Time Course of Hexachlorobenzene-induced Alterations of Lipid Metabolism and Their Relation to Porphyria

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A great deal of information concerning the effects of hexachlorobenzene on the haem metabolic pathway has been obtained but little is known about the effects of the drug on lipid metabolism. Consequently, the time course of phospholipid metabolism alteration caused by this xenobiotic was evaluated as related to changes in porphyrin metabolism with the aim to understand better the interregulation of both metabolisms. Female Wistar rats were treated with HCB (1 g/kg) over a 1–8 week period. Individual phospholipid content, [32P] incorporation, total lipid content, lipid peroxidation, uroporphyrinogen decarboxylase activity, its inhibitor generation and porphyrin content, were the parameters measured in the liver of treated rats. Phospholipid metabolism—with the exception of sphingomyelin—presents a biphasic behaviour, in both the endogenous contents and de novo synthesis. The turning point between both phases is the time at which levels of porphyrin and conjugated dienes increase, the latter compounds being involved in oxidative processes. On the other hand, sphingomyelin decreases continuously during the 8 weeks of treatment. It was also found that the malondialdehyde content increased during the early stages. The time sequence for haem metabolism parameters showed that the accumulation of porphyrins occurs after the decrease in uroporphyrinogen decarboxylase activity and the enzyme inhibitor formation, which are early events (first and second weeks). Porphyrins could not by themselves exacerbate uroporphyrinogen decarboxylase impairment or inhibitor generation. This study shows that hexachlorobenzene alters simultaneously phospholipid and porphyrin metabolisms from the early stages, and generates an oxidative environment that favours porphyrinogens and lipid oxidation at later stages. So, this oxidative environment links the alterations on both metabolisms.

Keywords: Porphyria Hexachlorobenzene Phospholipid Porphyrins Lipid peroxidation

INTRODUCTION

Hexachlorobenzene (HCB) is a fungicide of well known porphyrinogenic ability that caused a massive outbreak of porphyria cutanea tarda (PCT) in Turkey in 1959 (Schmid, 1960) and which is capable of inducing porphyria in several animal species (San Martin de Viale et al., 1977).

Uroporphyrinogen decarboxylase (URO-D)
is the key enzyme of the haem metabolic pathway which is blocked in cases of porphyria, not only in humans but also in the experimental HCB-induced model. This block impairs the regulation of the haem pathway (San Martín de Viale et al., 1977; Elder et al., 1976). The presence of an URO-D inhibitor has been reported in the liver of HCB-treated rats (Rios de Molina et al., 1980), and of mice treated with other polyhalogenated chemicals (Cantoni et al., 1984; Smith and Francis, 1987).

Despite the fact that a great deal of information concerning the effects of HCB on the haem metabolic pathway has been obtained (Courtney, 1979; Wainstok de Calmanovici et al., 1984; Wilson and Kueberuwa, 1994), the mechanism of HCB-induced porphyria is not yet fully elucidated. On the contrary, little is known about the effects of the drug on lipid metabolism and its relationship with porphyrin metabolism, despite the frequent observation of large numbers of lipid droplets revealed by electron microscopy examinations in liver, as well as proliferation of the smooth endoplasmic reticulum and increased hepatocyte size and liver weight, in accordance with the degeneration of liver fat commonly observed in HCB-exposed animals (Courtney, 1979; Boger et al., 1979; Kószo et al., 1982).

Increases in cytoplasmatic cholesterol content and its hepatic synthesis have been reported in mice treated with HCB (Wada et al., 1969). In contrast, cholesterol content remained unchanged in microsomal membranes of rats treated with this drug for a relatively short time. Nevertheless, such membranes show an increased phospholipid (PL) content and a decrease in free fatty acid content (Cantoni et al., 1987).

On the other hand, in experimental allyliso-propylacetamide (AIA)-induced porphyria, haem and fatty acid synthesis both increased about two-fold, a fact which suggests some metabolic relationship between both pathways, for which malonyl CoA has been postulated as the intermediate (Labbe et al., 1961).

Since the above suggests a link between lipid and porphyrin metabolisms, and considering that a complete and integrative study of the time course of the main events induced by HCB on both these pathways had not been performed, the present studies were undertaken in order to: (1) characterize the alterations in phospholipid metabolism; (2) study the time course of these processes as well as that of lipid peroxidation, in parallel to a complete time course study of the effects of HCB on key parameters of the haem pathway important in this type of porphyria; and (3) attempt to establish a correlation between both kinetic studies with the aim to shedding light on the mechanism of induction of this type of experimental cutanea tarda porphyria, as well as to increase the knowledge of the interregulation of both metabolisms.

Therefore, the present study not only evaluates individual PL content, its de novo synthesis and lipid peroxidation parameters in terms of HCB treatment time, but also evaluates the effects of this drug on the haem metabolic pathway, which are: a decrease in URO-D activity, formation of URO-D inhibitor and accumulation of hepatic porphyrins.

MATERIALS AND METHODS

Chemicals

HCB (commercial grade) was a gift from Compañía Quimica S.A., Buenos Aires, Argentina. [32P] Orthophosphate (specific activity: 25 mCi/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Uroporphyrin III was from Porphyrin Products, Logan, UT, U.S.A. Silica Gel G plates (0.25 mm thick), Fiske-Subarow reagent, Sephadex G-25 and thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). X-ray film for autoradiography was obtained from Eastman Kodak Co. (Rochester, NY, U.S.A.).

Animals and treatment

Female Wistar rats (200 g) were intoxicated with HCB at a dose of 1 g/kg body weight. The drug was suspended in water (40 mg/ml) with
the aid of Tween 20 (0.5 ml/100 ml of suspension) and administered by a stomach tube 5 days a week for 1, 2, 3, 4 or 8 weeks. No deaths or external alterations resulted from treatment with this dose. The low order of toxicity was indicative of the minimal absorption of HCB across the intestinal tract when administered in water (Courtney, 1979).

In our experience no changes were observed between animals treated with vehicle and non-treated animals (Table 1). For this reason vehicle-treated controls were omitted from this study.

**Enzyme preparation**

Enzyme was prepared as previously described (San Martin de Viale et al., 1977). Normal rats and animals treated for different intoxication times were killed by decapitation. Livers were removed and homogenates prepared with 0.154 M KCl (1:5 w/v). Followed by centrifugation at 11,000 g at 0-2°C for 20 min. The supernatants from normal rats and those treated with HCB for 1 and 2 weeks, were used as a source of enzyme. Supernatants from intoxicated animals (3-8 weeks of treatment) were filtered through Sephadex G-25 columns (2.4 x 30 cm) equilibrated and eluted with 0.134 M potassium phosphate buffer pH 6.8. Eluates containing no fluorescence were pooled and used as a source of porphyrinic enzyme.

**Inhibitor preparation**

Supernatants from normal and intoxicated rats (4 ml) were filtered through Sephadex G-25 columns as described above. Eluates were heated 5 min at 100°C, cooled and then centrifuged at 1,000 g for 10 min (Rios de Molina et al., 1980). Supernatants from treated animals (0.75 ml) were used as a source of inhibitor and those from untreated animals as controls. Inhibitor preparations showed no absorption in the Soret band of the spectrum.

**Uroporphyrinogen decarboxylase (URO-D) activity**

Incubations were carried out in a mixture (final volume 1.5 ml) containing: 0.067 M potassium phosphate buffer pH 6.8, 1 mM reduced GSH, 0.1 mM EDTA, 4.7 μM uroporphyrinogen III (Uro‘gen), enzyme from normal or intoxicated rats (3 mg/ml) and, when required, inhibitor or its corresponding control. URO-D activity was determined according to Wainstok de Calmanovici et al. (1984), except that the porphyrins formed were separated and quantified as methyl esters by high performance liquid chromatography as described by Billi de Catabbi et al. (1991). Results are expressed as coproporphyrinogen III formation (nmol of Copro‘gen per mg protein in 30 min or 1 hr).

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Non-treated rats</th>
<th>Vehicle-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>URO-D activity (nmol coprogen/30 min/mg prot)</td>
<td>0.360 ± 0.090 (6)</td>
<td>0.370 ± 0.070 (3)</td>
</tr>
<tr>
<td>Porphyrins content (μg/g liver)</td>
<td>2.6 ± 0.8 (8)</td>
<td>2.3 ± 1.0 (4)</td>
</tr>
<tr>
<td>Conjugated diene content (absorbance 230 nm)</td>
<td>0.404 ± 0.090 (5)</td>
<td>0.420 ± 0.100 (3)</td>
</tr>
<tr>
<td>MDA content (nmol MDA/g liver)</td>
<td>218 ± 38 (6)</td>
<td>240 ± 35 (4)</td>
</tr>
<tr>
<td>Lipid content (mg/g liver)</td>
<td>36 ± 11 (6)</td>
<td>38 ± 10 (3)</td>
</tr>
<tr>
<td>CerPtdCho.</td>
<td>48 ± 10 (5)</td>
<td>50 ± 12 (3)</td>
</tr>
<tr>
<td>(ng Pi/mg wet tissue) PtdCho.</td>
<td>414 ± 92 (5)</td>
<td>398 ± 100 (3)</td>
</tr>
<tr>
<td>(ng Pi/mg wet tissue) PtdIns.</td>
<td>82 ± 18 (5)</td>
<td>89 ± 20 (3)</td>
</tr>
<tr>
<td>(ng Pi/mg wet tissue) PtdSer.</td>
<td>52 ± 15 (5)</td>
<td>58 ± 18 (3)</td>
</tr>
<tr>
<td>(ng Pi/mg wet tissue) PtdEtn.</td>
<td>145 ± 34 (5)</td>
<td>139 ± 30 (3)</td>
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Rats with vehicle received 5 ml of Tween 20 0.5% in water administered by stomach tube, 5 days a week during 8 weeks. Values are expressed as the mean ± SD. Numbers between parentheses represent the number of animals used. Each experiment was performed twice.
**Porphyrim content**

Hepatic porphyrin content was determined spectrophotometrically in 0.1-1 ml aliquots of whole homogenates as total free porphyrins in 5% (w/v) HCl, as described by San Martín de Viale et al. (1977).

**Lipid content**

Lipids were extracted according to the method detailed by Recknagel and Ghoshal (1966), modified for liver homogenates. For this purpose, 1 g liver was homogenized with 0.154 M KCl (1:5 w/v) and 20 ml chloroform:methanol (2:1 v/v) were then added. The mixture was heated 2 min at 50°C, then shaken for 20 min, followed by the addition of another 10 ml of chloroform: methanol and 5 ml of water. After centrifugation, the lower phase was removed and filtered. Chloroform was evaporated under nitrogen on tared vessels. Lipid content was then evaluated gravimetrically.

**Malondialdehyde content (MDA)**

MDA was assayed as described by Ohkawa et al. (1979) except that the red pigment produced was extracted only with butanol to avoid interference by endogenous porphyrin. The reaction mixture contained: 0.2 ml homogenate (1:10 w/v), 0.2 ml 8.1% SDS, 20% acetic acid adjusted to pH 3.5, and 1.5 ml 0.8% thiobarbituric acid in a final volume of 4 ml. The mixture was heated for 60 min at 100°C. Results are expressed as nmol of malondialdehyde per gram of liver using an extinction coefficient of 156 000 M⁻¹cm⁻¹.

**Conjugated diene**

Hepatic mitochondria were obtained by differential centrifugation of homogenates. Lipids were extracted and quantified as above. Conjugated diene determination was performed as described by Recknagel and Ghoshal (1966). Results are expressed as absorbance of 1 mg/ml lipid solution at 230 nm.

**Preparation of tissue slices**

Portions of each liver were maintained in an ice-cold solution of 10 mM buffer Tris pH 7.4, containing 140 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 2 mM MgSO₄ (Tris electrolyte buffer), and 5 mM glucose. Tissue slices (ca. 0.5 mm thick) were obtained with a Stadie-Riggs microtome in the same solution. “De novo” phospholipid synthesis

For each experiment, 5 mg of tissue slices were collected in 0.5 ml Tris electrolyte buffer containing 5.5 mM glucose. Samples were incubated at 37°C with [³²P] orthophosphate (25 mCi/mmol), in a metabolic shaking bath for different periods of time. Although time-course experiments were performed, results shown in the figures correspond to the time of the steady-state equilibrium (120 min).

**Extraction and separation of lipids**

Incubations were stopped on ice and samples washed three times by centrifugation in ice-cold incubation medium. Following the addition of four parts of chloroform:methanol (1:2 v/v) (Bligh and Dyer, 1959), samples were homogenized in glass tubes using a Teflon pestle at 3 000-5 000 rpm. Phases were separated by adding one part of chloroform and one of water (Skipski et al., 1964), and the lower chloroform phase was removed. The inorganic phase was washed twice with chloroform and the organic phases were pooled and dried under a stream of nitrogen at 25°C. This extraction procedure ensures 80-85% recovery of lipids. The extracts were resuspended in chloroform and applied on precoated thin layer plates (0.25 mm thick). Lipids were separated by one-dimensional, two-solvent thin-layer chromatography. The first solvent system used was a mixture of chloroform:methanol-acetic acid–water (40:10:10:1, v/v). Plates were dried and developed to a level 0.6 cm below the first solvent front in a solvent system consisting of chloroform–methanol-acetic acid–water (120:46:19:3, v/v). Rf values for this system were 0.20, 0.30, 0.47, 0.55 and 0.70 for sphingomyelin (CerPtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn), respectively. The free fatty acids migrated with the solvent front (Weiss et al., 1982). Lipid fractions were detected with iodine vapor or by autoradiography. The zones on the thin-layer chromatographic plates corresponding to the various phospholipid fractions were scraped off and added to 6.0 ml of toluene-omnifluor (0.4%). Phospholipids were quantified in a liquid scintillation counter.

**Endogenous phospholipid determination**

For phospholipid quantification, specific areas of the plates were scraped off and digested.
with 70% perchloric acid in the presence of ammonium molibdate (0.5%). The resulting inorganic phosphate was assayed with the Fiske-Subbarrow reagent (Bartlett, 1959). Recovery of PtdCho added before extraction was 85%.

Proteins

Proteins were determined according to the method of Lowry et al. (1951).

Statistical treatment of results

Results are expressed as the mean ± SD, and mean values were compared by Student’s t-test or ANOVA. In order to obtain the value of p, a degree of freedom \( n_1 + n_2 - 2 \) was used. Differences were considered statistically significant when p values were less than 0.05.

RESULTS

Lipid peroxidation and total lipid content

Two parameters of lipid peroxidation were determined in order to detect the presence of reactive oxygen species in the liver of HCR-treated animals: MDA content and conjugated diene formation (Table 2). The first is more sensitive, though less specific. MDA tended to increase in the first weeks, reaching statistically significant values at the second week and maintaining these high levels until the end of the treatment. Conjugated diene formation started to increase in the third week and became significant during the fourth week of drug intoxication.

The total lipid content presented a statistically significant increase in the second week of treatment and then returned to almost normal values.

Phospholipid studies

Table 3 shows the hepatic PL distribution from a normal liver and the specific activity of \(^{32}P\) incorporation. PtdCho is the main PL, followed by PtdEtn. PtdIns and PtdSer account for 11 and 6.9% of the total PL, respectively,
Changes in phospholipid composition

Figure 1 shows the results obtained when the PL composition was studied as a function of HCB intoxication time and expressed as the percentage of change compared to normal animals. Beginning at the first week of treatment, all individual PL were significantly modified. CerPtdCho, a minor choline-containing PL, gradually decreased from the first to the eighth week of treatment. Thus, the greatest change was during the eighth week when the content decrease reached 78%. In contrast, PtdCho, the major choline-containing PL, presented a time-dependent increase between the first and third week of treatment followed by a drop without reaching control values in the fourth week. Consequently, after 8 weeks, 31% less PtdCho was found when compared to controls of the same age. PtdSer behaved similarly. PtdEtn also showed an increase during the first week and its levels remained unchanged until the third week, while after the fourth week its concentration decreased dramatically attaining almost control levels at this time. PtdEtn levels showed a 61% decrease by the eighth week. PtdIns' changes differed from those of the other PL, since this minor acidic PL increased during the first 2 weeks of treatment. Subsequently, a time-dependent decrease in concentration was observed. This drop tends to nullify the previous positive changes (i.e. increases). Following this, changes become negative, reaching 28% below control values by 8 weeks.

Changes in phospholipid $[^{31}P]$ incorporation

As shown in Fig. 2, after treatments of the first and second weeks, $[^{31}P]$ incorporation increased for all the PL studied, including phosphatidic acid, an indicator of Kennedy pathway activation. After 3 weeks of treatment, stimulation of $[^{31}P]$ incorporation is less evident, and at the fourth week, the decrease was greater than 50% for almost all PL. After the eighth week, the drop in radioactivity associated with PtdCho and PtdEtn was even more marked, while that for PtdIns and phosphatidic acid remained at 50% of control values.
Time course of hexachlorobenzene-induced alterations of lipid metabolism

Fig. 3. Hepatic porphyrins, uroporphyrinogen decarboxylase and inhibitor effects in terms of intoxication time. Rats were killed at the indicated times, their livers removed and porphyrins isolated. Total free porphyrins were determined in 5% (w/v) HCl. URO-D activity was measured as described in Section 2. The inhibitor was obtained from the same rats which were used to evaluate the other parameters at the different intoxication times. Its activity was tested on normal URO-D. URO-D activity is expressed as nmols of Copro'gen formed/1 hr/mg of proteins. Porphyrin content is expressed as µg of porphyrins/g liver. Values represent the mean of five to eight animals ± SD. (*) Significantly different from normal rats (p < 0.05). 0 weeks: normal rats. (■) URO-D; (☐) inhibitor + normal URO-D; (□) porphyrins.

**URO-D activity, inhibitor formation and endogenous porphyrin content**

Figure 3 shows the effects of HCB treatment on hepatic URO-D activity, the generation of its inhibitor and porphyrin content. URO-D activity, expressed as Copro'gen III formation, began to decrease in the first week of treatment (36% inhibition). The effect on this enzyme increased gradually and its activity then dropped dramatically by the fourth week with a percentage of inhibition of over 94%. After 8 weeks of fungicide treatment, severe porphyrina could be observed and URO-D activity had decreased further (97% inhibition).

Inhibitor formation owing to HCB intoxication could be detected from the first week and became statistically significant by the second week (25% inhibition). Inhibitor activity remained practically unchanged until the third week and was more evident from the fourth week on.

Hepatic porphyrins began to increase in the second week of HCB treatment (3.9 vs 2.7 µg porphyrin/g liver), and became statistically significant by the third week (30 vs 2.7 µg porphyrin/g liver). At this point, such an increase was coincident with the shift in the Soret band from 401 to 405 nm, indicating that higher carboxylated porphyrins had been accumulated. After 8 weeks of treatment, there was a remarkable increase in porphyrin content (330 vs 2.7 µg porphyrin/g liver).

Thus the inhibitor was present from the very first week, while endogenous porphyrins remained at control values. Between the second and third weeks of treatment, porphyrin increased 11-fold, while inhibitor activity remained unchanged. Between the third and fourth weeks there were no major changes in porphyrin content while the inhibitor action was more noticeable. These results indicate that there is no apparent correlation between porphyrin content and inhibitor activity.

**DISCUSSION**

This is the first study to show the time course of HCB-induced alterations of PL metabolism as related to changes in porphyrin metabolism. PL metabolism—with the exception of CerPtdCho—presents a biphasic behaviour, not only in PL levels but also in their de novo synthesis. By comparing Figs 1 and 2 we can conclude that the changes in PL de novo synthesis precede and account for changes observed in PL content in both phases. The first phase, i.e. that of increase in PL content, can be ascribed to a proliferation of membranes as a consequence of the important and rapid induction of drug-metabolizing enzymes (Lissner et al., 1975) in response to the foreign drug. The second phase, i.e. that of decrease in PL levels at longer intoxication times, that leads to a noticeable and in some cases dramatic loss of PL content, can be seen as a toxic effect of the drug leading to membrane destruction. Possibly this phase can be associated to the disorganization of the membranes clearly ascribed not only to the primary fluidizing effects of HCB, which is incorporated between the fatty acid chains of membrane lipids, but also to the effects of an altered lipid metabolism striving to eliminate the damaging effects of HCB on the membranes (Koszo et al., 1982).

The alterations in PL metabolism found in this study in the first phase of kinetic, are in
accordance with the results of Cantoni et al. (1987), who studied the endogenous contents of different phospholipids at a definite intoxication time (2 weeks) despite the fact that the HCB dose used was approximately two-fold lower than the one employed in the present study. Studies on de novo synthesis of PL have not been previously reported for this drug.

Taking into account that we have previously reported a biphasic behaviour for cytochrome P450 in response to HCB intoxication time (Wainstok de Calmanovici et al., 1984) similar to that reported here for PL (with a similar turning point), it might be possible that the increases and decreases in cytochrome P450 elicited by the drug as a function of intoxication time could be in part ascribed to changes in PL content. This would be in agreement with the idea that PL, especially PtdCho, play an essential role in the activity of the microsomal

![Diagram](image-url)

Fig. 4. Scheme for the mechanism of HCB action. ETC: microsomal electron transport chain. PL: phospholipids. (−): feedback negative regulation. W: week of treatment with HCB. Arrows beside the compounds indicate increase (↑) or decrease (↓) in levels or activity.
drug metabolism system (Stroeb et al., 1970). Taking into consideration the important role of cytochrome P450 in the induction of porphyria through metabolism of HCB (Wainstok de Calmanovici et al., 1984) and the incidence of PL on the activity of the cytochrome above emphasized, a possible indirect relationship between PL and porphyria induction could be suggested.

The differential behaviour of CerPtdCho, which decreases continuously in contrast to the other PLs which exhibit biphasic kinetics, can be interpreted as follows: while there is a proliferation of membranes associated with an increase in total lipid and PL contents, CerPtdCho might decrease so as to maintain the level of choline-containing PL, since there is a reverse correlation between the amounts of CerPtdCho and PtdCho in many membranes (Merrill and Jones, 1990). When later there is a destruction of membranes as mentioned above, a decreased synthesis of all PL occurs. All this is reflected in the remarkable decrease observed in the levels of CerPtdCho.

The results reported here demonstrate increases in the two lipid peroxidation parameters, thus reflecting rises in oxygen reactive species levels. The presence of increased amounts of MDA during the early stages agrees with the results reported by Goel et al. (1988), who found that MDA could be detected 9 days after a toxic dose of HCB. In this line, and supporting our findings at longer exposure times, are the results of Feldman and Bacon (1989) who found that in rats receiving HCB during 8 weeks, total hepatic porphyrins and conjugated diene had increased. It is worth mentioning that reactive species could play some role in the inhibitory process leading to porphyrin accumulation by formation of an URO-D inhibitor involving such reactive species. This is also in agreement with the observation that HCB administration induces a specific condition of imbalance in the liver between formation and inactivation of reactive species, that is associated with hepatic porphyrin accumulation (Rizzardini et al., 1988).

Concerning the haem metabolic pathway it is important to point out that the accumulation of porphyrins precedes neither the decrease of URO-D activity nor the enzyme inhibitor formation, which are early events.

In brief, present results show that, at very early stages, HCB produces alterations not only in haem metabolism but also in PL metabolism. Moreover, the drug causes an increase in hepatic reactive substances. All these effects could accompany HCB action at the microsomal level since HCB is known to be a cytochrome inducer (Wainstok de Calmanovici et al., 1984). This induction parallels an increase in PL metabolism with a proliferation of endoplasmic reticulum membranes in an effort to metabolize the foreign drug, as seen for other lipophilic drugs (Ishidate and Nakazawa, 1976). Furthermore, it has been proposed that HCB could act as a lipid-soluble drug that binds to cytochromes and is not readily metabolized, thus uncoupling the electron transport chain from mono-oxygenase activity and consequently favouring the production of reactive species (Ferioli et al., 1984). These reactive species could by themselves attack both the Uro'gen and lipids: the Uro'gen, leading to URO-D inhibitor generation according to the idea that the inhibitor could arise from a porphyrin ring (Francis and Smith, 1988), thus causing URO-D activity impairment, and lipids producing their peroxidation, which is probably overcome by the higher PL synthesis observed at this stage.

In later stages an increase in porphyrin levels occurs, since the haem pathway becomes deregulated because URO-D activity decreases by over 50% while aminolevulinic acid synthase activity is induced (Wainstok de Calmanovici et al., 1984). This situation leads to a massive accumulation of Uro'gen that is not further metabolized, but is instead readily oxidized by the oxidative environment created by HCB. In this respect, porphyrinogens were reported to be able to scavenge free radicals, thus limiting deleterious free radical reactions in vivo (Miller and Woods, 1993). Porphyrins could not by themselves exacerbate the impairment of URO-D or inhibitor generation. However, they might contribute, together with reactive species, to membrane destruction by diminishing the PL content due to PL synthesis alteration as well as to an increased lipid peroxidation. It is interesting to note that the turning point between both phases, observed in PL metabolism, is the time at which the levels of porphyrin and conjugated diene, both compounds involved in oxidatives processes, rise. The sequence of some events discussed above are summarized in Fig. 4.
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