THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 128, No. 2, December 1959
Printed in U.S.A.

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

(Received for publication, August 24, 1959)

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.* (5). 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 7.8 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 1 shows average 4-day excretion values of various urinary metabolites both before and after ingestion of biphenyl and of 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, 639 mg and 647 mg. Approximately 26% or 263 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 213 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 150°, respectively.

* This work was supported in part by United States Public Health Service Grant RG-791. A preliminary report has been presented (1).

† Biphenyl was obtained from Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York. 4-Chlorobiphenyl was obtained through the courtesy of Monsanto Chemical Company, St. Louis, Missouri.

DSW 026615

STLCOPCB4010576

TABLE I

Effect of ingestion of biphenyl and 4-chlorobiphenyl on excretion of urinary metabolites by the rabbit

Compound	Amount fed	Ethereal sulfate		Mercapturic acid		Glucosiduronic acid		Free (phenolic) compounds		Total increment as compound fed	
		Total	Increment as compound fed	Total	Increment as compound fed	Total	Increment as compound fed	Total	Increment as compound fed		
	g	mg*	mg	mg*	mg	mg*	mg	mg*	mg	mg	%
Control	0	37		71		510		311			
Biphenyl	1	67	134	75		854	262	384	213	609	61
Control	0	41		118		542		291			
4-Chlorobiphenyl	1	58	112	89		1182	803	306	30	617	65

* The values represent the total 1 day excretion. Each value is the average of results obtained on six different animals.

4-Chlorobiphenyl Metabolites 4-(*p*-Chlorophenyl)phenol (m.p. 146°) 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 glucosiduronide 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 glucosiduronide derivative for excretion because only a small fraction of the ingested 4-chlorobiphenyl (4%) 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 glucosiduronic acid isolated from the urines of animals fed either compound indicates 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 these compounds are dissimilar. Cornish and Block (2) reported 6% 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 could be detected, and, as mentioned before, free phenolic derivatives were an important excretory product. Cornish and Block (2) did find that more 1-chloronaphthalene and dichloronaphthalene than naphthalene was excreted as the glucosiduronide. The present study would confirm the report that glucosiduronide formation becomes more important in the metabolism of the ingested hydrocarbon by the rabbit when a compound is halogenated, and that apparently the rabbit has a mechanism for the dehalogenation in vivo of such compounds (2).

SUMMARY

Twenty-four-hour urine samples were collected for a 5 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 ethereal sulfates.

These metabolites in the urine accounted for 61% of the ingested biphenyl or 4-chlorobiphenyl. 4-Chlorobiphenyl was excreted mainly as the glucosiduronide (50%); free phenolic compounds (24%) and glucosiduronides (26%) in the urine accounted for most of the biphenyl. Thus the presence of a chlorine atom in the biphenyl molecule altered the metabolic pathway in the rabbit.

Isolation studies indicated that the glucosiduronic acid derivatives of both compounds were formed by conjugation in the 4- or *para*-position.

REFERENCES

1. Cornish, H. H., and Block, W. D., *Federation Proc.*, 15 (1956).
2. Cornish, H. H., and Block, W. D., *J. Biol. Chem.*, 231, 5 (1958).
3. Smyth, S. W., *J. Endocrinol.*, 2, 55 (1940).
4. West, H. D., Lawson, J. R., Mueller, J. H., and Matthews, G. R., *Arch. Biochem. Biophys.*, 60, 11 (1956).
5. Hanson, S. W. F., Mills, G. T., and Williams, R. T., *Biochem. J.*, 38, 271 (1944).
6. Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, 73, 627 (1927).
7. Folin, O., *Am. J. Physiol.*, 13, 45 (1905).
8. Folin, O., *J. Biol. Chem.*, 17, 469 (1914).
9. Chemnons, N. D., and Entmiker, J. B., *Semi-micro qualitative organic analysis*, 2nd edition, Interscience Publishers, Inc., New York, 1957.
10. Savoy, C. M., and Abernathy, J. L., *J. Am. Chem. Soc.*, 34, 2219 (1912).

DSW 026616

STLCOPCB4010577