HEPATIC METABOLISM OF HEXACHLOROBENZENE IN RATS

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1. Introduction

Hexachlorobenzene (HCB) has found agricultural use in the past as a fungicide and is a by-product of industrial aromatic chlorination procedures. The chemical is a significant environmental contaminant with long biological half-life and for that reason it has been of interest to environmental agencies. In addition, HCB was responsible for an epidemic of cutaneous porphyria which occurred in Turkey in the late 1950's when HCB-treated seed wheat was consumed during a period of famine [1]. The mechanism of this toxic form of porphyria cutanea tarda is of interest in part as a manifestation of hepato-toxicity of chlorinated hydrocarbons and in part because the unique biochemical lesion produced is very similar to that seen in man following exposure to ethanol, synthetic estrogens and progestogens, and other hepato-toxins.

In rats, the addition of 0.25% of HCB to the diet will produce an experimental porphyria in from 6 to 12 weeks [2]. In these animals, liver weight increases significantly; there is a large increase in smooth endoplasmic reticulum with marked induction of cytochrome *P*-450. Also, there is evidence both at a light microscopic and electron microscopic level of hepatocellular damage with necrosis of centrilobular cells [3]. It would be of particular interest if such striking morphologic and biochemical changes could be produced by a compound which is entirely inert, but when Parke and Williams studied the metabolism of chlorinated hydrocarbons some years back [4], they were unable to demonstrate metabolites of HCB and a survey of more recent literature has failed to produce a different view. In this communication we report evidence that HCB is not inert but is metabolized to pentachlorophenol (PCP) and also to more highly polar derivatives.

2. Materials and methods

Technical grade HCB was obtained from Eastman Kodak and recrystallised from hot benzene to yield fine colourless needles with a melting point of 225–226°C. This material was mixed with powdered rat chow to a concentration of 0.25% in the diet. Male Long Evans rats weighing 150–200 g housed in metabolic cages were allowed access *ad lib* to powdered rat chow containing HCB and to drinking water.

2.1. *Extraction of urine*

Although it was likely that phenolic derivatives of HCB might be conjugated prior to excretion in the urine, we found no greater yield of PCP if urine was submitted to acid hydrolysis prior to extraction. Various extraction sequences were tried; optimal recovery of PCP followed initial extraction of 20 ml of urine with 100 ml of chloroform: after chloroform had been washed with an equal volume of 0.1 N NaOH and the aqueous phase was back-extracted with chloroform after acidifying with 60 ml of 1 N HCl. The chloroform fractions were combined, dried over calcium chloride and the solvent distilled off using a rotary evaporatory.

2.2. *Thin-layer chromatography*

Extracts were taken up in chloroform and spotted on 0.25 ml TLC plates (5 × 20 cm) coated with 0.25 mm silica gel. Development was with benzene

using HCB and PCP as reference substances. The R_F of PCP was 0.56 and that of HCB was 0.91

2.3. *Gas Chromatography*

Analyses were performed on a Varian aerograph 2100 gas chromatograph utilizing a 4 ft × 1/4 inch column of 1.5% OV-17 on chromosorb-W. Nitrogen was the carrier gas (except on the GC-MS equipment) at a flow rate of 40 ml/min and a column temperature of 170°C.

3. Results

Thin-layer chromatography identified a single significant spot in the urine of HCB treated rats which was absent from urine of control animals and had an R_F of 0.56 matching precisely that of authentic PCP. In addition, in the treated animals, a considerable amount of polar material remained close to the origin.

Gas chromatography similarly yielded a peak with retention time matching that of PCP. Quantitative analysis was performed on the urine of rats consuming roughly 50–100 mg of HCB daily; using the extraction procedure outlined above we found 12.5 μg PCP/ml urine with a daily urine output of about 15 ml. The identity of the GC peak corresponding to PCP in retention time was confirmed using combined gas chromatography-mass spectrometry (Varian model CH-7 mass spectrometer). The mass fragmentation peaks of the supposed PCP chromatographic peak matched closely with the mass fragmentogram of the standard material thus establishing a positive identification.

In addition to PCP, other more polar compounds presumed to be derivatives of HCB were detected by on-column methylation using 0.2 M trimethyl anilium hydroxide as the derivatizing agent. This procedure eluted a complex pattern of derivatives from the column and further studies are in progress to identify the nature of at least some of these peaks and thus the most likely pathway(s) followed in the degradation of HCB.

4. Discussion

Although porphyria induced by HCB has been extensively studied, little attention has been paid to the mechanism of HCB toxicity. Further, HCB is a by-product of industrial aromatic chlorination procedures and disposal of HCB waste has led to environmental contamination; again, the mechanism of HCB toxicity is of interest. We have demonstrated what is probably an early step in HCB metabolism, namely the introduction of a single OH group. While HCB has low acute toxicity, PCP has an oral LD_{50} in rats of 180 mg/kg [5]. Before it will be profitable to speculate on the mechanisms of HCB hepatotoxicity, more information on the pathway of metabolism is required. While PCP is itself hepatotoxic, formation from HCB is likely to proceed either through a free-radical mechanism or by initial formation of an arene oxide. In either event, reactive intermediates may form covalent bonds with cellular constituents leading to irreversible cell damage. Further study of HCB metabolites should demonstrate a trans-dihydrodiol or mercapturic acid if an epoxide is formed initially.

References

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