2. *o*-Dichlorobenzene also gives rise to 3:4-dichlorophenylmercapturic acid as a minor metabolite (5% of the dose).

3. *p*-Dichlorobenzene (0.5 g./kg.) is mainly oxidized to 2:5-dichlorophenol which is excreted conjugated. 2:5-Dichloroquinol is also formed (about 6% of the dose) but in contrast with *o*-dichlorobenzene no mercapturic acid or dichlorocatechol is formed.

4. The excretion of the metabolites of *o*-dichlorobenzene is slow and appears to be complete in 5–6 days after dosing. With the *para* isomer, the excretion of metabolites is not complete and is still appreciable after 6 days.

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Studies in Detoxication

63. THE METABOLISM OF HALOGENOBENZENES. (a) *META*-DICHLOROBENZENE. (b) FURTHER OBSERVATIONS ON THE METABOLISM OF CHLOROBENZENE

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Baumann (1883) concluded from experiments on dogs that *m*-dichlorobenzene did not form a mercapturic acid. Later experiments by Callow & Hele (1926), however, suggested the opposite and they concluded that in dogs *m*-dichlorobenzene formed about the same amount of mercapturic acid as monochlorobenzene. In the present work we shall show that in the rabbit *m*-dichlorobenzene does form a small amount of 2:4-dichlorophenylmercapturic acid, but the major metabolites are conjugates of 2:4-dichlorophenol. This phenol is a specific inhibitor of catalase (Goldacre & Galston, 1953).

EXPERIMENTAL

Materials and reference compounds

m-Dichlorobenzene (Light and Co.) was fractionally distilled. Three fractions were obtained: b.p. 169–171° (40% of the material), b.p. 171–172° (55%) and b.p. >172° (5%). The fraction, b.p. 171–172°, which was used in some experiments, was subsequently found to contain about 5% monochlorobenzene and will be referred to in the text as 'impure *m*-DCB'. Purified *m*-dichlorobenzene ('pure *m*-DCB') containing only traces of chlorobenzene was prepared as follows. Chlorobenzene is more readily sul-

phonated than *m*-dichlorobenzene. The 'impure *m*-DCB' (25 ml.) was heated on the water-bath with conc. H₂SO₄ (25 ml.) for 8 hr. with constant shaking (during this period equal volumes of chlorobenzene and conc. H₂SO₄ formed a single phase). The mixture was poured into water and the hydrocarbon layer, washed with water, dried over anhydrous Na₂SO₄ and distilled. The 'pure *m*-DCB' had b.p. 172–173°, *d*₄²⁰ 1.288 and *n*_D²⁰ 1.544 (literature values, b.p. 172°, *d*₄²⁰ 1.288 and *n*_D²⁰ 1.5457).

Reference compounds. The following compounds were either purchased or prepared by standard methods (melting points are corrected): 2:4-dichlorophenol (British Drug Houses Ltd.) m.p. 44° from ligroin, its benzoate, m.p. 97° (Mosso, 1887) and its toluene-*p*-sulphonate, m.p. 121° (Groves, Turner & Sharp, 1929); 3:5-dichlorophenol, m.p. 55° from ligroin was made from 3:5-dichloroaniline (Hodgson & Wignall (1927) give m.p. 68°), its benzoate had m.p. 66° from ethanol (Hodgson & Wignall (1926) give m.p. 55°) and its toluene-*p*-sulphonate had m.p. 104° (needles from ethanol) (Found: C, 49.2; H, 3.3; Cl, 23.1. C₁₃H₁₀O₃Cl₂S requires C, 49.2; H, 3.2; Cl, 22.4%); 2:6-dichlorophenol, m.p. 65° (Holleman, 1917), was converted into its toluene-*p*-sulphonate, m.p. 72° (needles from ethanol), (Found: C, 49.5; H, 3.5; Cl, 22.1%); 3:5-dichlorocatechol, m.p. 84° (Dakin, 1909), was prepared from 2:4-dichlorophenol via 3:5-dichlorosalicylaldehyde (Duff, 1941) and converted into its ditoluene-*p*-sulphonate, m.p. 145° (short needles from

ethanol), (Found: Cl, 14.1. $C_{20}H_{16}O_6Cl_2S_2$ requires Cl, 14.4%); 2,6-dichloroquinol, m.p. 164°, was prepared according to Dakin (1909) from 3:5-dichloro-4-hydroxybenzaldehyde (Biltz, 1904), and its dibenzoate, m.p. 113°, according to Levy (1883) who gives m.p. 105°.

2,4-Dichlorothiophenol, m.p. 22° (Baddeley & Bennett (1933) give m.p. 20°) was prepared by alkaline hydrolysis of synthetic 2:4-dichlorophenylmercapturic acid (Parke, 1955) followed by steam distillation of the acidified hydrolysate. It was oxidized to 2:4:2':4'-tetrachlorodiphenyldisulphide, m.p. 84° (Baddeley & Bennett, 1933), with ethanolic iodine solution.

Reference compounds related to monochlorobenzene. Chlorobenzene (British Drug Houses Ltd.), b.p. 131–132°, was redistilled. 4-Chlorocatechol, m.p. 91°, chromatographically free from catechol and 3-chlorocatechol (cf. Willstätter & Müller, 1911) was prepared from 5-chlorosalicylaldehyde according to the method of Dakin (1909); 3-chlorocatechol, m.p. 45° (Willstätter & Müller, 1911), 4-chlororesorcinol, m.p. 89° (British Drug Houses Ltd.) and chloroquinol, m.p. 103° (British Drug Houses Ltd.), were prepared or purchased, and purified.

2-Chlorothiophenol, b.p. 205°, 3-chlorothiophenol, b.p. 205°, and 4-chlorothiophenol, m.p. 54° (cf. Dacomo, 1891), were prepared by hydrolysis of the corresponding mercapturic acids (cf. Parke, 1955) with alkali followed by steam distillation after acidification. 4:4'-Dichlorodiphenyldisulphide, m.p. 71° (cf. Otto, 1868) was obtained by oxidation of 4-chlorothiophenol with ethanolic iodine.

Methods

Animals and general analytical methods are as described in the preceding paper (Azouz, Parke & Williams, 1955).

Determination of 2:4- and 3:5-dichlorophenols. 3:5-Dichlorophenol gives a red colour with Brentamine Fast Red B salt in the presence of ammonia, which is proportional to the amount of phenol, whereas 2:4-dichlorophenol gives

no appreciable colour with this reagent. These phenols are the only chlorinated monophenols present in *m*-dichlorobenzene urine. They were therefore estimated together by the method used for 2:3- and 3:4-dichlorophenols described in the preceding paper (Azouz *et al.* 1955). The spectra in acid and alkali of 2:4- and 3:5-dichlorophenols are shown in Fig. 1 (ϵ at 244 $m\mu$. for 2:4-dichlorophenol is 9500 in NaOH and 300 in HCl, for 3:5-dichlorophenol, 6600 in NaOH and 150 in HCl). When 2:4- and 3:5-dichlorophenols were added to normal urines their recovery by this method was satisfactory; thus, for example, when 250 mg. of 2:4-compound and 25 mg. of 3:5-compound were added to 200 ml. normal urine, the recovery was $98 \pm 5\%$ and $93 \pm 5\%$ of the respective compounds.

Chromatography

The possible phenolic metabolites of *m*-dichlorobenzene are 2:4-, 2:6- and 3:5-dichlorophenols, 3:5-dichlorocatechol and 2:6-dichloroquinol. The three monophenols can be separated from the polyphenols by steam distillation and then on paper in solvent system *B* (Table 1). They are also distinguishable by colour reactions (see Table 2). The polyphenols are readily separable in solvent system *A*.

The 2:4- and 3:5-dichlorophenylmercapturic acids are only just separable in solvent system *B* (see Table 1). The 2:6- isomer is readily distinguished from the other two. They were detected by the indicator reagents described by Fewster & Hall (1951). Better separation of these mercapturic acids was obtained after conversion into the corresponding thiophenols. The dichlorophenylmercapturic acids (50 mg.) were heated at 100° for 30 min. in 5 ml. *N*-NaOH. The solutions were acidified with 2*N*- H_2SO_4 and steam distilled, and the volatile dichlorothiophenols extracted into ether. They were chromatographed on paper in about 50 μ g. quantities in solvent system *B* and detected by spraying first with aqueous 0.01 *N*- I_2 containing 10% 2*N*- H_2SO_4 and then with 2% starch solution. The thiophenols showed up as white spots on dark blue background (see Table 1).

The possible dihydric phenols of monochlorobenzene, 3- and 4-chlorocatechol, 4-chlororesorcinol and chloroquinol

Fig. 1. The spectra of 2:4- and 3:5-dichlorophenols. *A*, 2:4-dichlorophenol in 0.1*N*-NaOH (λ_{max} 244, 304 $m\mu$; ϵ_{max} 9500, 3400) and *B* in 0.1*N*-HCl (λ_{max} 285 $m\mu$; ϵ_{max} 2000); *C*, 3:5-dichlorophenol in 0.1*N*-NaOH (λ_{max} 243–244, 295, 300 $m\mu$; ϵ_{max} 6600, 4100, 4200) and *D* in 0.1*N*-NaOH (λ_{max} 223–224, 279, 287 $m\mu$; ϵ_{max} 7000, 2300, 2100).

Fig. 2. The spectra of 2:6-dichlorophenol. *A* in 0.1*N*-NaOH (λ_{max} 238, 301 $m\mu$; ϵ_{max} 7100, 5250) and *B* in 0.1*N*-HCl (λ_{max} 276 $m\mu$; ϵ_{max} 2250).

Table 1. R_F values of chlorophenols and mercapturic acids related to chlorobenzene and *m*-dichlorobenzene

Descending chromatography on Whatman no. 1 paper. In solvent *A* (benzene-acetic acid-water, 1:1:2, v/v) values are after 6 hr. and in *B* (ethanol-*n*-butanol-3*N* ammonia-ammonium carbonate buffer (11:40:19, v/v); Fewster & Hall, 1951) after 16 hr. R_F values followed by *A* were determined in solvent *A*, the others in solvent *B*.

Compound	R_F values	
2:4-Dichlorophenol	0.85	0.75* (buff)
2:6-Dichlorophenol	0.73	0.77* (pale yellow)
3:5-Dichlorophenol	0.94	0.80* (buff)
4-Chlorocatechol	0.33 <i>A</i>	—
3-Chlorocatechol	0.41 <i>A</i>	—
4-Chlororesorcinol	0.19 <i>A</i>	—
Chloroquinol	0.17 <i>A</i>	—
3:5-Dichlorocatechol	0.51 <i>A</i>	—
2:6-Dichloroquinol	0.39 <i>A</i>	—
L-2-Chlorophenylmercapturic acid	0.56	—
L-3-Chlorophenylmercapturic acid	0.61	—
L-4-Chlorophenylmercapturic acid	0.60	—
L-2:4-Dichlorophenylmercapturic acid	0.61	—
L-2:6-Dichlorophenylmercapturic acid	0.53	—
L-3:5-Dichlorophenylmercapturic acid	0.65	—
2-Chlorothiophenol	0.50	—
3-Chlorothiophenol	0.61	—
4-Chlorothiophenol	0.59	—
2:4-Dichlorothiophenol	0.02	—
2:6-Dichlorothiophenol	0.56	—
3:5-Dichlorothiophenol	0.39	—

* The phenols were first coupled with diazotized *p*-nitraniline and the product was chromatographed.

Table 2. Colour reactions on paper of various chlorophenols

The figures in parentheses are approximate pH values; — means not tested.

Phenol	Gibb's reagent*	Brentamine Fast Red B salt†	Diazotized sulphanilic acid‡	Diazotized <i>p</i> -nitraniline§	FeCl ₃	Ammoniacal AgNO ₃
2:4-Dichlorophenol	Pale blue (8–10), olive green (12)	Pale yellow	Orange-red slowly	—	—	—
2:6-Dichlorophenol	Pale green (8), deep green (10–12)	Red	Lemon-yellow	—	—	—
3:5-Dichlorophenol	Deep blue (8–10), blue (12)	Red	Orange-yellow	—	—	—
3-Chlorocatechol	No colour (7), blue → brown (9)	—	—	—	Green (7), blue (9)	Pale brown
4-Chlorocatechol	Blue (7), blue (9)	—	Red	Blue-violet	Green (7), violet (9)	Pale brown
3:5-Dichlorocatechol	Blue (>8)	—	—	—	Green (7), violet (9)	Pale brown
4-Chlororesorcinol	Violet (7), blue-violet (8)	—	Deep yellow- brown	Deep yellow- brown	Brown (7)	Pale brown
Chloroquinol	Pink (7), green (8)	—	Greyish pink	Pale yellow	Brown (7)	Brown
2:6-Dichloroquinol	Green (7–10)	—	—	—	—	Brown

* 2% ethanolic solution of 2:6-dichloroquinonechloroimide followed by saturated NaHCO₃ for pH 8; saturated borax solution for pH 9 and 2*N*-Na₂CO₃ for pH > 10.

† 0.1% aqueous solution of Brentamine Fast Red B salt followed by 2*N* ammonia.

‡ Diazotized sulphanilic acid followed by 2*N*-Na₂CO₃.

§ Diazotized *p*-nitraniline followed by 2*N*-Na₂CO₃.

|| 1% aqueous FeCl₃ followed by saturated NaHCO₃.

are separable on paper in solvent system *A* (see Table 1) and are detectable by their colour reactions (Table 2). The 2-, 3- and 4-chlorophenylmercapturic acids were not readily separated on paper, although the *ortho* could be distinguished from the *meta* and *para* isomers. No better separation could be achieved on conversion of the mercapturic acids into the thiophenols, except that the *ortho* is more readily distinguished from the *meta* and *para* isomers (see Table 1). The thiophenols were detected on the paper by spraying with iodine and starch as for the dichlorothiophenols, or by Folin & Ciocalteu's reagent (British Drug Houses Ltd.) followed by $2N\text{-Na}_2\text{CO}_3$, which gives a blue spot with the thiophenols, or by spraying with a mixture of 20% (w/v) aqueous hydroxylamine hydrochloride and a solution of CuCl_2 (1.5 g.), NH_4Cl (3 g.) and ammonia (sp.gr. 0.88; 3 ml.) in 50 ml. water (Feigl, 1954), whereby the thiophenols show up as yellow-brown spots on a white background.

Isolation and detection of metabolites

Phenols. A rabbit was fed 1.5 g. 'pure *m*-DCB' and the urine collected during 4 days. The urine was made $3N$ with respect to HCl and heated under reflux for 3 hr. After cooling, it was continuously extracted with ether for 8 hr. The extract was washed with $2N\text{-NaHCO}_3$ and the washings were discarded and then the phenols transferred to $2N\text{-NaOH}$. After acidifying with $2N\text{-H}_2\text{SO}_4$, the monophenols were separated from the solution by steam distillation and taken up in ether, and the dihydric phenols extracted from the residue with ether.

The monophenol extract gave a deep blue colour with Gibb's reagent at pH 8–14 and a red colour with Brentamine Fast Red B salt. These tests indicated the presence of 3:5- or 2:6-dichlorophenol or both. Chromatography in solvent system *B* revealed two spots of R_F 0.85 and 0.93 not present in normal rabbit urine. Spraying with diazotized sulphanilic acid and Brentamine Fast Red B salt, showed the spot of R_F 0.85 to be 2:4-dichlorophenol and the one with R_F 0.93 to be 3:5-dichlorophenol. The 2:6- isomer was not present. The presence of these two phenols was confirmed by coupling the extract with diazotized *p*-nitraniline before chromatography. Chromatography in solvent system *B* gave two buff spots with R_F 0.75 (2:4- compound) and 0.81 (3:5-compound) (cf. Table 1).

Chromatography of the dihydric phenol fraction in solvent system *A* revealed two spots (FeCl_3 spray), one of R_F 0.51 corresponding to 3:5-dichlorocatechol and a weak one of R_F 0.33 corresponding to 4-chlorocatechol. When 'impure *m*-DCB' was used, the spot corresponding to 4-chlorocatechol was much more intense. Chromatography in this and other systems failed to reveal the presence of any 2:6-dichloroquinol.

Isolation of 2:4-dichlorophenol. The urine from three rabbits which had received 1.5 g. each of 'impure *m*-DCB' was collected for 4 days, made $3N$ with respect to HCl and heated under reflux for 3 hr. The phenols were extracted with ether as above. The monophenol fraction was separated by steam distillation. The 3:5-dichlorophenol, as indicated by the red colour given by the distillate with Brentamine Fast Red B salt, appeared in the first fractions, but we failed to obtain a derivative of it. The phenols in the distillate were converted into toluene-*p*-sulphonates (1.72 g. or 19% of the dose) and recrystallization from ethanol yielded 2:4-dichlorophenyl toluene-*p*-sulphonate, m.p. and mixed m.p. 119° . (Found: Cl, 22.7. Calc. for $\text{C}_{13}\text{H}_{10}\text{O}_3\text{Cl}_2\text{S}$: Cl,

22.4%.) In a second experiment 1.45 g. (25% of the dose of 3 g.) of 2:4-dichlorophenyl toluene-*p*-sulphonate were obtained.

Working up the monophenol fraction chromatographically using powdered cellulose did concentrate the 3:5-isomer, but no pure derivative of it could be isolated.

3:5-Dichlorocatechol. The phenol fraction not volatile in steam was chromatographed on cellulose powder (Whatman, standard grade) in solvent system *A*. Two catechol bands were formed. These were eluted with the same solvent and the catechols in each transferred to a little $2N\text{-NaOH}$. Each fraction was then treated with toluene-*p*-sulphonyl chloride and the sulphonates formed isolated in the usual manner. From the first band a small quantity of crystalline material was isolated which on repeated crystallization from ethanol and ethanol-ethyl acetate mixtures yielded a few crystals of 3:5-dichlorocatechol ditoluene-*p*-sulphonate, m.p. and mixed m.p. 143° . The second fraction which presumably contained 4-chlorocatechol yielded no crystalline material.

The mercapturic acid fraction. The urine of rabbits fed with 0.5 g./kg. of 'pure *m*-DCB' (total 3 g.) was collected for 6 days and adjusted to pH 2 with $2N\text{-H}_2\text{SO}_4$. It was then continuously extracted with ether for 8 hr. The extract was washed with water and the carboxylic acids present transferred to saturated NaHCO_3 . The bicarbonate extract was washed twice with ether to remove any contaminating phenols and then acidified and the free acids were transferred to ether. The ether extract was chromatographed using solvent system *B* (Table 1) and acid spots were detected with the buffered methyl red-bromophenol blue indicator of Fewster & Hall (1951). The extract gave one spot of R_F 0.61. No crystalline mercapturic acid was isolated, but the fraction, on alkaline hydrolysis, acidification and steam distillation, yielded a thiophenol (30 mg. or 8% of dose) m.p. $15\text{--}20^\circ$ (2:4-dichlorothiophenol has m.p. 22°) and R_F 0.03 in solvent system *B* (see Table 1). On oxidation with ethanolic iodine, 2:4:2':4'-tetrachlorodiphenyldisulphide, m.p. 75° was obtained. On recrystallization the m.p. was raised to 84° , not depressing the m.p. of the authentic disulphide, m.p. 84° . (Found: Cl, 39.2. Calc. for $\text{C}_{12}\text{H}_6\text{Cl}_4\text{S}_2$: Cl, 39.8%.) These results show that the mercapturic acid is 2:4-dichlorophenylmercapturic acid.

Experiments with monochlorobenzene

The dihydric phenol fraction. Three rabbits were fed 1.5 g. each of chlorobenzene and the urine was collected for 5 days. One-half of the urine was hydrolysed by refluxing for 3 hr. with 0.5 vol. of concentrated HCl . After cooling, the urine was extracted with ether continuously for 8 hr. The phenols in the extract were transferred to $2N\text{-NaOH}$. The alkaline extract was acidified and monophenols removed by steam distillation. The non-volatile phenols in the residue were then extracted with ether. This ether extract was then used for paper chromatography in solvent system *A* (see Table 1). One catechol only was detected of R_F 0.33, which corresponds to 4-chlorocatechol. No other dihydric phenol was demonstrable in amounts greater than 0.2% of the dose (the limit of detection).

The mercapturic acid fraction. The other half of the above urine was acidified to pH 2 with $2N\text{-H}_2\text{SO}_4$ and continuously extracted with ether for 16 hr. Carboxylic acids in the extract were extracted into $2N\text{-Na}_2\text{CO}_3$ and this was re-extracted with ether to remove any phenols. The solution was then acidified and the carboxylic acids were transferred

to ether. Paper chromatography of this ether extract in solvent system *B* (see Table 1) at 22° revealed one spot (R_F 0.59) detected by the buffered methyl red-bromophenol blue indicator mixture. This result did not permit us to decide whether *o*-, *m*- or *p*-chlorophenylmercapturic acid or all three were present.

o- and *m*-Chlorophenylmercapturic acids are more soluble in water than the *para* isomer (Parke, 1955), hence these isomers if they occurred in small amounts would be readily lost on recrystallization of the mercapturic acid fraction from chlorobenzene urine. However, on alkaline hydrolysis these acids are converted into the chlorothiophenols, which can be recovered almost quantitatively by steam distillation. The presence of small amounts of *o*- and *m*-chlorothiophenol in *p*-chlorothiophenol are readily detected, since the former are liquids at ordinary temperatures and affect the m.p. of the *para* isomer. For example, a mixture of 10 mg. *o*- and 100 mg. *p*-chlorophenylmercapturic acid was hydrolysed with 5 ml. *N*-NaOH for 0.5 hr. at 100°. After acidification and steam distillation the chlorothiophenol isolated had m.p. 45–50° (pure *para* isomer, m.p. 54°) and on oxidation gave a disulphide, m.p. 55–60° raised to 67–68° on recrystallization from ethanol (4:4'-dichlorodiphenyldisulphide has m.p. 71°).

The ether extract of mercapturic acids from chlorobenzene urine was evaporated to dryness and the residue hydrolysed with 10 ml. *N*-NaOH for 0.5 hr. at 100°. The hydrolysate was acidified and steam distilled. The solid product (0.4 g.) was filtered off and dried. It had m.p. 54° (without recrystallization) not depressing the m.p. of 4-chlorothiophenol, m.p. 54°. On oxidation with ethanolic iodine it yielded pure 4:4'-dichlorodiphenyldisulphide, m.p. and mixed m.p. 72° without recrystallization. Paper chromatography of the thiophenol fraction (see Table 1) yielded one

spot of R_F 0.60, which excludes the *ortho* isomer (R_F 0.50). We conclude therefore that chlorobenzene urine contains no detectable *o*- or *m*-chlorophenylmercapturic acid.

RESULTS

The quantitative results are expressed in Table 3. Measurable metabolites of *m*-dichlorobenzene cease to be excreted in 5 days after dosing, but in this time not more than half the dose can be accounted for as total conjugates (Table 3). The major metabolites are glucuronides and ethereal sulphates which reach their peak excretion usually on the first day after dosing. On hydrolysis the urine yields 2:4- and 3:5-dichlorophenols and 3:5-dichlorocatechol. The first is the major phenol and accounts for at least 20 % of the dose. 3:5-Dichlorophenol is a relatively minor metabolite. 2:4-Dichlorophenylmercapturic acid and 3:5-dichlorocatechol are also minor metabolites and are excreted in greatest amount on the first day after dosing. Both catechol and mercapturic acid excretion continue until the fifth day.

DISCUSSION

The metabolites of *o*- and *m*-dichlorobenzenes are qualitatively similar but quantitatively completely different from that of monochlorobenzene (see Table 4). With the mono- compound, catechol and mercapturic acid are major metabolites, whereas with the *o*- and *m*-dichloro- compounds they are only formed in small amounts. *p*-Dichlorobenzene

Table 3. *Excretion of metabolites of m-dichlorobenzene*

Dose fed 0.5 g./kg. wt. of rabbit. Figures refer to percentage of dose excreted during 5 days after dosing. Figures in parentheses are mean values. Superior figures indicate the number of experiments.

	Glucuronide	Ethereal sulphate	Mercapturic acid	Total conjugates	Catechols	Dichlorophenols	
						2:4-	3:5-
'pure <i>m</i> -DCB'	38	8	8.3	54	2.1	24	4.6
	30	7	10.6	48	2.9	20	3.0
	40	6	13.8	60	2.9	19	3.2
	(36)	(7)	(10.9)	(54)	(2.6)	(21)	(3.6)
'impure <i>m</i> -DCB'	30-37 (32)*	9-16 (12)*	8.8-8.9 (8.9)*	49-53 (51)*	3.7-4.7 (4.1)*	—	—

Table 4. *Excretion of metabolites of dichlorobenzenes by rabbits*

Dose fed was 0.5 g./kg. body wt. Results are expressed as percentage of fed dose.

Metabolite	Dichlorobenzene			Monochlorobenzene*
	<i>ortho</i>	<i>meta</i>	<i>para</i>	
Glucuronide	48	36	36	25
Ethereal sulphate	21	7	27	27
Mercapturic acid	5	11	0	20
Total conjugates	74	51	63	72
Monophenols	39	25	35	3
Catechols	4	3	0	27
Quinols	0	0	ca. 6	—
Period of excretion	6† days	5† days	6‡ days	3 days

* From Azouz *et al.* (1952, 1953) and Spencer & Williams (1950).

† Excretion of metabolites apparently complete.

‡ Excretion of metabolites not complete in 6 days.

looked for dihydrodiols but failed to isolate any. However, it may be recalled that a dihydrodiol of monochlorobenzene has been isolated from urine (Smith, Spencer & Williams, 1950).

In the case of *p*-dichlorobenzene the formation of a 1:4-dihydro-1:4-diol (IV) would explain the metabolites better than a 1:2-dihydro-1:2-diol, since a quinol is formed rather than a catechol, thus:

Direct hydroxylation, however, could explain these reactions of the *para* compound equally well. It is to be noted that quinols were not detected as metabolites of *o*- and *m*-dichlorobenzenes.

It is to be expected that the output of total phenols would be equal to the sum of the glucuronide and ethereal sulphates. But an examination of Table 4 shows that the total phenols fall short of the total *O*-conjugates (*ortho*: *O*-conjugates, 69 %, phenols, 43 %; *meta*: *O*-conjugates, 43 %, phenols, 28 %; *para*: *O*-conjugates, 63 %, phenols, ca. 41 %). We believe these differences are due in part to loss of phenols during acid hydrolysis possibly due to their interaction with a variety of compounds which occur in urine, and also to the errors in the methods of estimating glucuronic acid and ethereal sulphates which are liable to an error of ± 10 %.

Orientation of the mercapturic acids

When monochlorobenzene is fed to animals the mercapturic acid excreted contains an *N*-acetylcysteine group which is *para* to the chlorine atom. This is also true for fluoro-, bromo- and iodo-benzene. An examination of the mercapturic acid fraction of chlorobenzene urine from rabbits failed to reveal any of the *ortho* or *meta* isomer. The small mercapturic acid fraction of *o*-dichlorobenzene contains only one such acid and this has been proved to be 3:4-dichlorophenylmercapturic acid by conversion into 3:4:3':4'-tetrachlorodiphenyl-disulphide, which was isolated and identified. (The mercapturic acid is probably of the L- series, since all naturally formed mercapturic acids belong to this configuration.)

Similarly, it has been proved that *m*-dichlorobenzene yields only 2:4-dichlorophenylmercapturic acid, and this in small amounts. Since *p*-dichlorobenzene does not yield a mercapturic acid, it appears that, in the mono- and di-chlorobenzene series, acetylcysteine conjugation only occurs in a free position which is *para* to a chlorine atom. Furthermore, it appears that the extent of this conjugation

diminishes as the number of chlorine atoms substituted into the benzene ring increases (at least up to three) (see also Table 4), since other work in this laboratory has shown that the three isomeric trichlorobenzenes do not result in the excretion of appreciable amounts of mercapturic acid, although free *para* positions are available in all three (Jondorf, Parke & Williams, unpublished results). Mercapturic acid formation in polychlorinated benzenes however is known to occur, since Bray, Hybs, James & Thorpe (1953) found 2:3:5:6-tetrachloronitrobenzene to yield 2:3:5:6-tetrachlorophenylmercapturic acid in large amounts in rabbits, the nitro group being eliminated. Other work in this laboratory has shown that 1:2:4:5-tetrachlorobenzene does not form a mercapturic acid in rabbits in amounts greater than 0.8 % of the dose, which is the limit of our method. This fact makes the mechanism of mercapturic acid formation from 2:3:5:6-tetrachloronitrobenzene highly interesting and suggests substitution of the nitro group by acetylcysteine (cf. Bray *et al.* 1953).

SUMMARY

1. *m*-Dichlorobenzene is oxidized in rabbits mainly to 2:4-dichlorophenol which is excreted conjugated with glucuronic and sulphuric acid. Small amounts of conjugated 3:5-dichlorophenol and 3:5-dichlorocatechol are also excreted.

2. *m*-Dichlorobenzene is excreted to a minor extent as 2:4-dichlorophenylmercapturic acid.

3. The excretion of these metabolites is slow and is virtually complete in about 5 days after dosing.

4. The mercapturic acid formed from monochlorobenzene in rabbits has been carefully examined and it has been shown to contain only the *para* isomer; the *o*- and *m*-chlorophenylmercapturic acids are not formed.

5. These results have been compared with those from chlorobenzene and *o*- and *p*-dichlorobenzene and discussed.

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Studies in Detoxication

64. THE SYNTHESIS OF THE ISOMERIC DICHLOROPHENYLMERCAPTURIC ACIDS

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Recent work in this laboratory on the metabolism of the *o*-, *m*- and *p*-dichlorobenzenes in rabbits (Azouz, Parke & Williams, 1953, 1955; Parke & Williams, 1955) has shown that mercapturic acids are minor metabolites (*ca.* 5% of the dose) of the *ortho* and *meta* isomers. In order to identify these acids, the synthesis of the six isomeric L-dichlorophenylmercapturic acids became necessary. The present paper describes the synthesis and properties of these compounds and the corresponding dichlorophenyl-L-cysteines. At the same time, the mercapturic acid fraction obtained after feeding chlorobenzene to rabbits was examined chromatographically to ascertain whether the *o*- and *m*-chlorophenylmercapturic acids were present in addition to the principal metabolite, *p*-chlorophenylmercapturic acid (Spencer & Williams, 1950*a, b*). The *o*- and *m*-chlorophenylmercapturic acids and the corresponding chlorophenyl-L-cysteines were therefore synthesized. Since the completion of this work, a paper by West & Mathura (1954) on the synthesis of these latter compounds has appeared.

EXPERIMENTAL

Melting points are corrected.

Materials. L-Cystine was purified by dissolving 10 g. in 200 ml. $N-H_2SO_4$, filtering, and reprecipitating with a saturated solution of ammonium acetate. After 1 hr. at 0°, the cystine was filtered off, washed with water, and dried in air. 2-Chloroaniline (b.p. 209°) and 3-chloroaniline (b.p. 230°) were purchased and purified by distillation. 2,3-Dichloroaniline (m.p. 24°), 2,4-dichloroaniline (m.p. 64°), 2,5-dichloroaniline (m.p. 52°), and 3,4-dichloroaniline (m.p. 72°) were purchased and were purified by distillation

in steam. 2,6-Dichloroaniline (m.p. 39°) was prepared from 3:5-dichlorosulphanilamide as described by Seikel (1944). 3:5-Dichloroaniline (m.p. 50°) was prepared from 3:5-dichloronitrobenzene (Roberts & Turner, 1927) according to Dyson, George & Hunter (1926), and was purified by steam distillation.

Preparation of dichlorophenylmercapturic acids. The isomeric *S*-dichlorophenyl-L-cysteines were synthesized from L-cystine (5 g.) and the corresponding dichloroanilines (7.5 g.) according to Parke & Williams (1951). Improved yields were obtained if the solution of cysteine cuprous mercaptide was filtered hot, to remove traces of copper oxides, before cooling to 0° and coupling with the diazotized dichloroanilines. The *S*-dichlorophenyl-L-cysteines (yield, 0.5–1.2 g.) were isolated by repeated extraction with 50% aqueous ethanol, in which cystine is sparingly soluble. These compounds were acetylated in pyridine with acetic anhydride to the corresponding mercapturic acids (Zbarsky & Young, 1943), which were then recrystallized from water or aqueous ethanol. A more efficient procedure for the isolation of pure dichlorophenyl-L-cysteines in quantity was to acetylate the crude dichlorophenylcysteine–cystine mixture to give the L-dichlorophenylmercapturic acid (yield, 1.5–4.5 g.) which was isolated, purified and subsequently hydrolysed with acid to the corresponding dichlorophenyl-L-cysteine.

Acid hydrolysis of dichlorophenylmercapturic acids. The pure L-dichlorophenylmercapturic acids (1 g.) were hydrolysed to the corresponding *S*-dichlorophenyl-L-cysteines by heating under reflux with 50 ml. $2N-H_2SO_4$ for 1–2 hr. 2:5-Dichlorophenylmercapturic acid was more resistant to acid hydrolysis than the other isomers, and 50 ml. of $5N-H_2SO_4$ was used in this case. The hydrolysed solutions were neutralized with $2N$ ammonia, and then adjusted to pH 5 with $2N$ acetic acid. The precipitated dichlorophenylcysteines were filtered off and recrystallized from aqueous ethanol. In no instance was there any evidence of racemization of the dichlorophenyl-L-cysteines, since the products obtained by acid hydrolysis of the mercapturic acids were