Direct Chloride Measurement of [4-^{36}Cl]Chlorobiphenyl and [4,4′-^{36}Cl]Dichlorobiphenyl Dechlorination in the Rat

M. T. SCOTT * **and J. E. SINSHEIMER** ˣ

Received December 27, 1982 from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication August 4, 1983. *Present address: Analytical Research and Development, Du Pont Pharmaceuticals, Garden City, NY 11530.

Abstract □ A reverse-phase HPLC system was used for the determination of inorganic chloride liberated *in vivo* from two biphenyl compounds in the rat. Oral administration of [4-^{36}Cl]chlorobiphenyl resulted in a total yield of 27.6% of the original dose excreted over 10 d in the urine and included 1.7% of the dose as inorganic chloride. For [4,4′-^{36}Cl]dichlorobiphenyl, the radioactivity of the original dose in the urine was 39.8% after 10 d, which included 11.5% of the dose as inorganic chloride. These results appear to represent the first direct determination of dechlorination by measurement of the inorganic chloride and suggest that biodechlorination plays a greater role in the metabolism of these compounds than previously expected.

Keyphrases □ Dechlorination—chloride in rat urine, 4-chloro- and 4,4′-dichlorobiphenyl □ Chloride determination—rat urine, 4-chloro- and 4,4′-dichlorobiphenyl □ 4-Chlorobiphenyl—*in vivo* dechlorination in rats □ 4,4′-Dichlorobiphenyl—*in vivo* dechlorination in rats.

The metabolism of polychlorinated biphenyls (PCBs), the ubiquitous environmental pollutants, leading to macromolecular binding (1-3) and mutagenicity (3) has been reported. Although metabolic activation appears to involve arene oxides (1-3), biodechlorination may play a role in the formation of bound metabolites and subsequent toxicity. Metabolic dehalogenation has been noted for polychlorinated biphenyls (4-6), bromobiphenyls (4-6), and iodobiphenyl (7). This paper describes an extension of these *in vivo* dehalogenation studies to ^{36}Cl-labeled 4-chlorobiphenyl and 4,4′-dichlorobiphenyl in an effort to assess the extent of biodehalogenation by direct chloride measurement.

EXPERIMENTAL SECTION

Copper(II) [^{36}Cl]Chloride—[^{36}Cl]Hydrochloride[1] (1.4 M, 1.0 mL, 50 μCi) and copper(II) oxide (55.9 mg, 1.7 mmol) were slowly heated to 115-125°C in a closed flask. This temperature was maintained for 8 h and then the vessel was cooled, opened, heated to dryness at 150°C for 3 h, and then stored in a desiccator. The brown product was redried immediately before use at 25°C and 0.3 mm pressure for 12 h.

[4-^{36}Cl]Chlorobiphenyl—In a modification of the general procedure of Doyle *et al.* (8) unlabeled copper(II) chloride (1.1976 g, 8.9 mmol) and isoamyl nitrate (2.812 g, 24.0 mmol) were added to [^{36}Cl]$CuCl_2$. After anhydrous acetonitrile (20 mL) was added, the mixture was maintained at 0°C to −5°C with stirring for 25 min before 4-aminobiphenyl (1.3528 g, 8.0 mmol) in anhydrous acetonitrile (10 mL) was slowly added. The mixture was stirred for 2 h at 0°C to −5°C and then for 3 h at room temperature before it was poured into 150 mL of 20% HCl and extracted with ether (3 × 150 mL). The ether was dried over sodium sulfate and the solvent was removed under reduced pressure. The aqueous phase was neutralized (pH 6-7) with 40% NaOH and extracted with ether. The ether was dried and the solvent removed as before. The resulting solid was then combined with the original solid. The product was isolated by column chromatography using silica gel and hexane as the mobile phase. After removal of the solvent, 925 mg (61.3%) of product with a specific activity of 2.24 μCi/mmol was obtained (mp 76-77°C). The IR, melting point, and NMR data of the product (synthesized in the same manner but with unlabeled $CuCl_2$) were identical with spectra of a known, pure (>99%) sample of 4-chlorobiphenyl[2].

[4,4′-^{36}Cl]Dichlorobiphenyl—Copper(II) oxide (62.0 mg, 1.89 mmol) and 1.0 mL of 1.54 M [^{36}Cl]hydrochloride (61 μCi) were mixed as described to produce labeled copper(II) chloride. Unlabeled copper(II) chloride (1.0819 g, 8.0 mmol) and isoamyl nitrite (2.812 g, 24.0 mmol) were added to labeled copper(II) chloride with the aid of 20 mL of anhydrous acetonitrile. Benzidine (740.2 mg, 4.0 mmol) in 15 mL of anhydrous acetonitrile was added slowly to the mixture which was at 0°C to −5°C. The mixture was stirred for 5 h at that temperature. Workup was as described for [4-^{36}Cl]chlorobiphenyl with a yield of 502.1 mg (56.3%) of white solid, mp 144.5-147.0°C (reference compound[2], 147-148°C). The specific activity was 4.61 μCi/mmol. The chromatographic behavior of the product that was synthesized in the same manner, except using unlabeled copper(II) chloride, matched the behavior of the known, pure (>99%) reference sample of 4,4′-dichlorobiphenyl[2]. Both labeled chlorobiphenyl compounds were found to be free of inorganic chloride-36 by HPLC using a C_{18} column and methanol-water (90:10).

***In Vivo* Studies**—Male Sprague-Dawley rats weighing 250 300 g were quarantined for 2 d prior to administration of substrates by gastric intubation. The dosing regimens of substrates in corn oil were as follows: three rats were dosed with 0.5 mL of [4-^{36}Cl]chlorobiphenyl, containing 197,000 dpm of substrate; three rats were dosed with 0.9 mL of [4,4′-^{36}Cl]dichlorobiphenyl, containing 60,000 dpm; another rat was given twice the dose of the others. Rats were allowed food and water *ad libitum*. Urine was collected daily and stored frozen.

Chlorine-36 Analysis—*Total Activity*—Urine (100 μL) was measured for total radioactivity by scintillation counting[3]. A storage period of 24 h in the dark, after addition of the cocktail[4] (10 mL) and before counting, was required to avoid chemiluminescence.

Inorganic Chloride—Urine was acidified to pH 6-7 with dilute sulfuric acid and centrifuged. The supernatant was combined with the supernatant resulting from recentrifugation of the washed pellet (0.5 mL of water), and 1.0 mL was injected into a C_{18} HPLC system (9). The mobile phase consisted of methanol-water-glacial acetic acid (12.5:86.5:1.0) at a flow rate of 1 mL/min. Inorganic chloride-36 was determined in the eluant 2-6 min after injection; methanol (1.0 mL) and cocktail[4] (14 mL) were added with counting for 10 min. Between injections the HPLC system was flushed with methanol (30 mL) and reequilibrated with mobile phase.

RESULTS AND DISCUSSION

Analytical System—The metabolism of the chlorinated biphenyls was expected to yield phenols (5) and their glucuronides (10). Therefore, an HPLC procedure that would separate inorganic chloride prior to highly polar metabolites was desired. We used the system devised by Mitchell *et al.* (11) for the separation of metabolic conjugates which happened to be similar to a system we have used for the determination of inorganic halides (9). In a test mixture of chloride in the presence of the glucuronide and sulfate of *p*-nitrophenol, chloride was recovered quantitatively well before completely resolved peaks for these conjugates.

***In Vivo* Dechlorination**—*[4-^{36}Cl]Chlorobiphenyl*—Figure 1 outlines the excretion of total urinary radioactivity expressed as an average of the percentage of dose for three rats. The excretion of radioactive substances (17.7 ± 6.45%) after the first day constituted at least half the total radioactivity over 10 d and agreed with the data in the literature [25.8 ± 13.3% (12)] for oral administration. Excretion of total radioactivity after intravenous administration has been reported (12, 13) to be even more rapid, with 80% of the dose excreted within 24 h and almost total excretion after 2 d. In the present study, urinary excretion of radioactivity decreased to 2.5 ± 1.5% for the second day and remained relatively constant through day 10.

Injection of an aliquot of a composite urine sample from each of the three rats indicated that 1.7 ± 0.5% (mean for three animals, 11 injections) of the dose of [4-^{36}Cl]chlorobiphenyl was excreted as inorganic chloride-36 over a 10-d period. This was less than the *in vivo* deiodination of [4-^{125}I]iodobiphenyl (14), as expected, due to the stronger carbon-chloride bond. The low levels of total radioactivity in daily urine samples prevented determination of liberated chloride-36 on a daily basis.

[1] New England Nuclear, Boston, Mass.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Model 9000 scintillation counter; Beckman, Berkeley, Calif.
[4] ACS; Amersham, Arlington Heights, Ill.

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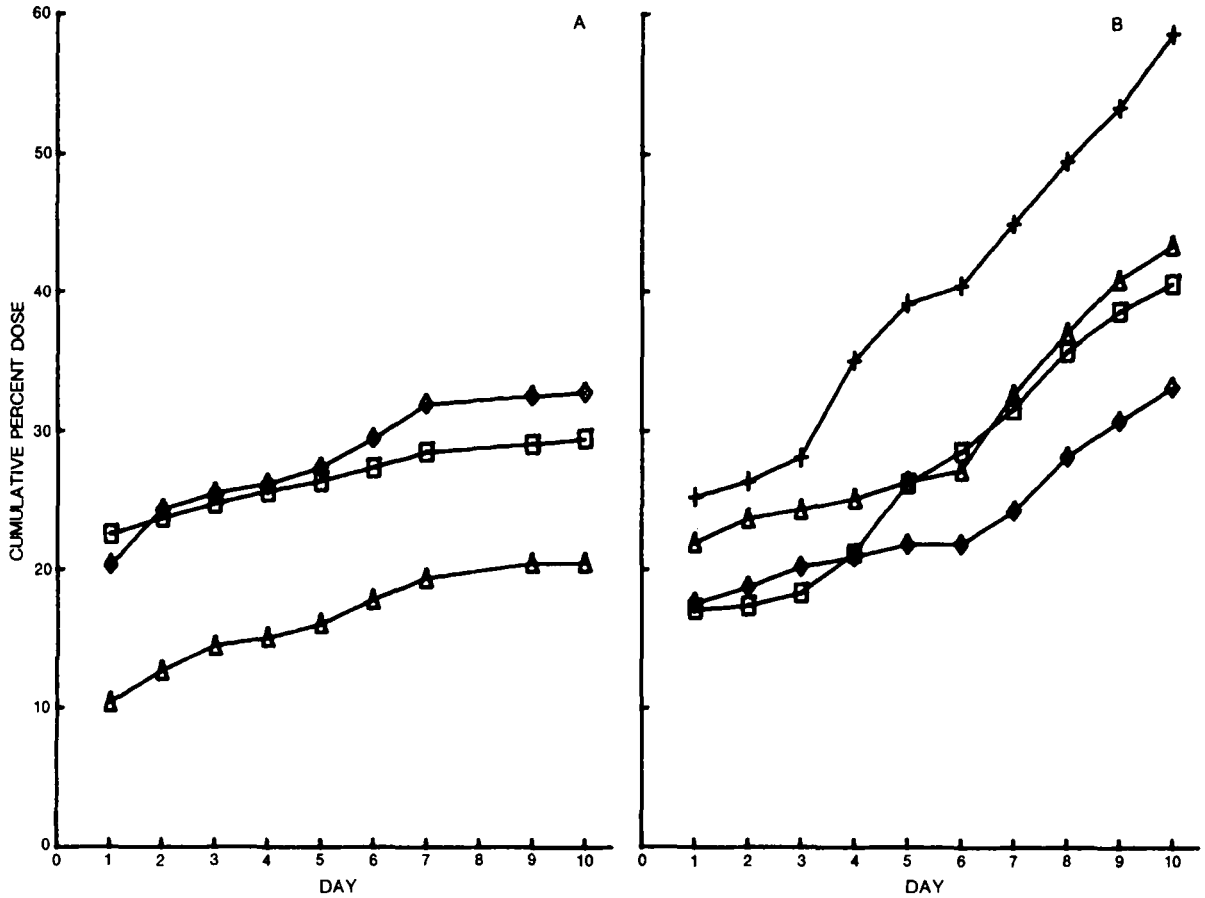

Figure 1—*Cumulative total urinary radioactivity expressed as a percentage of dose from [4-^{36}Cl]chlorobiphenyl (A) for 3 rats and [4,4'-^{36}Cl]dichlorobiphenyl (B) from 4 rats where the rat denoted with a "+" received twice the dose.*

These results appear to be the first to show dechlorination of 4-chlorobiphenyl and the first to follow dechlorination by direct determination of inorganic chloride. Previous studies in the rabbit (10) and in a variety of other species (5) reported no dechlorinated metabolites. The rapid excretion of total radioactivity coupled with the slow rate and low percentage of dechlorination may explain why no dechlorinated metabolites have been found in these *in vivo* studies.

[4,4'-^{36}Cl]Dichlorobiphenyl—The cumulative daily excretion of total radioactivity (percent of dose) for the four rats tested is summarized in Fig. 1. The total urinary radioactivity for the first day was 20.5 ± 3.9%, which agrees with the literature value of 22.9 ± 3.1% (12). Daily excretion of total radioactivity decreased rapidly, so that the day 2 through day 10 excretion ranged between 1 and 4.25% daily. This resulted in a small but continuous rise in the cumulative total urinary radioactivity for the dichloro compound in comparison to an earlier leveling off of radioactivity with 4-chlorobiphenyl for the 10-d period studied.

A composite urine sample for each of four rats was analyzed for chloride by HPLC, and the percentage of original dose was found to be 11.5 ± 2.7% (the mean of three measurements for each of four animals). This was 10-fold more dechlorination than found for [4-^{36}Cl]chlorobiphenyl. In addition to the twofold greater amount of original chloride, this increased dechlorination might have been a result of the increased importance of the dechlorination of an intermediate 3-hydroxy,4-chloro metabolite.

Tulp *et al.* (15) have shown that 4-chloro-3'-hydroxybiphenyl is an intermediate in the metabolism of 4,4'-dichlorobiphenyl and that the *ortho* hydroxy group in this metabolite and related compounds (16) activates dehalogenation. In the present study it is assumed that such activated dehalogenation would be more important in the 4,4'-dichlorobiphenyl than in the 4-chlorobiphenyl compound. In the former case there would be greater opportunity for the presence of a hydroxy group *ortho* to the increased number of chlorides. In the latter compound, there is the established (5, 10) hydroxylation of the unsubstituted ring with the elimination of the compound prior to the formation of 3-hydroxy-4-chlorobiphenyl.

The direct measurements of dechlorination for both 4-chloro- and 4,4'-dichlorobiphenyl would indicate that aromatic dechlorination plays a more important role in the metabolism of these compounds than previously noted.

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ACKNOWLEDGMENTS

This study was supported in part by Training Grant 5-T32 GM-07767 from the National Institutes of Health.