the partial functions. This conception could readily be elaborated, but whether such a modification in activity could be demonstrated is another matter.

SUMMARY

1. Haemolysis of horse and sheep erythrocytes by *Clostridium welchii* and *Cl. oedematiens* toxins was always preceded by decomposition of some of the phospholipin of the cell, indicating that haemolysis is primarily dependent upon the action of the lecithinases present.

2. The rates of hydrolysis of the phospholipin of intact erythrocytes of horse and sheep by the three immunologically distinct lecithinases—*Cl. welchii* α -toxin and *Cl. oedematiens* β - and γ -toxins—are different.

3. These differences appear sufficient to account for the known differences in sensitivity of horse and sheep cells to haemolysis by the immunologically specific lecithinases.

4. After exposure to *Cl. welchii* α -toxin at 37°, sheep erythrocytes undergo extensive haemolysis (hot-cold lysis) on cooling below a critical temperature. This effect is less marked with horse cells and does not occur with human or rabbit erythrocytes.

5. The significance of the selective action of the lecithinases on erythrocytes of different species is discussed with relation to the problem of virulence and biological differentiation.

I am indebted to Dr C. L. Oakley for the gift of toxins and antitoxins, and wish to thank Miss J. Brockwell for her technical assistance.

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Studies in Detoxication

33. THE METABOLISM OF HALOGENOBENZENES. A COMPARISON OF THE GLUCURONIC ACID, ETHEREAL SULPHATE AND MERCAPTURIC ACID CONJUGATIONS OF CHLORO-, BROMO- AND IODO-BENZENES AND OF THE *o*-, *m*- AND *p*-CHLOROPHENOLS. BIOSYNTHESIS OF *o*-, *m*- AND *p*-CHLOROPHENYLGLUCURONIDES

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It is believed that the halogenobenzenes are transformed in the body into phenols and into mercapturic acids. Because cysteine is involved in mercapturic acid formation, the sulphur conjugation of the halogenobenzenes has received considerable attention. There seems, however, to be little information as to the relative amounts of the halogenobenzenes excreted as sulphur conjugates (mercapturic acids) and as oxygen conjugates (glucuronides and ethereal sulphates) (Witter, 1945). Earlier work (e.g. Binkley, 1949; Coombs & Hele, 1926; Rhode, 1923; Stekol, 1936; Shiple, Muldoon & Sherwin, 1924) attempted to correlate mercapturic acid formation with sulphate conjugation, but this gave an incomplete picture because the glucuronic acid conjugation

was left out of account. The object of the present work was to find out how the halogenobenzenes are distributed over the three conjugations, sulphate, glucuronic and mercapturic acid. The view that sulphur conjugation of aromatic compounds may be the precursor of their oxygen conjugations (Quick, 1937) was kept in mind. A study of the conjugation of the chlorophenols (cf. Baumann & Preusse, 1879; Shiple *et al.* 1924; Coombs & Hele, 1926; Külz, 1883) was included for comparison, and at the same time the *o*-, *m*- and *p*-chlorophenylglucuronides were prepared biosynthetically as reference compounds and for further information on the anomalous optical rotations of acetylated *o*-substituted phenylglucuronides (Smith, 1949).

METHODS AND MATERIALS

Materials. The halogenobenzenes and *o*- and *p*-chlorophenols were commercial samples. *m*-Chlorophenol was prepared from *m*-chloraniline (Holleman & Rinkes, 1911). The halogenobenzenes and *o*-chlorophenol were purified by distillation; chlorobenzene, b.p. 132°; bromobenzene, b.p. 156°; iodobenzene, b.p. 188°; *o*-chlorophenol, b.p. 175–176°; *m*- and *p*-chlorophenols were recrystallized from ligroin and had m.p. 31.5 and 42.5°, respectively (all melting points and boiling points quoted in this paper are uncorrected).

Reference compounds. For identification purposes, the toluene-*p*-sulphonates of the chlorophenols were prepared and used. The *o*- (m.p. 75°) and *p*- (m.p. 71°) esters are described by Wild (1947). The 3-chlorophenyl toluene-*p*-sulphonate appears to be new. It formed colourless prisms, m.p. 42°, from ethanol. (Found: C, 55.5; H, 4.1. $C_{13}H_{11}O_3ClS$ requires C, 55.2; H, 3.9%.) These compounds have low melting points, but they could easily be obtained in 95–100% yields.

Animals. Chinchilla rabbits, kept on a carefully controlled diet of 75 g. Lever's cubes per day and water *ad lib.*, were used. This diet was found to contain 0.5% cystine. The compounds, emulsified with water, were administered by stomach tube.

Analytical methods. Ethereal sulphate was determined by the Folin gravimetric method, glucuronic acid according to Hanson, Mills & Williams (1944), and mercapturic acid, with slight modifications for rabbit urine, according to Stekol (1936). *O*-Acetyl was estimated according to the method of Alicino (1948) for acetylated sugars. In this method it was found that with the triacetyl glucuronidamides described below, 5 hr. in 0.01N-NaOH at room temperature was necessary for the quantitative removal of the acetyl groups.

Quantitative results

Halogenobenzenes. The results with these compounds are summarized in Table 1. In all cases the compounds were fed

Fig. 1. Excretion of glucuronic acid, ethereal sulphate and mercapturic acid by rabbit no. 13 which had received 345 mg. chlorobenzene orally on the fourth day of the experiment. —●—●, glucuronic acid; —×—×, ethereal sulphate; —○—○, mercapturic acid.

in amounts equivalent to 150 mg. chlorobenzene/kg., i.e. bromobenzene at 210 mg./kg. and iodobenzene at 272 mg./kg.

It was noted that the increase of ethereal sulphate and glucuronic acid above normal following the feeding of chloro- and iodo-benzene appeared only for 1 day, whereas the excretion of mercapturic acid lasted for 2 days (Fig. 1). In the case of bromobenzene, all three conjugations remained above normal for 2 days (Fig. 2). It is clear that, on the diet provided, just over 20% of all three halogenobenzenes are eliminated as the corresponding mercapturic acid. Ethereal sulphates (*E*) and glucuronides (*G*) are produced in equal amounts, i.e. the ratio (*G/E*) is nearly unity. Furthermore, the oxygen conjugation is 2.5–3 times the sulphur conjugation.

Fig. 2. Excretion of glucuronic acid, ethereal sulphate and mercapturic acid by rabbit no. 130 which had received 701.5 mg. of bromobenzene orally on the fifth day of the experiment. —●—●, glucuronic acid; —×—×, ethereal sulphate; —○—○, mercapturic acid.

Chlorophenols. In this case the metabolites are all oxygen conjugates, there being no sulphur conjugation (see Table 1). In the case of *o*- and *m*-chlorophenols about four times as much glucuronide is excreted as ethereal sulphate and with *p*-chlorophenol seven times. Thus the *G/E* ratio for the halogenobenzenes is entirely different from that of the halogenophenols.

Isolation of chlorophenylglucuronide derivatives

All urines from rabbits receiving chlorophenols were normal in appearance, faintly alkaline and non-reducing. The naphthoresorcinol test was strongly positive.

***o*-Chlorophenol and derivatives.** *o*-Chlorophenol was more toxic than its *m*- and *p*-isomers, and in doses greater than 0.3 g./kg. it caused twitching of the limbs and the animals became distressed. The *m*- and *p*-isomers showed these effects in higher doses.

o-Chlorophenol (9.5 g.) was fed to six rabbits and the 24 hr. urine (675 ml.), after making faintly acid with a few drops of conc. HCl, was continuously extracted with ether for 8 hr. until the extract no longer gave a colour with 2:6-dichloroquinone chloroimine. The extract was dried with anhydrous Na_2SO_4 and evaporated to dryness at 20°. The residual reddish brown gum (0.34 g.) was dissolved in NaOH (5 ml. of 10% w/v) and then shaken for 0.5 hr. with 5 ml. acetone containing 0.6 g. toluene-*p*-sulphonyl chloride. The mixture was poured into 75 ml. cold water and a crystalline precipitate separated during the next 24 hr. On crystallization from ethanol, the precipitate yielded 32 mg. (0.15% of dose) of 2-chlorophenyl toluene-*p*-sulphonate, m.p. 73°; mixed m.p. with authentic material, 74°.

Table 1. *Urinary excretion of ethereal sulphate, glucuronide and mercapturic acid by rabbits receiving halogenobenzenes and chlorophenols orally*

(All doses were molecular equivalents of 150 mg. chlorobenzene/kg. i.e. bromobenzene, 210, iodobenzene, 272 and chlorophenols, 171.3 mg./kg. Three rabbits used for each compound. Figures in columns 2, 3 and 4 give values for individual rabbits and means.)

Compound	Percentage of dose excreted as			Ratio G/E	Ratio: O -conjugation/ S -conjugation $\left(\frac{G+E}{M}\right)$	Total conjugation (percentage of dose) $G + E + M$
	Glucuronide (G)	Ethereal sulphate (E)	Mercapturic acid (M)			
Chlorobenzene	25.6	23.8	21.2	0.95	2.5	72.2
	29.1	27.5	19.8			
	21.0	28.4	20.2			
Bromobenzene	39.2	34.5	19.9	1.09	3.7	97.9
	41.2	39.1	21.9			
	40.2	36.8	20.9			
Iodobenzene	38.1	27.5	24.3	1.06	2.7	83.5
	27.8	34.3	20.7			
	28.0	27.7	23.8			
<i>o</i> -Chlorophenol	77.2	25.6	0	4.4	—	101.7
	85.7	14.5	0			
	84.4	15.9	0			
<i>m</i> -Chlorophenol	75.4	20.1	0	3.8	—	98.7
	83.2	16.4	0			
	75.7	24.4	0			
<i>p</i> -Chlorophenol	85.5	13.3	0	6.9	—	101.1
	89.5	15.9	0			
	90.0	9.1	0			

o-Chlorophenylglucuronidamide. A basic lead acetate precipitate was prepared from the 24 hr. urine of nine rabbits which had received, collectively, 10 g. of *o*-chlorophenol. The Pb was removed with H_2S and the glucuronide gum prepared as described by Garton & Williams (1948) for catechol glucuronide. The brown gum (17.3 g.) was purified by dissolving in 20 ml. water and pouring into 400 ml. absolute ethanol. A dirty white precipitate which formed was discarded and the solution was evaporated under reduced pressure to a clear yellow gum (11.7 g.). The precipitation with ethanol was repeated and finally *o*-chlorophenylglucuronide was obtained as a clear yellow non-reducing gum (8.9 g.) which could not be crystallized. It was methylated by repeated treatments of its ethanolic solution with ethereal diazomethane. Removal of the solvents left *o*-chlorophenyl-glucuronide methyl ester as a non-crystalline gum. (Found: OMe, 11.3. $C_{13}H_{15}O_7Cl$ requires OMe, 10.2%.)

The above ester (7.8 g.) was dissolved in 20 ml. methanol and the solution saturated at 0° with dry NH_3 . After keeping at 0° overnight in a stoppered flask, a small precipitate was filtered off and discarded. The filtrate was reduced *in vacuo* to a semi-crystalline gum (8 g.) which was dissolved in 20 ml. water, treated with charcoal and filtered. Addition of 20 ml. ethanol to the filtrate caused no crystallization. Treatment with a large volume of ether caused the separation of an oil which, on scratching, crystallized. The crystals (7 g.) were purified by dissolving in ethanol and precipitating with ether. The β -*o*-chlorophenyl- D -glucuronidamide hemihydrate was obtained as colourless needles, m.p. 202° , and $[\alpha]_D^{24.5} - 69.5^\circ$ (*c*, 0.3 in methanol). (Found: C, 45.9; H, 5.3; N, 4.7; Cl, 11.7. $C_{13}H_{14}O_6NCl$, $0.5H_2O$ requires C, 46.1; H, 4.8; N, 4.5; Cl, 11.3%.) On acid hydrolysis with 4*N*-HCl for 2.5 hr., the amide yielded *o*-chlorophenol which was identified as the toluene-*p*-sulphonate.

The above amide (0.2 g.) was added to a mixture of 1 ml. acetic anhydride and 1 ml. pyridine. The amide slowly dissolved in 1 hr. at room temperature. After keeping overnight, water was added to the mixture until a permanent opalescence appeared. On cooling to 0° crystals appeared, and crystallization was completed by further addition of water. The crystals (212 mg.) were recrystallized from aqueous ethanol. The triacetyl- β -*o*-chlorophenyl- D -glucuronidamide formed plates soluble in methanol, ethanol and $CHCl_3$. It melted at 126° and showed $[\alpha]_D^{21} - 45.3^\circ$ (*c*, 0.3 in ethanol). (Found: CH_3CO , 30.1. $C_{18}H_{20}O_9NCl$ requires CH_3CO , 30.1%.)

Derivatives of *m*-chlorophenylglucuronide

m-Chlorophenol (9.7 g.) was fed to six rabbits. From the 24 hr. urine, 0.29% of the dose was isolated by ether extraction as free *m*-chlorophenol and identified as toluene-*p*-sulphonate (m.p. and mixed m.p. 41°).

m-Chlorophenylglucuronidamide. *m*-Chlorophenol (10 g.) was fed to nine rabbits and from the 24 hr. urine, 14 g. of crude glucuronide gum was obtained as described for the *o*-isomer. After purification *m*-chlorophenylglucuronide was obtained as an ethanol-soluble gum (9.8 g.) which on methylation gave a gummy methyl ester. (Found: OMe, 10.9%.) The ester (7 g.) in methanol was saturated with NH_3 and the amide began to crystallize. After an hour at 0° , the crystals (5.3 g.) were filtered off and recrystallized from absolute ethanol. β -*m*-Chlorophenyl- D -glucuronidamide formed colourless needles, m.p. 211° , and showed $[\alpha]_D^{24} - 84.7^\circ$ (*c*, 0.15 in methanol). (Found: C, 47.6; H, 4.6; N, 4.5; Cl, 12.0. $C_{13}H_{14}O_6NCl$ requires C, 47.4; H, 4.7; N, 4.6; Cl, 11.7%.) On acid hydrolysis, the amide yielded *m*-chlorophenol which was identified as the toluene-*p*-sulphonate.

Triacetyl- β -*m*-chlorophenyl- D -glucuronidamide prepared as for the *o*-isomer and in similar yield (0.22 g. from 0.2 g.)

formed long colourless plates from aqueous ethanol, m.p. 154°, and $[\alpha]_D^{21} - 13.3^\circ$ (c, 0.3 in ethanol). (Found: CH_3CO , 30.1. $\text{C}_{18}\text{H}_{20}\text{O}_6\text{NCl}$ requires CH_3CO , 30.1%.)

Derivatives of *p*-chlorophenylglucuronide

After feeding 10.5 g. of *p*-chlorophenol to six rabbits, the acidified 24 hr. urine was extracted with ether. The ether extract contained free *p*-chlorophenol (0.21% of the dose), identified as the toluene-*p*-sulphonate, m.p. and mixed m.p. 70°.

p-Chlorophenylglucuronidamide. On feeding 10 g. of *p*-chlorophenol to six rabbits, there was obtained from the urine 8.5 g. of purified *p*-chlorophenylglucuronide as a gum (the crystalline glucuronide is described below). Methylation with diazomethane yielded 7.8 g. of gummy methyl ester (found: OMe, 11.4%), and 7.5 g. of this ester yielded 6.3 g. of crystalline β -*p*-chlorophenyl-*D*-glucuronidamide. On recrystallization from methanol and water, the amide formed colourless, long, narrow plates, m.p. 245–246° (decomp.), $[\alpha]_D^{24} - 85.6^\circ$ (c, 0.14 in methanol). (Found: C, 47.8; H, 4.5; N, 4.7. $\text{C}_{18}\text{H}_{20}\text{O}_6\text{NCl}$ requires C, 47.4; H, 4.7; N, 4.6%.) The amide was only slightly soluble in cold water, methanol and ethanol, but soluble in these solvents on heating. It was sparingly soluble in ether. On acid hydrolysis it yielded *p*-chlorophenol which was identified as the toluene-*p*-sulphonate, m.p. 70°.

Triacetyl β -*p*-chlorophenyl-*D*-glucuronidamide was obtained as for the other isomers (100 mg. yielding 98 mg. of acetate). It formed colourless plates, sparingly soluble in water, m.p. 159°, $[\alpha]_D^{21} - 10.7^\circ$ (c, 0.28 in ethanol). (Found: CH_3CO , 29.8. $\text{C}_{18}\text{H}_{20}\text{O}_6\text{NCl}$ requires CH_3CO , 30.1%.)

Phenolic metabolites of *p*-chlorophenol

Ethereal sulphate fraction. Four rabbits were fed a total of 6 g. of *p*-chlorophenol and the urine (500 ml.) collected for 36 hr. The urine was made 0.5N with respect to HCl by addition of conc. HCl and then heated on the boiling-water bath for 25 min. to hydrolyse ethereal sulphates. When cold, the urine was extracted continuously with peroxide-free ether until the extract no longer gave a positive test with Gibbs's 2:6-dichloroquinone chloroimine reagent (9 hr.). On cooling, the ether extract deposited white plates which were collected and washed with ether. Concentration of the filtrate and washings *in vacuo* yielded more of the plates. Eventually 2.51 g. of the plates were obtained and proved to be β -*p*-chlorophenyl-*D*-glucuronide monohydrate. It was recrystallized from water and formed long narrow plates, m.p. 151°, $[\alpha]_D^{19} - 82^\circ$ (c, 0.5 in water). (Found: C, 44.6; H, 4.8; H_2O , 5.6. $\text{C}_{18}\text{H}_{18}\text{O}_6\text{Cl}$, H_2O requires C, 44.7; H_2O , 5.6%.) The solution of the glucuronide in water was strongly acidic. On hydrolysis by boiling for 2 hr. under reflux with 4N-HCl, the glucuronide yielded *p*-chlorophenol which was identified as the toluene-*p*-sulphonate, m.p. and

mixed m.p. 70–71°. The ultraviolet absorption spectra of the glucuronide were determined in water, 0.1N-HCl and 0.1N-NaOH and found to be identical (Fig. 3), there being no change in the spectrum in alkali as compared with acid. This is in contrast with *p*-chlorophenol (Smith, Spencer & Williams, 1950). By means of these spectra the glucuronide can readily be distinguished from the free phenol, and the latter estimated in the presence of the glucuronide.

Fig. 3. Ultraviolet absorption spectrum of *p*-chlorophenylglucuronide monohydrate in water (λ_{max} 219.5, 275.5 μ . with ϵ_{max} 10,700 and 930).

The ether mother liquors, after removal of the glucuronide, on evaporation gave a dark oil which appeared to contain (by colour tests) *p*-chlorophenol and traces of chlorocatechol. The oil was shaken with 25 ml. water and the emulsion centrifuged to give an oil and a clear supernatant solution. The latter was now treated with an equal volume of 0.2M-acetate buffer at pH 6.75 and 10 ml. saturated normal lead acetate to precipitate the catechol derivative. The precipitate was collected, suspended in water and decomposed in the usual manner with H_2S . After filtering off PbS, the solution was extracted continuously with ether (3 hr.). The ether extract yielded a small amount of red oil from which 4-chlorocatechol was isolated as the di-toluene-*p*-sulphonate, m.p. and mixed m.p. 114–116°, after crystallization from ethanol (yield, 21 mg.). The oil remaining after the preliminary water extraction and centrifugation was dissolved in caustic soda and treated with 4 g. toluene-*p*-sulphonyl chloride in acetone. The product was poured into water and the precipitate crystallized from ethanol. The crystals (1.35 g.) were identified as 4-chlorophenol toluene-*p*-sulphonate, m.p. and mixed m.p. 70–71°.

Table 2. Phenols isolated from the ethereal sulphate and glucuronide fraction of *p*-chlorophenol urine

Fraction	Phenols isolated	Percentage of dose of <i>p</i> -chlorophenol
Ethereal sulphate	<i>p</i> -Chlorophenol as toluene- <i>p</i> -sulphonate	10.3
	4-Chlorocatechol as the di-toluene- <i>p</i> -sulphonate	0.1
Glucuronide	<i>p</i> -Chlorophenol as toluene- <i>p</i> -sulphonate	36.6
	<i>p</i> -Chlorophenol as glucuronide	17.5
		Total 64.5%

Table 3. *Optical rotations of chlorophenylglucuronidamides*

Compound	$[\alpha]_D$	$[M]_D/100$	Concentration (g./100 ml. solution)
β -o-Chlorophenylglucuronidamide hemihydrate	-69.5°*	-217	0.3
β -m-Chlorophenylglucuronidamide	-84.7°*	-257	0.15
β -p-Chlorophenylglucuronidamide	-85.6°*	-260	0.14
β -p-Chlorophenylglucuronide monohydrate	-82°†	-264	0.5
Triacetyl β -o-chlorophenylglucuronidamide	-45.3°‡	-195	0.3
Triacetyl β -m-chlorophenylglucuronidamide	-13.3°‡	-57	0.3
Triacetyl β -p-chlorophenylglucuronidamide	-10.7°‡	-46	0.3

* In methanol at 24°. † In water at 19°. ‡ In ethanol at 21°.

Glucuronide fraction. The residual urine after the removal of the *p*-chlorophenylglucuronide and the phenols (see preceding paragraph) was now warmed to remove dissolved ether and then made 3N with respect to HCl by the cautious addition of conc. HCl. The solution was heated under reflux for 2.5 hr., cooled and then extracted with ether. The phenolic material from this extract on treatment with toluene-*p*-sulphonyl chloride yielded 4.83 g. of crude 4-chlorophenol toluene-*p*-sulphonate, m.p. and mixed m.p. 70–71°, after crystallization from ethanol. No other phenol was detected.

It is thus clear that *p*-chlorophenol is oxidized in the body only to a very minor extent (about 0.1%). The oxidation product is 4-chlorocatechol which occurs only in the ethereal sulphate fraction (Table 2).

DISCUSSION

It is clear from Table 1 that the halogenobenzenes undergo three types of conjugation in the rabbit, and that mercapturic acids, glucuronides and ethereal sulphates are excreted roughly in the ratio 2 : 3 : 3. The oxygen conjugation is far more important quantitatively than the sulphur conjugation, although in the past only the latter has been considered in studies of the metabolism of the halogenobenzenes. The significance of these results and the relation between the oxygen and sulphur conjugations are discussed in the succeeding paper (Smith *et al.* 1950).

The metabolites of *p*-chlorophenol have been studied in detail and it appears that this compound is converted almost quantitatively into the corresponding glucuronide and ethereal sulphate, only about 0.1% being oxidized to 4-chlorocatechol. The latter was excreted as an ethereal sulphate, but was not found in the glucuronide fraction. It is interesting to note that the oxidation of *p*-chlorophenol occurs *ortho* to the hydroxyl group. This result would be expected on the ordinary rules of aromatic substitution, i.e. the hydroxyl group is more strongly *ortho-para* directing than the chloro group. It is also clear from Table 1 that the chlorophenols do not give rise to mercapturic acids, but undergo oxygen conjugation only.

In this paper the chlorophenylglucuronides are described for the first time, and a comment is needed on the optical rotations of their triacetyl derivatives. In a recent paper Smith (1949) has drawn attention to the anomalous rotation of triacetyl β -o-cyanophenylglucuronide methyl ester as compared with its *m*- and *p*-isomers. The same phenomenon can now be seen with triacetyl β -o-chlorophenylglucuronidamide which has a rotation some three to four times those of its *m*- and *p*-isomers, although the rotations of the unacetylated amides are of the same order (Table 3). This phenomenon, which appears to be due to restriction of rotation by *ortho*-substituents, will be further discussed with more examples in a future publication.

SUMMARY

1. A quantitative study has been made in the rabbit of the elimination of chloro-, bromo- and iodo-benzene as ethereal sulphates, glucuronides and mercapturic acids. Three times as much oxidation occurs as conjugation with acetylcysteine and the ratio ethereal sulphate : glucuronide : mercapturic acid is approximately 3 : 3 : 2.

2. A similar study has been made of the *o*-, *m*- and *p*-chlorophenols. These are excreted entirely as glucuronides and ethereal sulphates, there being no mercapturic acid formation.

3. *p*-Chlorophenol is converted almost quantitatively into the corresponding glucuronide and ethereal sulphate, only about 0.1% being oxidized to 4-chlorocatechol which appears in the ethereal sulphate fraction.

4. The glucuronides of the *o*-, *m*- and *p*-chlorophenols have been isolated from urine and characterized as the corresponding glucuronidamides and triacetyl glucuronidamides.

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Studies in Detoxication

34. THE METABOLISM OF CHLOROBENZENE IN THE RABBIT. ISOLATION OF DIHYDRODIHYDROXYCHLOROBENZENE, *p*-CHLOROPHENYLGLUCURONIDE, 4-CHLOROCATECHOL GLUCURONIDE AND *p*-CHLOROPHENYL-MERCAPTURIC ACID

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Baumann & Herter (1877) observed that when the urine of animals dosed with naphthalene was acidified and heated, free naphthalene was liberated. Later Bourne & Young (1933, 1934) suggested that this naphthalene arose from a labile precursor, possibly a dihydronaphthol. Eventually reduced derivatives of polycyclic hydrocarbons were isolated from the urine of animals receiving naphthalene (Young, 1946, 1947), anthracene (Boyland & Levi, 1935, 1936) and phenanthrene (Boyland & Wolf, 1948). These reduced derivatives were shown to be *trans*-1:2-dihydro-1:2-diols in the cases of naphthalene and anthracene (Booth & Boyland, 1949). In the case of phenanthrene, Boyland & Wolf (1948) have produced evidence for the formation of two diols, and there is information which suggests that diols are also metabolites of carcinogenic hydrocarbons such as benzpyrene (Weigert, 1945; Boyland & Weigert, 1947).

The evidence just summarized suggests that the formation of reduced diols may be a general metabolic reaction of polycyclic hydrocarbons, but as yet there is no proof that such a reaction also occurs during the metabolism of monocyclic benzene derivatives. Porteous & Williams (1949) and Garton & Williams (1949) expressed the view that a diol may be a metabolite of benzene on the grounds that benzene gives rise to more catechol than does phenol. It has also been suggested by Smith & Williams

(1949) that aniline forms reduced derivatives *in vivo*.

In the cases of naphthalene and anthracene the corresponding mercapturic acids were isolated as well as the diols. It appeared to us, therefore, that we would be more likely to achieve success in isolating a diol if we selected for study a benzene derivative known to give rise to a mercapturic acid. Chlorobenzene is such a derivative. The present paper deals, in part, with the isolation and characterization of a diol formed in the rabbit from chlorobenzene. *p*-Chlorophenylmercapturic acid was also isolated and further characterized.

The quantitative results of the preceding paper (Spencer & Williams, 1950) showed that the oxygen conjugates of chlorobenzene metabolism were far more important quantitatively than the mercapturic acid. Although the metabolism of the halogenobenzenes was studied as early as 1879 (Baumann & Preusse, 1879, 1881) the nature of these major metabolites has not, until now, been elucidated. Baumann & Preusse, from qualitative evidence not supported by actual isolation, suggested that both monohydric and dihydric halogen-substituted phenols were formed. We shall show that the major phenolic metabolite of chlorobenzene is, contrary to expectation, 4-chlorocatechol. The expected *p*-chlorophenol is quantitatively only a very minor metabolite.