thin-layer chromatography. The liver and kidneys exhibited microscopic fluorescence which was stronger in the kidneys than in the liver. Control animals were fed empty capsules.

[b] ND = not detectable; 2= protoporphyrin; 3 = 3-COOH porphyrin; 4= coproporphyrin; 5= penta-COOH porphyrin; 6 = hexa-COOH porphyrin; 7 = hepta-COOH porphyrin; 8 = uroporphyrin.

Table 8. Qualitative differentiation of porphyrin methyl esters from organs of Japanese quail receiving PBB by thin-layer chromatography.[a]

PBB dose, mg/kg	Duration of treatment, days	Number of methyl ester groups in the porphyrin[b]		
		Liver	Bile	Kidneys
500	8	4;8		4;7;8
	9	ND		ND
	10	2;4;8		7;8
	13	4	2;3;4	4;6;7;8
	13	2		ND
	14	ND		8
	16	8		4;7;8
	16	2;4		4
	16	ND		ND

[a] Japanese quail were subjected to 500 mg/kg of PBB in their feed. Bile, liver, and kidneys of the animals that died were examined for the porphyrin patterns by means of thin-layer chromatography.

[b] ND = not detectable; 2 = protoporphyrin; 3 = 3-COOH porphyrin; 4 = coproporphyrin; 6 = hexa-COOH porphyrin, 7 = hepta-COOH porphyrin.

The fact that the three sacrificed animals with microscopic fluorescence in the liver had a ALAS activity of 180–520 (nmole ALA/g liver/hr and that 12 of the 16 remaining PBB-treated animals had a ALAS activity ranging between 180 and 520 nmole ALA/g liver/hr while practically no microscopic fluorescence was found in the livers of these animals suggests that these ALAS activities are not correlated with hepatic porphyrin fluorescence (Table 1). Protoporphyrin (PROTO), coproporphyrin (COPRO), and uroporphyrin (URO) were present in the liver during treatment with the halogenated aromatics, HCB (*4*) and PBB. This suggests an increased heme synthesis, also because of an increased P-450 content (*4*) and a doubled ALAS activity. ALAS activity is fast, but slightly increased long before porphyria develops (see Fig. 1).

Porphyrin Accumulation

PBB-porphyria developed from completely fluorescent fragments or the margins in the liver so that the entire liver showed diffuse fluorescence. PBB porphyria is characterized by an accumulation of porphyrins with 5-8 COOH groups in the liver, bile, and kidneys (Table 7). Later porphyrinogens were found in the blood of these animals. The hepatic and renal porphyrins thus must have formed *in situ*. Porphyrins with 5-8 COOH groups normally are not found in the bile. The presence of these porphyrins in the bile of PBB-treated animals suggests a disturbance of the excretion of these porphyrins from the liver into the blood.

With PBB, porphyrin accumulation develops first in the kidneys and gall bladder before it occurs in the liver (Table 6).

The exclusive presence in the kidneys of porphyrins with 5, 6, and 7 COOH groups in the animals treated with PBB which died evidently indicates an uncoupling from the cytoplasmatic to the mitochondrial compartmentalization and an excretory disturbance of the kidneys. Porphyrin accumulation in the liver is the consequence of this.

Hepatic and renal porphyrin accumulation requires prolonged loading with HCB (*4*) and PBB and appears just before death. This was also found by de Matteis et al. (*9*) in rabbits, by Vos et al. (*10*) in Japanese quail, and by Simon et al. (*11*) in rats. Porphyrin accumulation in the bile occurred within a few days. The hepatic porphyrins appear much later, while renal porphyrins occur only when the animals are moribund. Evidently, the liver can excrete porphyrins via the bile at the start of the PBB loading period, but in a later stage this is no longer possible, because the liver and kidneys are then flooded with porphyrins.

The sensitivity of Japanese quail for the porphyrinogenic action of HCB (*4*) and PBB is remarkable when compared to rats and mice. Japanese quail can exhibit porphyrin accumulation in the bile and liver as a result of HCB (*4*) and PBB within a few days. In rats, the earliest porphyrin accumulation in the liver occurs only after 8 weeks of HCB treatment (*11*), and it can require more than 12 months in some cases (*12*). Two rats which we

Table 9. Activity of serum glutamic acid dehydrogenase (SGLDH) and of serum glutamic acid pyrotartaric acid transaminase (SGPT) during the porphyrinogenesis by PBB in the Japanese quail.[a]

No. of animals	Substance	Dose, mg/kg	Duration of treatment, days	SGPT, mU/ml	SGLDH, mU/ml	Positive fluorescence/n animals	
						Bile	Liver
6	Control	—	5	0.04 ± 0.02	2.52 ± 0.28	0/6	0/6
6	PBB	500	5	0.04 ± 0.02	3.10 ± 0.31	1/6	0/6
6	Control	—	10	0.05 ± 0.02	0.0	0/6	0/6
6	PBB	500	10	0.03 ± 0.01	0.58 ± 0.14[b]	4/6	0/6
6	Control	—	13	0.06 ± 0.02	0.48 ± 0.14	0/6	0/6
6	PBB	500	13	0.06 ± 0.01	2.23 ± 0.27[b]	3/6	0/6
6	Control	—	20	0.08 ± 0.02	1.33 ± 0.20	0/6	0/6
6	PBB	500	20	0.07 ± 0.02	4.62 ± 0.39[b]	1/6	0/6

[a] Male Japanese quail were fed 500 ppm PBB. On different days the activities of GLDH and GPT were determined in the pooled serum of three animals. The porphyrin accumulation in the bile, liver, and kidneys was detected with the fluorescence microscope.
[b] $p < 0.05$; means ± SEM

Table 10. Activity of SGLDH and of SGPT during porphyrinogenesis by PBB in Japanese quail.[a]

No. of animals	Substance	PBB dose, mg/kg	Duration of treatment, days	SGPT, mU/ml	SGLDH, U/ml	Positive fluorescence/n animals	
						Bile	Liver
6	Control	—	3	0.07 ± 0.01	0.98 ± 0.35	0/6	0/6
6	PBB	1000	3	0.08 ± 0.01	0.56 ± 0.41	6/6	0/6
6	Control	—	5	0.04 ± 0.03	0.36	0/6	0/6
7	PBB	1000	5	0.06 ± 0.01	0.96 ± 0.54[b]	3/7	3/7

[a] Japanese quail were daily dosed orally, with capsules with 100 mg/kg PBB. On different days the activities of GLDH and GPT were determined in the combined serum of 2 or 3 animals. The porphyrin accumulation in the bile, liver and kidneys was detected with the fluorescence microscope.
[b] $p < 0.05$; means ± SEM

loaded with 500–1000 mg PBB/kg-day for one month died without porphyrin fluorescence in the liver and kidneys. Five kestrels (*Falco tinnunculus*) loaded with PBB 250–1000 mg/kg for 9-16 days, did not develop porphyria. Mice and guinea pigs cannot be made porphyric with HCB but die with the loading dose employed (*9*).

Heme

The stimulated heme formation detectable by an elevated P-450 content in the liver after HCB loading (*13–15*) requires a higher ALA supply. After the application of PBB, a decrease of the heme content of liver can be observed. Not a single PBB-loaded animal exhibited porphyrin accumulation in the liver (Table 2).

The experiments in Table 3 show that porphyrin production in the liver, which is excreted through the bile and which was induced in the animals by a single dose of PBB, can be returned to a normal level by hemin. The heme deficiency induced by PBB at this stage is eliminated by hemin. The increased heme synthesis, detectable by the twofold ALAS excretion through the bile is inhibited by hemin. In this phase of PBB loading, heme plays the role of a regulator as was assumed by Granick (*16*). Heme can exert a feedback.

The polyhalogenated aromatic PBB produces a diminished heme content prior to the development of porphyria. This occurs parallel to increased heme synthesis. This can be detected by an increase or constant heme content per total but enlarged liver (Table 2). During treatment an immediate decrease of heme (Table 2) and a twofold increase of ALAS activity (Table 1) occur before hepatic porphyrin accumulation becomes manifest. The increased heme synthesis is relevant for the production of cytochrome P-450, which plays an important role in the metabolism of exogenous substances. The inducer thereby increases the possibilities for an accelerated turnover of itself. This cannot be accomplished, because PBB is only sparingly metabolized. For mixtures of Clophen A50 (PCB) and PBB in Japanese quail liver microsomes, very diffuse P-450 binding spectra (type I spectral binding) were found, probably due to interactions of several compounds which might counteract each other (*8*). A possible metabolism of PBBs by the liver P-450 oxidation system is very slow. Chlori-

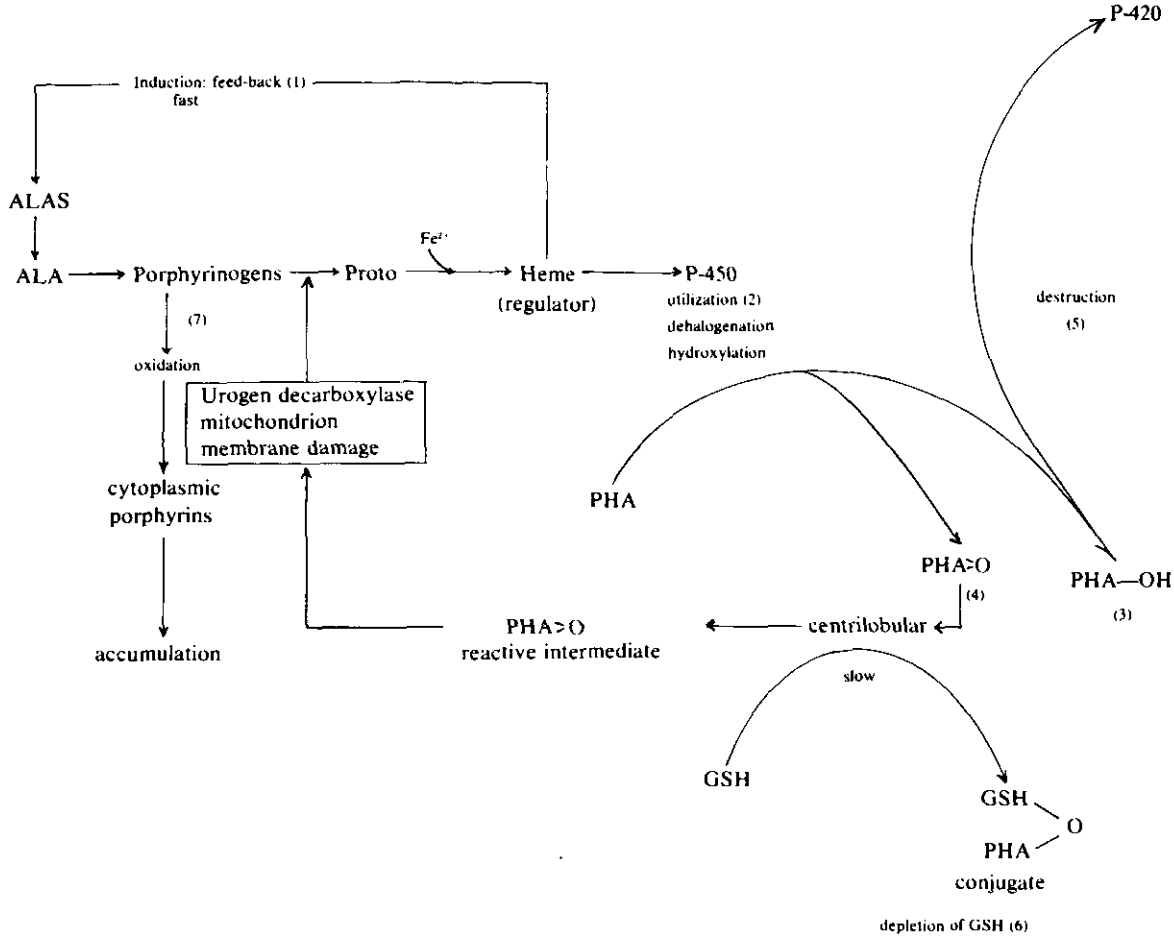

FIGURE 1. Mechanism of action of hepatic porphyria caused by polyhalogenated compounds (PHA).

nated phenols, such as metabolites of hexachlorobenzene (*17*) converted cytochrome P-450 into P-420 *in vitro* (*8*). Indications of increased heme utilization caused by polyhalogenated aromatics can also be found in the literature. Wada et al. (*13*) found an elevated P-450 content and a twofold increase of ALAS activity with 2 days of HCB loading. The same was observed by Sweeney et al. (*18*) (see Fig. 1). The experiments of Stonard (*14*), Stonard and Zenov (*15*), Rajamanickam et al. (*19*) and Strik and Koeman (*20*) confirm the increase of the hemoprotein P-450 content under the influence of HCB (*4*). The animals were in good condition during these experiments. There was no indication of hepatic porphyrin accumulation. Rajamanickam et al. (*19*) also found an elevated incorporation of glycine and ALA in heme and P-450. Polychlorobiphenyl (PCB) and phenobarbital induce an elevated P-450 content (*5, 21*) and some microsomal enzyme reactions, such as demethylation, reduction and hydroxylation in the liver (*21*). This indicates that the polyhalogenated aromatics (HCB, PBB, and PCB) stimulate the liver to increased heme synthesis as a reaction to the exogenous substances. This is necessary for the production of the hemoprotein P-450 which plays an important role in the metabolism of exogenous substances. This results in a heme deficiency which has a derepressing effect on the neosynthesis of ALAS or a deinhibiting effect on its activity. This increased ALAS activity is implicitly related with increased heme synthesis (Table 1). A continuous decrease of the heme content by the application of PBB (Table 2) may be due to a disturbance in protoporphyrin synthesis. Oxidized porphyrinogens accumulate and sufficient protoporphyrin is no longer produced. A consequence of this is a further decrease of the heme content, possibly paralleled by an accumulation of iron. This iron can no longer be effectively utilized by incorporation in protoporphyrin. According to Bach et al. (*22*), HCB porphyria in rats is paralleled by a storage of iron and accumula-

tion of porphyrins in the liver.

In contrast, the heme deficiency and excessive porphyrin accumulation in the liver, bile, and kidneys resulting from 7 days of PBB loading apparently can not be inhibited by hemin (Table 4).

Heme could not block uroporphyrin accumulation from added ALA in presence of polyhalogenated aromatic compounds in chick embryo liver cell culture (*23*). Heme can not exert a feedback when porphyria develops. Hemin does not play the role which it fulfills with a few doses of PBB when heme synthesis is increased in this stage, and there is no indication of porphyria. In this second phase of PBB loading, when porphyria is involved, porphyrin or heme synthesis as well as porphyrin byproduct elimination are damaged to such a degree that hemin is not capable of counteracting the porphyria and then can no longer exercise its feedback function. A disturbed protoporphyrin synthesis is also supported by the finding of Taljaard et al. (*24*) that uroporphyrinogen decarboxylation activity was reduced to zero in HCB rats with a completely porphyric liver. According to these authors (*24*) and Table 7, protoporphyrin synthesis is arrested in the stage of decarboxylation of the porphyrinogens.

Heme also plays a central regulatory function in PBB-stimulated heme synthesis. This is no longer the case when porphyria has developed due to these substances.

Reactive Intermediate

According to Sinclair and Granick (*23*), there is an induction of a metabolizing system. This requires protein synthesis, since it is blocked by cycloheximide. The polyhalogenated aromatic is converted by the induced drug metabolizing enzyme system to a metabolite (see Fig. 1), since known inhibitors of cytochrome P-450 action (e.g. SKF-525A) prevent uroporphyrin formation. Furthermore iron (P-450-dependent iron?) is required at some reaction step for the formation of the metabolite that causes uroporphyrin accumulation. The active compound is not a known metabolite, but is probably an intermediate (Fig. 1), because known metabolites of HCB (*17*) did not cause any porphyria in Japanese quail (*2*) but can convert P-450 *in vitro* into P-420 (*8*) (Fig. 1).

Brodie (*25*) indicates that centrilobular liver necrosis produced by bromobenzene is mediated through a chemically active metabolite, which is formed oxidatively by enzymes in liver microsomes. The active intermediate of bromobenzene is an epoxide which reacts with glutathione (GSH). After liver GSH has been sufficiently depleted, the epoxide then reacts with tissue macromolecules including proteins (Fig. 1). Experiments were carried out to test if GSH depletion correlates with liver porphyria caused by polyhalogenated aromatics in Japanese quail.

Mitochondrial Enzymes

PBB, administered in capsules, produce an immediate biochemically detectable liver lesion. This becomes manifest by an elevated SGLDH activity (*1*, *26*) (Tables 9 and 10).

Pietschmann and Raab (*27*) and Simon et al. (*11*) also found an elevation of SGPT, and Ivanov (*28*) found an increase in γ-glutamyltranspeptidase (γ-GT) in rats made porphyric with HCB. However, their experiments do not reveal whether the observed liver damage began before or after the onset of porphyria (i.e., hepatic porphyrin accumulation).

The liver lesion observed occurs before the animals exhibit hepatic porphyrin accumulation and before a morphological liver lesion can be detected. This may indicate that PBB-porphyria is a form which develops on or after damage of the liver has occurred. Because serum GLDH (SGLDH), as a typical mitochondrial enzyme, is elevated while SGPT is not, PBB may possibly act by damaging the membranes and particularly the mitochondrial membrane. A possible pathological change in the cell membrane permeability caused by HCB has also been postulated (*29*). The affinity of polyhalogenated aromatics for membrane structures which are rich in lipids may be assumed because of their lipophilic character. The affinity of PBB for lipid-rich membrane structures can also become manifest at the start of treatment with these substances. The membrane structure, of which cytochrome P-450 is a part, does not become functional by interaction of PBB with HCB (*4*). P-450 is released from its structure and the free P-450 is immediately metabolized by biliverdin. An increased degradation of P-450 demands a new supply. Heme availability or heme synthesis is adjusted to this. This may represent a possible explanation of the increased heme synthesis caused by polyhalogenated aromatics. It can be recognized by a two- to threefold increase in ALAS activity and increased release of by-products of heme synthesis (porphyrins) in the liver. This becomes manifest as an elevated porphyrin accumulation in the bile, which is the first to be detected.

The observation of Sweeney et al. (*18*) that hepatic mitochondria diminish during HCB loading also suggests a mitochondrial effect of HCB. The cytoplasmatic enzymes of heme synthesis can then still function normally and the cytoplasmatic porphyrins (with 4-8 COOH groups) accumulate in the liver (Table 7).

Uroporphyrinogen Decarboxylase

Polyhalogenated aromatic compounds evoke a cytoplasmic porphyria characterized by porphyrins with 7 and 8 carboxyl groups in the liver, 5, 6, 7, and 8 carboxyl groups in the bile, and 6, 7, and 8 carboxyl groups in the kidney (*2, 4*). Due to the marked increase in higher carboxylated porphyrins in the liver and urine in this experimental porphyria, it was felt that there may be a failure to decarboxylate uroporphyrinogen normally (*9*). In fact uroporphyrinogen decarboxylase activity could not be detected in the livers of HCB-treated animals (*24, 30*).

Evidence (Fig. 1) was obtained for an instable intermediate that is generated in the liver from chlorinated hydrocarbons which inhibits uroporphyrinogen decarboxylase, thus causing uroporphyrin to accumulate. The data suggest that the intermediate may be a hydroxylated derivative (*23*).

Sexual Hormones

The relative proportions of steroid hormones produced by the ovaries and the testes are possibly involved in sensitivity of organisms to porphyrinogenic compounds (*4, 31–33*). Nonfunctional dearrangement of smooth endoplasmatic reticulum (SER) is manifested in whorl formation. This phenomenon is found only in male rats (*20*). The formation of a possible porphyrinogenic intermediate may be slowed down by the nonfunctional rearrangement of SER.

Conclusion

PPBs (*4*) increase the heme utilization by cytoplasmic hemoproteins (the cytochromes). An increased demand for heme by these hemoproteins can be filled by increased heme synthesis, detectable by a more than about two fold increase in ALAS activity in the liver and porphyrin by-products in the bile. This increased heme synthesis can be inhibited by hemin.

PBB porphyria (*1*) is preceded by liver and kidney damage. Accumulation of porphyrins in the liver is not solely due to an increase of ALAS activity, but rather to drug enzyme induction and the formation of an reactive intermediate which causes centrilobular liver damage and ultimately porphyria. The hepatic mitochondria decrease in number and are damaged (elevated SGLDH); the proliferated endoplasmic reticulum, including the hemoprotein P-450, is no longer capable of normal activity; uroporphyrinogen decarboxylase activity is reduced to zero; renal porphyrins accumulate and are excreted in the liver and via the bile; in addition there are many morphological changes.

Porphyria in Humans

Some polyhalogenated aromatics are able to produce liver porphyria in experimental animals (*34*). Chronic hepatic porphyria in humans caused by polyhalogenated hydrocarbons is known. Chlorodibenzodioxins (*35, 36*), hexachlorobenzene (*37*), methyl chloride (*38*) and vinyl chloride (*39*) evoke increased total urinary porphyrin values of 81-8778 μg/l. and an abnormal porphyrin pattern in urine.

A group which has been exposed to PBB, a powerful porphyrinogen, over the past several years has been identified. The sole purpose of the investigation was to assess liver health, using urine porphyrin excretion as an indicator in a population of farm families which, was presumed, received the highest doses of PBB. No attempt has been made to analyze or assess any other PBB-produced conditions, symptoms, or illnesses unrelated to liver damage.

Because of the macroscopic fluorescence of the urines examined, total porphyrin values of more than 100 μg/l., and abnormal thin-layer chromatography porphyrin patterns (containing porphyrins with 7, 6, or 5 COOH groups), it was concluded that in 26 to 45% of the study group, members of the farm families exposed to PBB indications for the presence of coproporphyrinuria and chronic hepatic porphyria type A could be detected. These symptoms may be indicative of slight liver damage (*40*).

REFERENCES

1. Strik, J. J. T. W. A. Toxicity of hexachlorobenzene (HCB) and hexabromobiphenyl (HBB). Meded. Rijksfac. Landbouwwet. Gent 38: 709 (1973).
2. Strik, J. J. T. W. A. Porphyrinogenic action of polyhalogenated aromatic compounds with special reference to porphyria and environmental impact. In: Proceedings of the International Symposium on Clinical and Biochemical Diagnosis and Therapy of Porphyrias and Lead Intoxication. M. Doss, Ed., Springer, Berlin, in press.
3. Mendoza, C. E., et al. Effects of bromobenzene on the male rat. Toxicol. Appl. Pharmacol. 41: 127 (1977).
4. Strik, J. J. T. W. A. Experimental hepatic porphyria in birds. Ph.D. Thesis, University of Utrecht, 1973.
5. Vos, J. G., et al. Polychlorinated biphenyls as inducers of hepatic porphyria in Japanese quail, with special reference to delta-amino levulinic acid synthase activity, fluorescence and residues in the liver. Toxicol. Appl. Pharmacol. 20: 232 (1971).
6. Strik, J. J. T. W. A. Species differences in experimental porphyria caused by polyhalogenated aromatic compounds. Enzyme 16: 224 (1973).
7. Vos, J. G., et al. Toxicity of hexachlorobenzene in Japanese quail with special reference to porphyria, liver damage, reproduction and tissue residues. Toxicol. Appl. Pharmacol. 18: 944 (1971).

8. Förlin, L., and Strik, J. J. T. W. A. Spectral interaction of polyhalogenated aromatics with hepatic cytochrome P-450 in different species. In: Proceedings of the International Conference on *In Vivo* Aspects of Biotransformation and Toxicity of Industrial and Environmental Xenobiotics. I. Gut, Ed., Elsevier, Amsterdam, in press.
9. de Matteis, F., Prior, B. E., and Rimington, C. Nervous and biochemical disturbances following hexachlorobenzene intoxication. Nature 291: 363 (1961).
10. Vos, J. G., et al. Experimental studies with HCB in birds. TNO-Nieuws 27: 599 (1972).
11. Simon, N., Dobozy, A., and Berko, G. Porphyrinstoffwechsel-und klinisch laboratoriumsmässige Untersuchungen in Modellversuchen und an Patienten mit Porphyria Cutanea Tarda. Arch. Klin. Exp. Derm. 238: 38 (1970).
12. Elder, G. H. Identification of a group of tetracarboxylate porphyrins, containing one acetate and three propionate β-substituents in faeces from patients with symptomatic cutaneous hepatic porphyria and from rats with porphyria due to hexachlorobenzene. Biochem. J. 126: 877 (1972).
13. Wada, O., et al. Behavior of hepatic microsomal cytochromes after treatment of mice with drugs known to disturb porphyrin metabolism in liver. Biochem. Pharmacol. 17: 595 (1968).
14. Stonard, M. D. Early biochemical changes observed in rat liver during chronic feeding of the porphyrinogenic compound hexachlorobenzene. In: Abstr. Commun. Meet. Fed. Eur. Biochem. Soc. 8: 869 (1972).
15. Stonard, M. D., and Zenov, P. Effect of hexachlorobenzene on hepatic microsomal enzymes in the rat. Biochem. Pharmacol. 23: 2175 (1974).
16. Granick, S. The induction *in vitro* of the synthesis of δ-aminolevulinic acid synthetase in chemical porphyria: A response to certain drugs, sex hormones and foreign compounds. J. Biol. Chem. 241: 1359 (1966).
17. Koss, G., Koransky, W., and Steinback, K. Studies on the toxicology of hexachlorobenzene. II. Identification and determination of metabolites. Arch. Toxicol. 35: 107 (1976).
18. Sweeney, G. D., et al. Experimental porphyria. I. The experimental porphyrias—a group of distinctive metabolic lesions. S. Afr. J. Lab. Clin. Med. 45: 68 (1971).
19. Rajamanickan, C., et al. Effect of hexachlorobenzene on haeme synthesis. Biochem. J. 129: 381 (1972).
20. Strik, J. J. T. W. A., and Koeman, J. H. Porphyrinogenic action of hexachlorobenzene and octachlorostyrene. In: Porphyrins in Human Diseases. M. Doss, Ed., Karger, Basel, 1976, p. 418.
21. Litterst, C. L., and E. J. van Loon. Enzyme induction by polychlorinated biphenyls relative to known inducing agents. Proc. Soc. Exptl. Biol. Med. 141: 765 (1972).
22. Bach, P. H., et al. The effect of iron overload on the development of hexachlorobenzene (HCB) porphyria. S. Afr. J. Lab. Clin. Med. 17: 75 (1972).
23. Sinclair, P., and Granick, S. Uroporphyrin formation induced by chlorinated hydrocarbons (Lindane, polychlorinated biphenyls, tetrachlorodibenzo-*p*-dioxin). Requirements for endogenous iron, protein synthesis and drug metabolizing activity. Biochem. Biophys. Res. Commun. 61: 124 (1974).
24. Taljaard, J. J. F., Shanley, B. C., and Joubert, S. M. Decreased uroporphyrinogen decarboxylase activity in experimental symptomatic porphyria. Life Sci. 10: 887 (1971).
25. Brodie, B. Possible mechanisms of drug-induced tissue lesions. Chem.-Biol. Interactions 3: 247 (1971).
26. Strik, J. J. T. W. A. Chemical porphyria in Japanese quail (*Coturnix c. Japonica*). Enzyme 16: 211 (1973).
27. Pietschmann, H., and Raab, W. Hepatische Porphyrien: Veränderungen der Serumenzymaktivitäten und hämatologische Befunde beim Menschen und bei der Ratte. Congr. Int. Dermat. 13: 1293 (1967).
28. Ivanov, E., et al. Mechanism of the changes in serum and liver γ-glutamyltranspeptidase activity. II. Experimental hexachlorobenzene porphyria in rabbits. Enzyme 21: 17 (1976).
29. Kószó, F., Siklósi, C., and Simon, N. Liposome model experiment for the study of assumed membrane damage in porphyria cutanea tarda. Biochem. Biophys. Acta 363: 182 (1974).
30. Elder, G. H., Evans, J. D., and Matlin, S. A. The effect of the porphyrinogenic compound, hexachlorobenzene, on the activity of hepatic uroporphyrinogen decarboxylase in the rat. Clin. Sci. Mol. Med. 51: 71 (1976).
31. Ippen, H., Aust, D., and Goerz, G. Klinisch-experimentelle Untersuchungen zur Entstehung der Porphyrien. III. Wirkung einiger Steroid-Hormone auf die 'latente Hexachlorbenzol-Porphyrie' der Ratte. Arch. Dermatol. Forsch. 245: 305 (1972).
32. Simon, N., Berko, G., and Dobozy, A. Influence of sex hormones on porphyrin metabolism in model experiments. Borgyogy. Venerol. Szemle 49: 49 (1973).
33. Grant, D. L., Shields, J. B., and Villeneuve, D. C. Chemical (HCB) porphyria. Effect of removal of sex organs in the rat. Bull. Environ. Contam. Toxicol. 14: 422 (1975).
34. Strik, J. J. T. W. A., and Wit, J. G. Hepatic porphyria in birds and mammals. TNO-Nieuws 27: 604 (1972).
35. Bleiberg, J., et al. Industrially acquired porphyria. Arch. Dermatol. 89: 793 (1964).
36. Jirásek, L., et al. Chlorakne, Porphyria cutanea tarda und andere Intoxikationen durch Herbizide. Hautarzt 27: 328 (1976).
37. Cam, C., and Nigogosyan, G. Acquired toxic porphyria cutanea tarda due to hexachlorobenzene. J. Amer. Med. Assoc. 183: 88 (1963).
38. Chalmers, J. N. M., Gillam, A. E., and Kench, J. E. Porphyrinuria. In a case of industrial methyl chloride poisoning. Lancet: 806 (Dec. 28, 1940).
39. Lange, C. E., et al. Urinary porphyrins among PVC workers. In: Porphyrins in Human Diseases. M. Doss, Ed., Basel, Karger, 1976, p. 352.
40. Strik, J. J. T. W. A., et al. Coproporphyrinuria and chronic hepatic porphyria found in farm families in Michigan, U.S.A., exposed to polybrominated biphenyls (PBB). In preparation.