Metabolism of Biphenyl and 4-Chlorobiphenyl in the Rabbit*

WALTER D. BLOCK AND HERBERT H. CORNISH

From the Department of Dermatology, Medical School, The University of Michigan, Ann Arbor, Michigan

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The ability of the rabbit to metabolize naphthalene and chlorinated naphthalene was markedly influenced by the degree of halogenation of the compound according to Cornish and Block (2). Naphthalene is related structurally to biphenyl, in which a coaxial linkage connects two phenyl molecules. Whether the presence of a chlorine atom in biphenyl also alters the metabolism of this compound as it does in the case of naphthalene has not been reported. Stroud (3) and West et al. (4) have studied the metabolism of unsubstituted biphenyl respectively in rabbits and in rats. The present investigation is a comparative metabolic study of the urinary end-products of biphenyl and 4-chlorobiphenyl in the rabbit.

**EXPERIMENTAL**

The compounds biphenyl and 4-chlorobiphenyl were recrystallized from alcohol until the melting points were constant, 69-70° and 76-77°, respectively.

Six male albino rabbits (2 kg in weight), fed a diet of oats and cabbage, were used for each compound studied. Twenty-four hour urine samples were collected for a 4-day period both before and after administration of either 1 g of biphenyl or 1 g of 4-chlorobiphenyl in corn oil given by stomach tube. Each animal served as his own control.

The urine samples were analyzed for their content of the following metabolites: glucosiduronic acids, phenolic compounds, ethereal sulfates, and mercapturic acids (neutral sulfur). Creatinine content was also determined.

Glucosiduronic acids were assayed by the procedure of Hanson et al. (6). Phenolic compounds were determined with the Folin-Ciocalteu reagent (6), with 4-hydroxybiphenyl as a standard. Sulfur partitions were done by the barium sulfate precipitation procedure of Folin (7). Creatinine content was determined by a photometric modification of Folin's picric acid method (8).

The amount of the metabolite excreted by each rabbit during the 4 days when neither biphenyl nor 4-chlorobiphenyl was fed, was subtracted from the amount excreted during the 4 days after the compound was fed. The increment, which was presumed to be caused by feeding of the hydrocarbon, was calculated in terms of milligrams of either biphenyl or 4-chlorobiphenyl.

For studies in which several of the excretory products derived from biphenyl and 4-chlorobiphenyl were isolated from urine, 1 g of compound was fed (6 rabbits fed each compound) and the urine collected for 48 hours. Urine collected from animals fed the same compound was pooled and frozen until analyzed. An aliquot of the urine was adjusted to pH 7.2 to 7.3 with 10% NaOH and continuously extracted with ether for 24 hours. The ether extract was evaporated to dryness and the residue taken up in hot absolute alcohol and water added until the solution became turbid. The mixture was placed in the cold (15°) for 24 hours and a crystalline phenolic compound obtained. After several recrystallizations this compound gave a constant melting point. The ether-extracted urine was concentrated to one-half its original volume under reduced pressure, acidified to pH 2.0 with 10% HCl, and placed in the cold (15°) for 24 hours. A yellow precipitate high in glucosiduronic acid content was secured. This material was recrystallized from hot absolute alcohol until a constant melting point was obtained.

**RESULTS**

**Metabolic Studies**

Table I shows average 4-day excretion values of various urinary metabolites both before and after ingestion of biphenyl and 4-chlorobiphenyl. Because normal daily excretion of these metabolites is variable, changes in excretion of less than 20% are not considered meaningful. Amounts of ingested biphenyl and 4-chlorobiphenyl accounted for by the four urinary metabolites determined were essentially the same, 630 mg and 647 mg. Approximately 26% or 262 mg of the biphenyl was excreted as the glucosiduronic acid derivative, but 50%, or 505 mg of the ingested 4-chlorobiphenyl was excreted in this manner.

Free phenolic compounds in the urine accounted for 243 mg of the ingested biphenyl as contrasted to 30 mg of the 4-chlorobiphenyl. Ethereal sulfate excretion accounted for 13 and 11%, respectively, of the ingested biphenyl and 4-chlorobiphenyl. Neither biphenyl nor 4-chlorobiphenyl seemed to be excreted as mercapturic acid derivatives.

**Isolation Studies**

**Biphenyl Metabolites**—4-Hydroxybiphenyl (m.p. 165°) was isolated from the ether-soluble fraction of urine excreted by rabbits fed biphenyl. The acetate and benzoate derivatives of this compound were prepared and found to have melting points of 88° and 151°, respectively, which are consistent with values reported in the literature (9). Biphenylglucosiduronic acid (m.p. 183-184°) was isolated from the ether-insoluble fraction of the urine. Hydrolysis of the compound with acid or β-glucuronidase yielded 4-hydroxybiphenyl (m.p. 165°). Addition of known 4-hydroxybiphenyl did not depress the melting point. Acetate and benzoate derivatives of the isolated 4-hydroxybiphenyl had melting points of 87-88° and 149°, respectively.
Effect of ingestion of biphenyl and 4-chlorobiphenyl on excretion of urinary metabolites by the rabbit

<table>
<thead>
<tr>
<th>Compound</th>
<th>Etherable sulfates</th>
<th>Mercapturic acid</th>
<th>Glucosiduronate</th>
<th>Free phenolic compounds</th>
<th>Total increment as compared to control</th>
<th>mg/animal fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>37</td>
<td>74</td>
<td>510</td>
<td>311</td>
<td>0</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>1</td>
<td>85</td>
<td>75</td>
<td>851</td>
<td>202</td>
<td>175</td>
</tr>
<tr>
<td>4-Chlorobiphenyl</td>
<td>0</td>
<td>41</td>
<td>118</td>
<td>542</td>
<td>294</td>
<td>165</td>
</tr>
</tbody>
</table>

The values represent the total 4-day excretion. Each value is the average of results obtained on six different animals.

4-Chlorobiphenyl Metabolites—4-(p-Chlorophenyl)phenol (m.p. 140°) was isolated from the ether-soluble fraction of urine excreted by rabbits fed 4-chlorobiphenyl. Acetate and benzoate derivatives of this compound had melting points of 110° and 182°, respectively. These values are consistent with those reported in the literature (10). 4-Chlorobiphenyl glucosiduronide (m.p. 186°) was isolated from the ether-insoluble fraction of the urine. Hydrolysis of the compound with acid or β-glucuronidase yielded 4-(p-chlorophenyl)phenol (m.p. 145°) whose acetate and benzoate derivatives had melting points of 110-111° and 181°, respectively.

The isolation procedures used in this study do not rule out the presence of other metabolites of biphenyl and 4-chlorobiphenyl in the urine of rabbits fed these compounds.

DISCUSSION

The results of this study indicate that 4-chlorobiphenyl is metabolized as readily as biphenyl by the rabbit. The presence of a chlorine atom in the 4-position of biphenyl, however, does alter the distribution of the urinary metabolites of the compound. Essentially twice as much 4-chlorobiphenyl as biphenyl was excreted as the glucosiduronic acid derivative. The isolation studies indicated that the glucosiduronic acids of both 4-chlorobiphenyl and biphenyl were formed by conjugation in the 4- or para-position. Oxidation of the 4′ position of the 4-chlorobiphenyl to a phenol group must have taken place in order for the glucosiduronate to be formed, and 4-(p-chlorophenyl)phenol was isolated from the ether-soluble fraction of urine collected from rabbits fed 4-chlorobiphenyl. Nevertheless, the animal preferentially formed the glucosiduronate derivative for excretion because only a small fraction of the ingested 4-chlorobiphenyl (3%) was excreted as the free phenolic compound. This pathway was a major one for the excretion of biphenyl (24%) and was equally as important as the formation of glucosiduronides in biphenyl metabolism.

Hydrolysis by β-glucuronidase of the glucosiduronides isolated from the urine of animals fed either compound indicated a beta linkage with the glucosiduronic acid moiety.

Although biphenyl is related structurally to naphthalene in that both are composed of two phenyl groups but with different linkages, the pathways for metabolism, by the rabbit, of the two compounds are dissimilar. Cornish and Block (2) reported 19% of the ingested naphthalene was excreted as mercapturic acid derivatives, and 2% as free phenolic compounds. In the present study, no increment in mercapturic acid excretion caused by feeding biphenyl could be detected, and, as mentioned before, free phenolic derivatives were an important excretory product. Cornish and Block (2) did find that more l-chloronaphthalene and dichloronaphthalene than naphthalene was excreted as the glucosiduronide. The present study would confirm the finding that glucosiduronide formation becomes more important in metabolism of the ingested hydrocarbon by the rabbit when the compound is halogenated, and that apparently the rabbit lacks a mechanism for the dehalogenation in two of such compounds (2).

SUMMARY

Twenty-four-hour urine samples were collected for a 4-day period both before and after oral administration of either 1 g of biphenyl or 1 g of 4-chlorobiphenyl to rabbits. The urine samples were analyzed for their content of glucosiduronic acids, free phenolic compounds, mercapturic acids, and etheral sulfates.

These metabolites in the urine accounted for 64% of the ingested biphenyl or 1 g of 4-chlorobiphenyl to rabbits. The urine samples were analyzed for their content of glucosiduronic acids, free phenolic compounds, mercapturic acids, and etheral sulfates.

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