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Comparison of the Porphyrinogenic Activity of Hexabromobenzene and Hexachlorobenzene in Primiparous Wistar Rats

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Hepatic porphyria in various animal species has been reported to be induced by hexachlorobenzene (HCB) (STRIK 1973). One of the symptoms is a high porphyrin level in the liver and urine. Excretion of porphyrins in rats was enhanced by estradiol administration and was further enhanced by administration of a combination of estrogen and androgen, or gestagen (IPPEN and AUST 1972). Rat pups breast feeding on dams fed diet containing 80-ppm HCB accumulated liver porphyrin higher than those nursing on the control dams (MENDOZA et al. 1975).

A closely related compound, hexabromobenzene (HBB), is a fire retardant used in plastics, textiles, and woods (NEGISHI et al. 1972, RALEY 1972, MISCHUTIN 1974, PASHIN et al. 1974). In adult male Wistar rats, no significant effects were observed after feeding ad libitum on 160 ppm HBB in the diet for 12 weeks (MENDOZA et al. 1977). As far as we know, no report is available on the porphyrinogenic activity of HBB.

In humans, another symptomatic type of porphyria, acute intermittent porphyria, has been associated with pregnancy. Acute intermittent porphyria has been reported to become severe during pregnancy (REIDENBERG and FARBER 1955, DURST and KREMBS 1956, HUGHES 1957, ZIMMERMAN et al. 1966). On the contrary, FREEDMAN et al. (1952) observed that a 24-year old woman with acute porphyria recovered clinically to a remarkable degree during pregnancy and after delivery. Two other porphyria cases that have been reported improved in the last trimester of pregnancy and following delivery (LINAS et al. 1947, COLEMAN 1948). It is, therefore, interesting to investigate the porphyrinogenic activity of HBB and HCB in rats post partum.

This study was then initiated to compare the porphyrinogenic potential of HBB with HCB in the liver of primiparous rats. To determine the degree of HBB and HCB effects on the other systems, during the test, esterase activities and body and organ weights were also measured.

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MATERIALS AND METHODS

Female Wistar rats, about 250 g, (Woodlyn Laboratories, Ltd.) were fed ad libitum powdered rat cubes containing 80 ppm HBB (98% purity, m.p. >300°C), (Aldrich Chemical Co.) or HCB (BDH Chemicals, Ltd.) and 4% corn oil two weeks before mating until the termination of the experiment. The control received diets containing 4% corn oil only. After mating, they were placed in individual cages. The dams gave births within a period of one month. The females were allowed to whelp only once and to nurse their pups for 16 to 23 days. (The pups were used in another experiment.)

The dams were sacrificed 101 days after initiation of test diets or 35-66 days after weaning. The body and liver weights were determined. The liver was rinsed with a cold physiological saline solution, blotted before weighing and stored in a freezer pending analysis. HCB and HBB residues were determined in the livers.

HCB was extracted by homogenizing the liver (20%, w/v) in redistilled water. A 0.2 ml of homogenate was diluted with 0.8 ml of distilled water and was subsequently shaken with 5 ml of hexane for 20 min. The hexane layer was analyzed by gas chromatography (MENDOZA et al. 1975).

For HBB extraction, 0.2 ml of 20% liver homogenate was mixed with 5 ml of acetonitrile before adding 5 ml of distilled water and was shaken with 5 ml of hexane for 1 hr. The hexane layer was analyzed by gas chromatography (MENDOZA and LAVER 1977).

Esterase activity was determined using thiophenyl acetate (TPA), (Polysciences, Inc.); indophenyl acetate (IPA), (Eastman Kodak) and p-nitrophenyl acetate (PNPA), (Sigma Chemical Co.). The rate of substrate hydrolysis was determined spectrophotometrically. The method of (ELLMAN et al (1966), modified by AUGUSTINSSON et al. (1972) was used to measure esterase hydrolysis of TPA. Our previously published methods of MENDOZA et al. (1976) were used to determine the rate of PNPA and IPA hydrolysis.

The tissue protein was determined by the biuret method (GORNALL et al. 1948) after solubilization with 0.8% sodium desoxycholate. Crystalline albumin from bovine serum (General Biochemicals) was used as a protein standard.

The porphyrin concentration in the liver was determined according to the method of ABBRETTI and

DE MATTEIS (1971). Coproporphyrin (Sigma Chemical Co.) was used as a standard reference.

RESULTS AND DISCUSSION

It should be noted that no marked difference in external appearance could be discerned between the control and HCB, or HBB, groups. There was no statistical difference shown between the body or liver weights in both experiments. The mean body weights were 328±5 g for control, 322±5 g for HCB and 317±5 g for HBB groups. The mean liver weights were approximately 12 g for all groups.

Reproduction and survival statistics

Table 1 shows that the mean number of pups per litter in HCB and HBB treated groups was comparable to that of the control. The gestation indices were similar in controls and the treated groups. Only 0.7, 2.6, and 1.6% stillborn were observed in the control, HCB and HBB groups, respectively. The mean numbers of pups at parturition were 12.6 for control, 12.1 for HCB and 12.0 for HBB. Even during the nursing period, only a 0.4% death rate was observed in either HCB or control groups. No death was observed in HBB groups during this period. These results suggest that HCB or HBB at the 80-ppm level in the diet for 101 days has no marked effects on gestation and neonatal survival.

Porphyrin level in the liver

Table 2 shows liver porphyrin levels in the control, HBB and HCB groups. The HBB group has a significantly higher level of porphyrin than or HCB control groups. Possibly, the 2% impurity in the HBB preparation could also be porphyrinogenic. However, its contribution to the porphyrinogenic effect may be insignificant since the total amount per liver expected is very small, assuming that its retention in the tissues was similar to HBB. The contaminants indentified in the HBB preparation used were pentabromobenzene and pentabromochlorobenzene, which were confirmed by mass spectrometry.

It should be noted in Table 2 that the HCB group has to be divided into two sub-groups: (A), without and (B), with elevated porphyrin levels. The non-porphyric rats were equally exposed to HCB as the susceptible rats and their HCB levels in liver (see Table 3) were comparable. These observations indicate that some rats were genetically resistant to porphyrin accumulation or had a remission of porphyria during

TABLE 1

Reproduction and survival statistics for rats fed control, 80 ppm HCB or 80 ppm HBB diet[a]

Treatment	Number of litters observed	Mean number of pups/ litter ± S.E.	Mean number alive at birth/litter	Gestation index[b]
Control	16	12.6 ± 0.9	12.0	0.98
HBB	15	12.1 ± 1.2	11.8	0.98
HCB	14	12.0 ± 0.9	11.8	0.98

[a]S.E. = standard error of the mean.

[b]Mean number alive/mean number of pups per litter.

TABLE 2

Liver porphyrin concentration (nmole/g of wet tissues) in dams fed control, 80 ppm HCB or 80 ppm HBB diet

Treatment	n	Porphyrin (nmole/g of wet tissue)
Control	8	0.45 ± 0.07
HBB	10	534.9 ± 80.9[b,c]
HCB[a]	16	6.62 ± 2.75[d]
(A)	(8)	(0.48 ± 0.06)
(B)	(8)	(12.76 ± 4.66)[d,e]

[a]HCB group was subdivided into two subgroups (A) and (B), in order to differentiate the non-porphyric from porphyric rats.

[b]Statistically different from the control, $p < 0.01$.

[c]Statistically different from HCB (B), $p < 0.01$.

[d]Statistically different from the control, $p < 0.05$.

[e]Statistically different from HCB (A), $p < 0.05$.

gestation, lactation and intervening period prior to sacrifice. The proportions of those rats observed with elevated porphyrin levels agreed with that reported by DE MATTEIS et al. (1961) for rabbits. These workers observed that female rabbits fed a diet containing 0.5% HCB did not respond uniformly to the treatment. Out of 4 rabbits tested, two showed marked disturbance of porphyrin metabolism, one less marked and one only slight disturbance. GRANT et al. (1975) and KUIPER-GOODMAN et al. (1977) also noted the variability of liver porphyrin concentrations in rats fed HCB. GOLDSTEIN et al. (1976) observed the heterogeneity of response in chicks to porphyrinogenic activity of some hexachlorobiphenyl isomers.

HCB and HBB residues in the liver

Table 3 shows HBB and HCB residue levels in livers of rat dam 35-66 days after weaning. There was approximately 90-fold and >50-fold increase in HCB and HBB residues, respectively, over the control. The data clearly indicate that HCB accumulated in the liver approximately 8 times that of HBB.

TABLE 3

HCB and HBB residues (µg/g of wet tissues ± S.E. in livers of rat dams after 101 days in control, 80 ppm HCB or 80 ppm HBB diet[a]

Treatment	n	HCB (ppm)	HBB (ppm)
Control	8	0.46 ± 0	0.1
HBB[b]	15	-	4.82 ± 0.70
HCB[b]	16	39.78 ± 2.59	-
(A)	(8)	(42.6 ± 4.3)	-
(B)	(8)	(37.0 ± 2.8)	-

[a] S.E. = standard error of the mean. n = number of individuals.
[b] HCB group was divided into two subgroups (A) and (B) based on the porphyrin levels in Table 2.

Liver esterase activity

The liver esterase activities toward TPA, PNPA and IPA were measured to determine if the levels of HBB or HCB found in the tissues have any effects on this system. Activities towards TPA and PNPA were observed significantly higher in the HBB group than in HCB or control groups. These studies demonstrated that perhaps HBB is a persistent inducer of esterases in the dams. The data also showed that possibly the amount of HCB was reduced by lactation to a level which was not effective to induce the enzyme. The low values for esterase activity toward TPA and PNPA as compared to the control is inexplicable at this moment. No significant difference was noted in the esterase activity towards IPA between control, HCB and HBB groups. It should be noted that the esterases studied are microsomal whereas porphyrin synthesis is mitochondrial in origin. These parameters, the esterase activity and porphyrin level, must not be compared directly.

TABLE 4

Liver esterase activity (μmol/min/g of protein ± S.E.) in rat dams after 101 days in a diet containing 80 ppm HCB[a]

Treatment	n	TPA	PNPA	IPA
Control	16	332 ± 25	882 ± 84	99 ± 4
HCB	32	243 ± 8[b]	411 ± 20[b]	99 ± 3
HBB	28	514 ± 20[c]	1190 ± 50[c]	77 ± 3

[a] S.E. = standard error of the mean; see the text for chemical observations. n = number of analysis, 2 analyses per sample.

[b] Significantly different from the control or HBB treated at $p < 0.01$.

[c] Significantly different from the controls $p < 0.01$.

SUMMARY and CONCLUSION

It can be concluded that HBB used in this study was more porphyrinogenic than HCB. The results suggest that HBB was an effective porphyrinogen even at a concentration in the liver of only 1/8 that of HCB. Further studies should be encouraged to determine the biochemical and pharmacological significance of HBB in the development of hepatic phorphyria. The effects of lactation on the body burden of HBB or HCB should also be investigated.

This study showed that HCB-treated primiparous rats did not show elevated esterase activity after over three months of continous feeding on a diet containing 80 ppm HCB. Some of these dams showed elevated levels of porphyrin although the HCB residue levels in the liver were similar to those of the non-porphyric, HCB-fed dams. This discrepancy in the response suggests genetic heterogeneity of predisposition to, or ability to recover from, acquired porphyria in the rat. The low porphyrin levels in some cases may also be due to reduction of HCB concentrations during lactation. This, perhaps, suggests that lactation may have exerted some therapeutic effects on certain porphyric animals but it could consequently be deleterious to the nursing pups (MENDOZA et al. 1975).

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