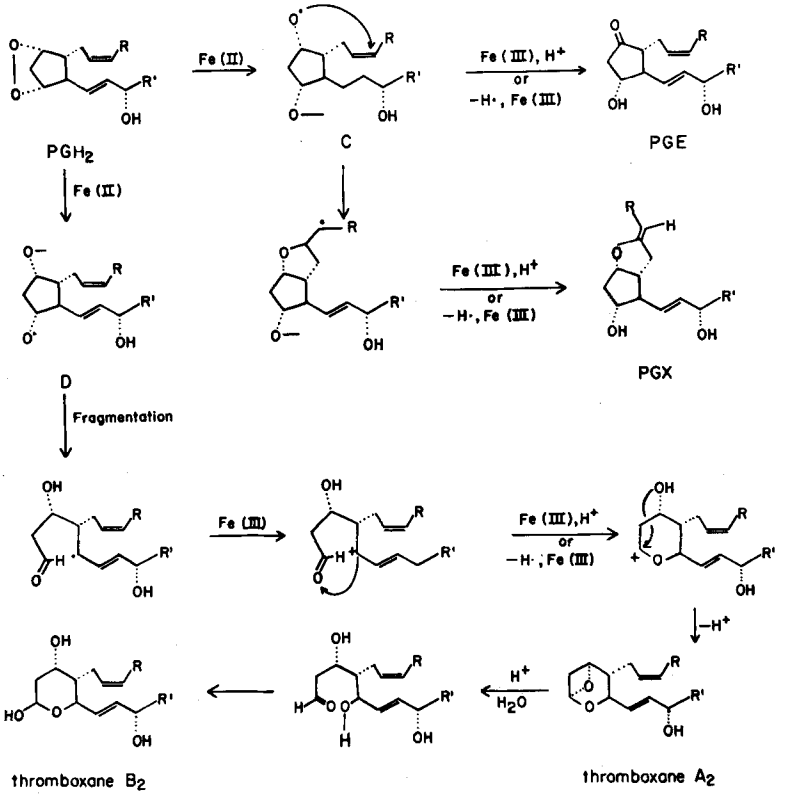
On the basis of these observations we suggested[8b] that the Fe(II)-induced isomerization **1**→**2** might serve as a model for the transformation of the prostaglandin endoperoxides to the PGE's in vivo under the influence of Fe(II)-based enzyme systems as shown in the first row of Scheme II. The lower part of the Scheme adumbrates our suggestion for the biosynthesis of PGX and the thromboxanes, using as a model the 2 other reactions of endoperoxides induced by the Fe(II)-Fe(III) redox system which have been discussed in the preceding paragraphs.

Thus in the anion radical C, produced by one-electron reduction of PGH_2, an alternative to oxidation to PGE is attack by the C-9 oxyradical on the double bond of the side chain attached to C-9 in a manner analogous to the reaction A→B of Scheme I. This is consistent with the alteration of double bond geometry (cis→trans). Subsequent oxidation by the enzyme-based redox system would explain the formation of PGX.

Thromboxane biosynthesis requires cleavage of the 11, 12-bond of a precursor derived from PGH_2[4,9,10]. This can be rationalized by invoking one-electron reduction of PGH_2 to the isomeric anion radical D whose fragmentation (arrows) and subsequent oxidation by the enzyme-based redox system is analogous to the loss of an isopropyl group (and oxidation of the latter) exhibited by dihydroascaridole on treatment with $FeSO_4$[18]. The circumstance that ring cleavage leads to an allylic radical may conceivably assist the mode of fragmentation of D. The subsequent steps leading to thromboxane A_2 and A_3 are self-explanatory.



Reductive dechlorination of chlorobiphenylols by rats

M. Th. M. Tulp[1], W. A. Bruggeman and O. Hutzinger

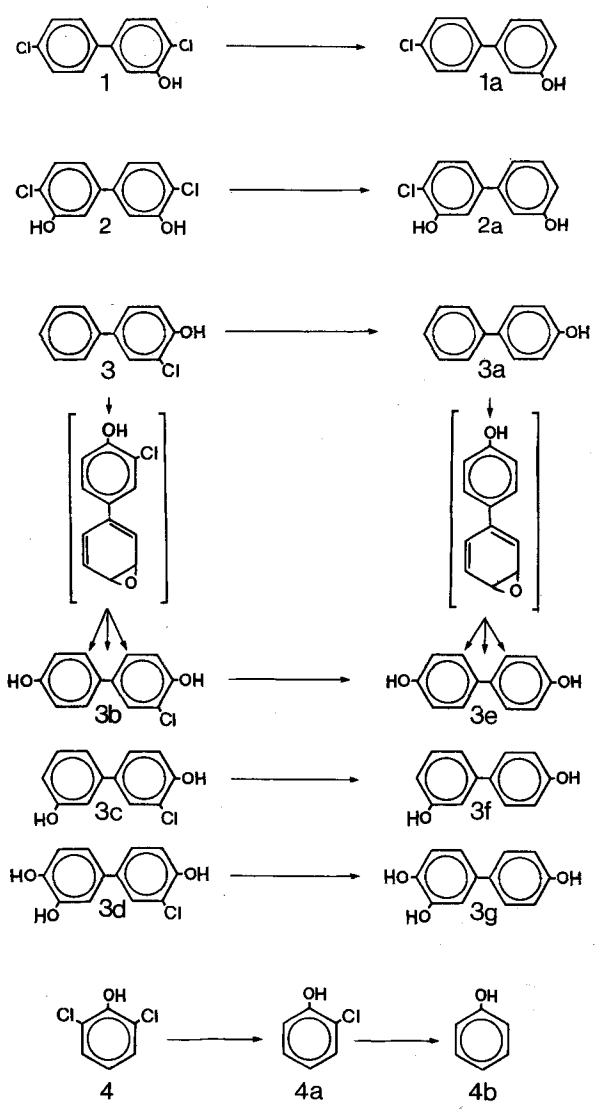
Laboratory of Environmental Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (the Netherlands), 17 December 1976

Summary. Dechlorinated products were isolated from the urine of rats that were administered chlorobiphenylols, the primary hydroxylated metabolites of PCB in mammals. The mechanism of chlorine loss from chlorobiphenylols is different from the mechanism of dechlorination via arene oxides whereby concomitant hydroxylation is always observed.

Although extensive metabolic dechlorination is known to occur with compounds in which chlorine is not bound to an aromatic carbon[2], this reaction is much less important in aromatic chlorine compounds[3]. The possibility of reductive dechlorination of an aromatic chlorine compound was firstly reported in 1973[4] for hexachlorobenzene (HCB), and recently unambiguously demonstrated: pentachlorobenzene, tetrachlorobenzene and a number of polychlorophenols were found as metabolites from HCB in rats[5,6]. This reductive dechlorination was shown to be catalysed by an enzyme located in the microsomal fraction of liver, lung, kidney and intestine[5].

Reductive dechlorination of polychlorinated biphenyls (PCB) is known only as a photochemical pathway[7]. All documented cases of metabolic chlorine loss from PCB involve concomitant hydroxylation via an arene oxide

intermediate[3,8]. During a study on the metabolism of 4,4'-dichlorobiphenyl in rats[9], we recently detected 4'-chloro-3-biphenylol as minor metabolite. This compound was also found in the urine of rats after feeding 4,4'-dichloro-3-biphenylol, the major metabolite of 4,4'-dichlorobiphenyl in this animal. Such a metabolic reaction cannot be explained by an arene oxide mechanism but suggests direct metabolic dechlorination.



Cumulative relative urinary excretion 7 days after an oral dose of 100 mg/kg

Compound	Unchanged compound* (%)	Hydroxylated metabolites (%)	Dechlorinated metabolites (%)
1	16	72	12
2	37	56	7
3	70	25	5
4	64	21	15

*Percentages were calculated from peak areas in the total ion chromatogram.

In this study with 4 structurally related compounds (all have a chlorine atom in the ortho position to a hydroxy group), we show that such dechlorination reactions are apparently more common.

Materials and methods. 4,4'-Dichloro-3-biphenylol, 4,4'-dichloro-3,3'-biphenyldiol, 3-chloro-4-biphenylol and 2,6-dichlorophenol of high purity (99.9% by GC-MS), were dissolved in peanut oil (oleum arachidis) and administered orally to male Wistar rats as a single dose of 100 mg/kg. The animals were housed in individual metabolic cages and supplied with water and food ad libitum during the experimental period of 7 days. Faeces and urine were collected separately in 4 N sulphuric acid to prevent microbial metabolism after excretion. Isolation, purification and methylation of the metabolites were performed as described[9,10]. We used a Hewlett-Packard 5982 A GC-MS system operating in the EI mode at 70 eV, equipped with an 0.4×180 cm all glass column, packed with 0.2% Carbowax 20 M on Chromosorb W 100–120 mesh. Starting material and the methyl ethers of the metabolites were synthesized by reacting an aniline with amyl nitrite in the presence of an excess of aromatic reactant[10–13].

Results and discussion. GC-MS investigation of the methylated urine extracts showed that 4,4'-dichloro-3-biphenylol (compound **1**, see scheme), 4,4'-dichloro-3,3'-biphenyldiol (compound **2**), 3-chloro-4-biphenylol (compound **3**) and 2,6-dichlorophenol (compound **4**) were dechlorinated to form 4'-chloro-3-biphenylol (**1a**), 4-chloro-3,3'-biphenyldiol (**2a**), 4-biphenylol (**3a**) and 2-chlorophenol (**4a**) respectively.

Loss of chlorine in compounds **1a** to **4a** was evident from the change in the specific isotope clusters of the molecular ions. The position of the hydroxy groups in these compounds was clear from the specific fragmentation patterns of their methyl ethers[9,10,13], and was further ascertained by synthesis. In all cases the dechlorinated products were minor metabolites (see table) and, except for **2a**, could only be detected in the urine.

Since direct dechlorination products were never observed in studies with chlorobiphenyls[3], it seems that a hydroxy group in the aromatic nucleus is necessary for the loss of a chlorine atom via reductive dechlorination.

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In all compounds studied, the hydroxy group and the chlorine atom were in the ortho position relative to each other and no further relationship (e.g. meta and para) was investigated. All compounds also gave hydroxylated metabolites formed by either direct hydroxylation or hydroxylation via arene oxide intermediates. Compound **4a** was further dechlorinated to phenol (**4b**). From compound **3** 3 more metabolites formed via reductive dechlorination (compounds **3e**, **3f** and **3g**, see scheme) were isolated. By studying the specific fragmentation pattern, the relative intensities and the retention times and by comparison with data of similar compounds from earlier studies[9,10,13], the structures of the compounds shown in the Scheme could be determined. In all cases final proof was obtained by synthesis. Because the compounds **3b**, **3c** and **3d** are major metabolites, whilst **3a**, **3e**, **3f** and **3g** represent only a few percent of the total amount of metabolites formed, most likely **3e**, **3f** and **3g** were formed via reductive dechlorination of **3b**, **3c** and **3d** respectively, since the latter all have a hydroxy group ortho to a chlorine atom. The present data however, do not rule out formation of **3e**, **3f** and **3g** via the 3′, 4′-epoxide of **3a**, similar to 3′, 4′-epoxidation of **3**. The relative amounts of the unchanged compounds, their hydroxylated and dechlorinated metabolites isolated from the urine are given in the table.

Biosynthesis of phosphatidylethanolamine from CDP-ethanolamine by the Golgi complex of rat liver in vitro[1]

Patricia L. Chang[2], Jennifer M. Sturgess and M. A. Moscarello[3]

Research Institute, Departments of Pathology and Biochemistry, The Hospital for Sick Children, Toronto (Ontario, Canada), 4 February 1977

Summary. A Golgi-rich fraction from rat liver has been shown to synthesize phosphatidylethanolamine from CDP-ethanolamine in vitro. The implications of the existence of such a pathway for the membrane flow hypothesis are discussed.

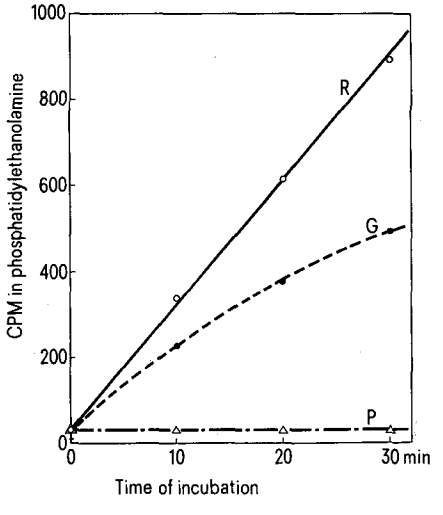
The biosynthesis of phospholipids has been shown to occur in endoplasmic reticulum[4–6] and in mitochondria[7], whereas the Golgi complex has been reported to lack some of the enzymes required in such biosynthesis[5,6]. However, this report presents evidence that the Golgi-rich fractions from rat liver can synthesize phosphatidylethanolamine from CDP-ethanolamine in vitro, demonstrating that at least the terminal enzyme is present. The rough endoplasmic reticulum showed similar synthetic activity while the plasma membrane showed no incorporation of CDP-ethanolamine.

The reaction under study was as follows:

1,2-diacyl-sn-glycerol + CDP-ethanolamine*
ethanolamine phosphotransferase
→ phosphatidylethanolamine* + CMP

When CDP-ethanolamine was radioactively labelled with ^{14}C in the ethanolamine moiety, the recovery of radioactive phosphatidylethanolamine measured the activity of ethanolamine phosphotransferase, an enzyme responsible for the final step of phosphatidylethanolamine de novo synthesis according to the Kennedy pathway.

Materials and methods. All subcellular fractions were prepared from the livers of male Wistar rats weighing 200–230 g. The rough microsomal fractions were prepared by Dallner's method[8] and Golgi-rich fractions by the method of Sturgess, et al.[9]. The plasma membrane frac-



Recovery of radioactive phosphatidylethanolamine from CDP-ethanolamine vs time of incubation with rough microsomal, Golgi-rich and plasma membrane fractions of the rat liver.
Rough microsomal (R, 232 μg of protein), Golgi-rich (G, 139 μg of protein) or plasma membrane (P, 117 μg of protein) fractions were each incubated at 37 °C in a medium containing CDP-[^{14}C]-ethanolamine and diglyceride (see Materials and methods) in quadruplicate. At each time interval: 0, 10, 20 and 30 min, incubation was terminated, phospholipids were extracted from the total incubation mixture and separated by TLC. The spot corresponding to phosphatidylethanolamine (PE) was scraped and counted in a liquid scintillation system.

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2 Present address: Department of Paediatrics, McMaster Medical Center, Hamilton, Ontario, Canada.
3 Reprint requests to be addressed to Dr M. A. Moscarello, Research Institute, Hospital for Sick Children, Toronto, M5G 1X8, Ontario, Canada.
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