The observation that one type of antiovalbumin is produced first and a different type later is reminiscent of the results found by Kekwick & Record (1941) in an electrophoretic study of horse serum during immunization with diphtheria toxoid. The antitoxin originally confined to the γ-globulin later appeared in a new β_2 or T fraction. Variation in distribution of antibodies with different characteristics, against different antigens has been observed in studies of rabbit sera by electrophoresis-convection (Cann, Campbell, Brown & Kirkwood, 1951) and of human sera by zone electrophoresis (Kuhns, 1954), but there was no evidence of progressive changes during immunization with the same antigen. The diversity of antibodies has been discussed by Kabat (1953). It remains to be investigated whether any immunological distinction, such as combining ratio, can be detected between the two chromatographically different types of antiovalbumin found in this work.

SUMMARY

1. Rabbit γ-globulin has been fractionated by partition chromatography using the two liquid phases produced by a mixture of potassium phosphate, water and ethyl and butyl cellosolves, with Celite 545 used to hold the stationary phase.

2. γ-Globulin from normal rabbits appeared to be a complex mixture of very similar components, none of which could be isolated individually.

3. Immune γ-globulin could be fractionated to give a partial separation of inert globulin from antibody.

4. One type of antiovalbumin appeared to be formed in the earlier stages of immunization, to be largely replaced by a chromatographically distinct second type as immunization continued.

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Studies in Detoxication

62. THE METABOLISM OF HALOGENOBENZENES. *ORTHO*- AND *PARA*-DICHLOROBENZENES*

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Both *o*- and *p*-dichlorobenzenes are employed as insecticides. The *ortho* compound is used chiefly in soil against subterranean termites and to protect wood against beetles. The *para* compound is employed on a very large scale against the clothes moth and against insects infesting hides, furs and museum specimens, and also as a deodorant in block form (Shepard, 1951). *o*-Dichlorobenzene also has numerous other industrial uses, and a review of its toxic effects, which on the whole are relatively mild, has been given by Browning (1953). Little, however, is known about the metabolism of these

* Part 61, Robinson & Williams (1955).

compounds in animals. According to Callow & Hele (1926) the *ortho* isomer forms in dogs a mercapturic acid whose constitution was not determined. The *para* isomer, on the other hand, does not form a mercapturic acid (Baumann, 1883). More recently we have shown that *o*-dichlorobenzene yields 4:5-dichlorocatechol in the rabbit (Azouz, Parke & Williams, 1953), but we shall show in this paper that catechol formation is not a major path of metabolism of this compound. This is in contrast with monochlorobenzene, which yields 4-chlorocatechol as a major metabolite in rabbits (Smith, Spencer & Williams, 1950; Azouz *et al.* 1953).

EXPERIMENTAL

Materials and reference compounds

All melting points are corrected.

o-Dichlorobenzene, b.p. 178–179°, was purified according to Cohen & Hartley (1905). The following compounds were prepared by standard methods: 2:3-dichlorophenol, m.p. 57° (Holleman, 1917) and its *benzoate*, m.p. 81°, needle from ethanol (Found: C, 58·4; H, 3·2; Cl, 26·5. $C_{13}H_8O_2Cl_2$ requires C, 58·4; H, 3·0; Cl, 26·6%), and *toluene*-p-*sulphonate*, m.p. 95°, needles from methanol (Found: C, 49·0; H, 3·2. $C_{13}H_{10}O_3Cl_2S$ requires C, 49·2; H, 3·2%); 3:4-dichlorophenol, m.p. 71° (Holleman, 1917), and its *benzoate*, m.p. 97°, needles from ethanol (Found: C, 58·5; H, 3·0. $C_{13}H_8O_2Cl_2$ requires C, 58·4; H, 3·0%), and *toluene*-p-*sulphonate*, m.p. 111°, plates from ethanol (Found: C, 49·2; H, 3·2. $C_{13}H_{10}O_3Cl_2S$ requires C, 49·2; H, 3·2%); 4:5-dichlorocatechol, m.p. 105° (Peratoner, 1898); 2:3-dichloroquinol, m.p. 145° (Conant & Fieser, 1923).

3:4-Dichlorocatechol was synthesized as follows. 2:3-Dichlorophenol was converted into 3:4-*dichlorosalicylaldehyde* as described by Duff (1941) for the preparation of *o*-hydroxyaldehydes. The aldehyde, m.p. 94°, formed yellow needles from ethanol (yield 20%). (Found: C, 44·3; H, 2·0; Cl, 36·8. $C_7H_4O_2Cl_2$ requires C, 44·0; H, 2·1; Cl, 37·1%.) The 2:4-*dinitrophenylhydrazone* formed red needles from ethanol and yellow prisms from dioxan, both having m.p. 305–306° (decomp.). (Found: N, 15·6; Cl, 19·0. $C_{13}H_8O_5N_4Cl_2$ requires N, 15·1; Cl, 19·1%). The aldehyde (0·6 g.) in N-NaOH (5 ml.) was treated with 4 ml. of 20 vol. H_2O_2 (2 moles) (cf. Dakin, 1909.) The mixture darkened and became hot. After an hour, the cooled mixture was acidified with 2N-H_2SO_4 and then treated with a slight excess of saturated $NaHCO_3$ solution. The dichlorocatechol was extracted with ether, the ethereal solution dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue (0·5 g.) was recrystallized from light petroleum (b.p. 60–80°), whereby 3:4-*dichlorocatechol* was obtained as colourless needles, m.p. 99°. (Found: C, 40·1; H, 2·4; Cl, 39·7. $C_6H_4O_2Cl_2$ requires C, 40·3; H, 2·3; Cl, 39·6%.) It gave the typical catechol colour reactions with aqueous $FeCl_3$, being green in neutral and blue in $NaHCO_3$ solution. Its *ditoluene*-p-*sulphonate* formed colourless rhombs, m.p. 132°, from ethanol. (Found: Cl, 15·1. $C_{20}H_{16}O_6Cl_2S_2$ requires Cl, 14·6%.)

For the identification of the mercapturic acid of *o*-dichlorobenzene, 3:4-dichlorothiophenol and 3:4:3′:4′-tetrachlorodiphenyldisulphide were prepared. Synthetic 3:4-dichlorophenylmercapturic acid (0·2 g.) (Parke, 1955) in 2N-NaOH (5 ml.) was heated under reflux for 0·5 hr. The solution was acidified with 2N-H_2SO_4 and the thiophenol separated by steam distillation. 3:4-Dichlorothiophenol (0·1 g.) was obtained as a colourless solid, m.p. 28° after crystallization from ethanol. (Found: Cl, 39·9. $C_6H_4Cl_2S$ requires Cl, 39·6%.) The thiophenol (0·06 g.) in ethanol (5 ml.) was oxidized with ethanolic iodine solution and the mixture poured into water (10 ml.). The white precipitate of 3:4:3′:4′-*tetrachlorodiphenyldisulphide* was recrystallized from ethanol and formed colourless needles, m.p. 83–84°. (Found: C, 40·8; H, 1·9; Cl, 39·9. $C_{12}H_6Cl_4S_2$ requires C, 40·5; H, 1·7; Cl, 39·8%.)

p-Dichlorobenzene, m.p. 53°, was purified by recrystallization from ethanol. The following reference compounds were made: 2:5-dichlorophenol, m.p. 58° (Noelting & Kopp, 1905), and its benzoate, m.p. 68° (Holleman, 1917); 2:5-dichloroquinol, m.p. 168°, and its dibenzoate, m.p. 186° (Levy & Schultz, 1881).

Methods

Animals. Chinchilla rabbits, kept on a diet of 60 g. of rat cubes (diet 41; Associated London Flour Millers) and 100 ml. water per day, were used throughout this work. The dichlorobenzenes were administered by stomach tube, the *ortho* compound suspended in water and the *para* compound as 25% (w/v) solution in olive oil. Urine was collected daily.

Analytical methods. The urine was analysed daily for glucuronic acid by the modified naphthoresorcinol method of Paul (1951), ethereal sulphate by the turbidimetric method of Sperber (1948), total catechols according to Azouz *et al.* (1953) and mercapturic acid by the iodine titration method of Stekol (1936).

Determination of 2:3- *and* 3:4-*dichlorophenols.* 2:3-Dichlorophenol gives a red colour ($\lambda_{max.}$ 500 mμ.) when coupled with Brentamine Fast Red B salt (I.C.I. Ltd.; diazo salt from 5-nitro-*o*-anisidine with naphthalene 1:5-disulphonic acid) in alkaline solution. 3:4-Dichlorophenol does not give a red colour with this reagent, but could be estimated spectrophotometrically by a determination of the difference between its acid and alkaline spectra at 244 mμ., a correction being made for any 2:3-dichlorophenol present. The spectra of these phenols are shown in Fig. 1.

The urines from rabbits fed with *o*-dichlorobenzene were collected daily and each diluted to 200 ml. with water. The diluted urine (10 ml.) mixed with 5 ml. conc. HCl was heated under reflux for 3 hr. and the mixture then steam distilled, 100 ml. of distillate being collected. To 20 ml. of the distillate, containing <1 mg. of 2:3-dichlorophenol, 2 ml. of a freshly prepared aqueous solution of Brentamine Fast Red B salt (1 mg./ml.) were added, followed by 2 ml. of 2N-NH_4OH. The solution was mixed, diluted to 25 ml. with water and the colour density measured at 500 mμ. in a Unicam Spectrophotometer SP. 500. A blank was prepared by substituting 20 ml. water for the steam distillate. The standard curve for 0·1–1·0 mg. of the phenol in 25 ml. solution was a straight line. This procedure determined the 2:3-dichlorophenol present. The 3:4-dichlorophenol was estimated by diluting two 5 ml. portions of the distillate to 25 ml. with 0·1N-HCl and 0·1N-NaOH respectively, measuring the difference in absorption of the two solutions at 244 mμ. and then correcting for the absorption at 244 mμ. due to 2:3-dichlorophenol (ϵ at 244 mμ. being 8250 in 0·1N-NaOH and 200 in 0·1N-HCl, see Fig. 1). Blank estimations for 2:3- and 3:4-dichlorophenol were carried out on normal rabbit urines for several days before dosing

with *o*-dichlorobenzene. Using this method, the recovery, for example of 5 mg. of 2:3- and 25 mg. of 3:4-dichlorophenol added simultaneously to 20 ml. rabbit urine, was about 104±5% and 92±5% respectively.

Determination of 2:5-dichlorophenol. This steam-volatile phenol shows a maximum absorption at 241 mμ. ($\epsilon_{max.}$ 8500) in 0·1 N-NaOH. In 0·1 N-HCl the ε value at 241 mμ. is 500 (see Fig. 2 for spectra). The urines of rabbits which had received *p*-dichlorobenzene were collected daily and diluted to 200 ml. with water. The diluted urine (10 ml.) was mixed with 5 ml. conc. HCl, heated under reflux for 3 hr. and then steam distilled, 100 ml. of distillate being collected. Two 5 ml. portions of the steam distillate were then diluted to 25 ml. with 0·1 N-NaOH and 0·1 N-HCl respectively and the extinction at 241 mμ. was measured. The amount of 2:5-dichlorophenol excreted per day was then calculated from the expression

$$\text{mg. phenol/day} = 203{\cdot}75\ (E_{alk.} - E_{acid}),$$

where $E_{alk.}$ and E_{acid} are the observed extinctions in NaOH and HCl respectively and the factor 203·75 includes the dilutions, the mol.wt. of the phenol and the ε values in acid and alkali. Analyses were carried out on normal rabbit urine for 4 days before administering the *p*-dichlorobenzene, and normal urines contained the equivalent of 6–11 mg. dichlorophenol/day. The recovery of 100 mg. of 2:5-dichlorophenol added to 100 ml. rabbit urine was 93±5%.

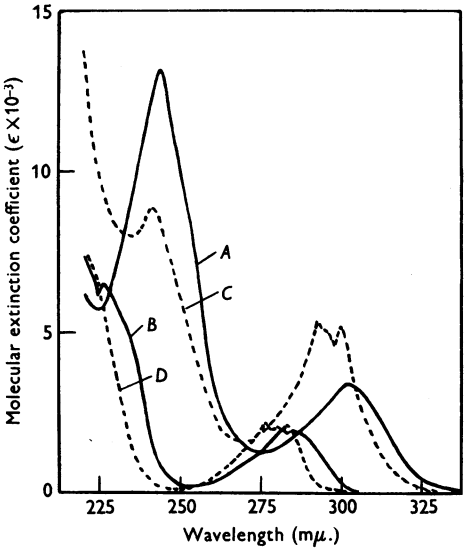

Fig. 1. The absorption spectra of 2:3- and 3:4-dichlorophenols. *A*, 3:4-Dichlorophenol in 0·1 N-NaOH ($\lambda_{max.}$ 244, 302 mμ.; $\epsilon_{max.}$ 13 250, 3250); *B*, 3:4-dichlorophenol in 0·1 N-HCl ($\lambda_{max.}$ 226, 282, 285 mμ.; $\epsilon_{max.}$ 6500, 1950, 1900); *C*, 2:3-dichlorophenol in 0·1 N-NaOH ($\lambda_{max.}$ 241, 292, 300 mμ.; $\epsilon_{max.}$ 8800, 5250, 5000); *D*, 2:3-dichlorophenol in 0·1 N-HCl ($\lambda_{max.}$ 277, 280, 283 mμ.; $\epsilon_{max.}$ 2150, 2000, 2050).

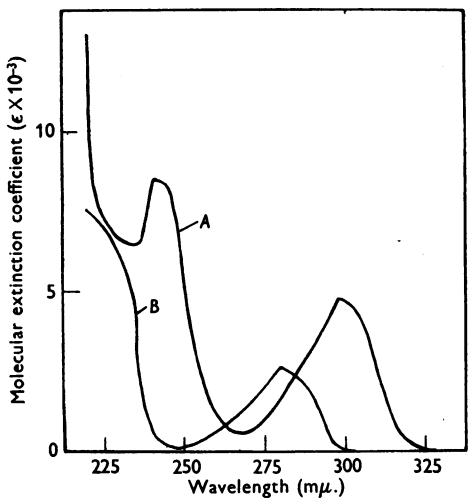

Fig. 2. The absorption spectra of 2:5-dichlorophenol. *A*, in 0·1 N-NaOH ($\lambda_{max.}$ 241, 298 mμ.; $\epsilon_{max.}$ 8500, 4800) and *B* in 0·1 N-HCl ($\lambda_{max.}$ 280 mμ., $\epsilon_{max.}$ 2600).

Chromatography

o-Dichlorobenzene metabolites. The possible phenolic metabolites of *o*-dichlorobenzene are 2:3- and 3:4-dichlorophenol, 3:4- and 4:5-dichlorocatechol and 2:3-dichloroquinol. The monophenols could be separated from the polyphenols by steam distillation, and therefore it was not necessary to separate all five phenols on the same chromatogram. Two solvent systems were used: *A*, benzene–acetic acid–water (1:1:2, v/v) and *B*, the *n*-butanol–ethanol–ammonia–ammonium carbonate buffer system of Fewster & Hall (1951). The two monophenols were not separated satisfactorily in these solvents, but the 2:3-dichlorophenol could be detected by colour reactions (see Tables 1 and 2). The three polyphenols were readily separated in both systems and detected by the colour reactions given in Table 2. The convenient amounts of the phenols used for the chromatograms were of the order of 50 μg.

Two mercapturic acids, namely 2:3- and 3:4-dichlorophenylmercapturic acids, are also possible metabolites. The pure compounds could be separated in the solvent system *B* (see Table 1). They were detected by spraying the paper with the buffered methyl red–bromophenol blue indicator of Fewster & Hall (1951) and they showed up as pink spots on a grey background. Proof of the nature of the mercapturic acid was obtained by other means (see below).

Table 1. *R_F values of dichlorophenols and dichlorophenylmercapturic acids*

Descending chromatography on Whatman no. 1 paper. In solvent *A* (benzene–acetic acid–water, 1:1:2, v/v) R_F 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) 16 hr.

Compound	R_F values in solvent *A*	*B*
2:3-Dichlorophenol	—	0·84
3:4-Dichlorophenol	—	0·95
3:4-Dichlorocatechol	0·54	0·62
4:5-Dichlorocatechol	0·42	0·52
2:3-Dichloroquinol	0·38	—
2:5-Dichlorophenol	0·93	—
2:5-Dichloroquinol	0·33	—
2:3-Dichlorophenylmercapturic acid	—	0·70
3:4-Dichlorophenylmercapturic acid	—	0·75

Table 2. *Colour reactions of certain chlorinated phenols*

Phenols, 10–100 μg., spotted on Whatman no. 1 filter paper and the paper sprayed with the solutions described below. Figures in parentheses are pH values.

Compound	Gibb's reagent*	Brentamine Fast Red B† salt	Ferric chloride‡	Diazotized *p*-nitraniline§	Ammoniacal $AgNO_3$
2:3-Dichlorophenol	Blue-green (8–14)	Red	—	Orange	—
3:4-Dichlorophenol	None (<12) blue-green slowly (14)	None	—	Orange	—
3:4-Dichlorocatechol	Blue (9)	None	Green, neutral blue, $NaHCO_3$	—	—
4:5-Dichlorocatechol	Blue (9)	None	Green, neutral violet, $NaHCO_3$	—	Slight brown
2:3-Dichloroquinol	Blue (9)	None	Brown	Green→yellow	Brown
2:5-Dichlorophenol	Blue-green (8–14)	Red	—	Red	—
2:5-Dichloroquinol	Violet (8)	—	Brown	Orange-brown	Brown

* 2% ethanolic solution of 2:6-dichloroquinonechloroimide followed by saturated $NaHCO_3$ for pH 8; saturated borax solution for pH 9; Na_2CO_3 for pH > 10.
† 0·1% aqueous solution of Brentamine Fast Red B salt followed by 2N ammonia.
‡ 1% aqueous $FeCl_3$, followed by saturated $NaHCO_3$.
§ Diazotized *p*-nitraniline followed by 2N-Na_2CO_3 (cf. Bray, Thorpe & White, 1950).

p-Dichlorobenzene metabolites. Preliminary tests had shown that mercapturic acids and catechols were not metabolites. 2:5-Dichloro-phenol and -quinol were readily separated chromatographically (see Table 1).

Isolation of metabolites

o-Dichlorobenzene

2:3- and 3:4-Dichlorophenols. *o*-Dichlorobenzene (1·25 g.) was fed to each of two rabbits and a 48 hr. urine collected. The urine was refluxed for 3 hr. with 0·5 vol. of conc. HCl. After cooling, the urine was continuously extracted with ether for 8 hr. The extract was evaporated and the residue steam distilled. The steam-volatile phenols were extracted from the distillate with benzene, which on evaporation left 1·2 g. of crude phenols. On benzoylation, the phenols yielded 0·7 g. (35% of dose) of crystalline benzoate, which on recrystallization from ethanol gave 3:4-dichlorophenyl benzoate, m.p. 96° and mixed m.p. 97°. (Found: C, 58·8; H, 3·0%.)

The 2-day urine of four rabbits which had collectively received 6 g. of *o*-dichlorobenzene was hydrolysed and the phenols were collected by steam distillation as above. 3:4-Dichlorophenol is less volatile in steam than its 2:3- isomer, which distils first. The crude phenols were therefore steam distilled until the distillate gave only a weak colour for the 2:3- isomer with Brentamine Fast Red B salt. This steam distillation was repeated 10 times and the phenol finally extracted into ether. The phenol was transferred to 2N-NaOH (5 ml.) and the solution treated with toluene-*p*-sulphonyl chloride (0·2 g.) in acetone (5 ml.). On pouring the product into water, 2:3-dichlorophenyl toluene-*p*-sulphonate was obtained (20 mg.), m.p. and mixed m.p. 93° after repeated recrystallization from ethanol and finally methanol. (Found: C, 49·1; H, 3·1%.)

The catechol fraction. 4:5-Dichlorocatechol has already been proved by isolation to be a metabolite of *o*-dichlorobenzene (Azouz *et al.* 1953). Paper chromatography, however, showed that 3:4-dichlorocatechol, but not 2:3-dichloroquinol, was also present. The urine of three rabbits which had collectively received 4·5 g. of *o*-dichlorobenzene was collected for 6 days. One-third of the urine was hydrolysed by heating for 3 hr. under reflux with 0·5 vol. of conc. HCl. The liberated phenols were separated by continuous extraction with ether for 8 hr. The extract was washed with water and the phenols transferred to 2N-NaOH. The alkaline solution was acidified and the monophenols were removed by steam distillation. The phenols not volatile in steam were now extracted from the residue into ether and then chromatographed in solvent systems *A* and *B* (see Table 1). Spraying with 2% aqueous $FeCl_3$ revealed two catechol spots, R_F 0·54 and 0·42 in the first solvent and 0·62 and 0·52 in the second. The faster spot corresponded to 3:4-dichlorocatechol and the other to the 4:5- isomer. From the size of the spots it was estimated that roughly 1% of the dose was appearing as 3:4- and 4% as 4:5-dichlorocatechol. (In the earlier publication (Azouz *et al.* 1953) it was reported that from *o*-dichlorobenzene urine, 4:5-dichlorocatechol ditoluene-*p*-sulphonate, m.p. 201° and an unidentified toluene-*p*-sulphonate of m.p. 98° were isolated. The latter ester has now been examined and appears to be a mixture of the esters of 3:4- and 2:3-dichlorophenol. On repeated recrystallization from ethanol it yielded pure 3:4-dichlorophenyl toluene-*p*-sulphonate, m.p. and mixed m.p. 111°.) Derivatives of 3:4-dichlorocatechol were not isolated in a pure state.

The mercapturic acid fraction. The urine (1900 ml.) of four rabbits which had collectively received 4 g. of *o*-dichlorobenzene was collected for 4 days. It was adjusted to pH 2 with 2N-H_2SO_4 and extracted continuously with ether for 16 hr. The extract and deposited gum were then extracted with saturated $NaHCO_3$, whereby phenols were left behind in the ether. The bicarbonate extract was then acidified with 2N-H_2SO_4 and the mercapturic acid transferred to chloroform (three extractions). Paper chromatography of this extract in solvent system *B* revealed one spot with R_F 0·75 corresponding to 3:4-dichlorophenylmercapturic acid (see Table 1). Attempts to crystallize the acid failed. The mercapturic acid fraction was therefore evaporated to dryness and the residue hydrolysed by refluxing for 0·5 hr. with 10 ml. 2N-NaOH. The solution was acidified with 2N-H_2SO_4 and the 3:4-dichlorothiophenol separated by steam distillation. The colourless crystalline thiophenol (60 mg. or 1·2% of the dose) was collected. It had m.p. 26° and did not

Table 3. *Quantitative excretion of metabolites of* o- *and* p-*dichlorobenzenes*

These results were obtained on a group of three rabbits studied simultaneously. Dose fed was 0·5 g./kg. body wt. Figures refer to percentage of dose excreted during 5–6 days after dosing; mean values are given in parentheses.

Compound fed	Glucuronide G	Ethereal sulphate E	Mercapturic acid M	Total conjugates (G+E+M)	Catechols	Dichlorophenols 2:3-	3:4-	2:5-
o-Dichlorobenzene	42	23	6·0	71	4·9	7·9	29	—
	51	15	3·2	69	2·2	9·8	29	—
	50	25	5·7	81	4·7	8·1	30	—
	(48)	(21)	(5·0)	(76)	(3·9)	(8·9)	(30)	—
p-Dichlorobenzene	32	30	0	62	0	—	—	35
	43	20	0	63	0	—	—	34
	34	30	0	64	0	—	—	36
	(36)	(27)	—	(64)	—	—	—	(35)*

* In addition to 2:5-dichlorophenol, about 6% of the dose of *p*-dichlorobenzene was excreted as 2:5-dichloroquinol.

depress the m.p. of the authentic compound. (Found: Cl, 39·5. Calc. for $C_6H_4Cl_2S$: Cl, 39·6%.) On oxidation of the thiophenol with ethanolic iodine solution and diluting with water, 3:4:3′:4′-tetrachlorodiphenyldisulphide was obtained as a colourless solid, m.p. and mixed m.p. 83°. (Found: Cl, 39·5. Calc. for $C_{12}H_6Cl_4S_2$: Cl, 39·8%.) These results show that *o*-dichlorobenzene is partly metabolized to 3:4-dichlorophenylmercapturic acid.

p-*Dichlorobenzene*

2:5-Dichlorophenol and 2:5-dichloroquinol. The urine of three rabbits, each of which had been given 1·5 g. of *p*-dichlorobenzene dissolved in olive oil, was collected for 6 days. A portion of the urine (equivalent to a dose of 4·1 g. *p*-dichlorobenzene) was heated under reflux with 0·3 vol. of conc. HCl for 3 hr. The urine was cooled and continuously extracted with ether for 8 hr. The phenols in the extract were transferred to N-NaOH, which was then acidified with 2N-H_2SO_4 and steam distilled. 2:5-Dichlorophenol, m.p. and mixed m.p. 56° was collected from the distillate by filtration (yield, 1·85 g. or 40% of dose). (Found: Cl, 43·1. Calc. for $C_6H_4OCl_2$: Cl, 43·5%.) 2:5-Dichlorophenyl benzoate was prepared and had m.p. and mixed m.p. 67°. (Found: Cl, 26·3. Calc. for $C_{13}H_8O_2Cl_2$: Cl, 26·6%.)

The aqueous residue after steam distillation was continuously extracted with ether for 8 hr. Evaporation of the ether extract left a dark brown residue. This was dissolved in 20 ml. 2N-NaOH and treated with 1 ml. benzoyl chloride. From the product there was isolated 2:5-dichloroquinol dibenzoate, m.p. and mixed m.p. 186° after crystallization from ethanol–benzene. (Found: Cl, 19·2. Calc. for $C_{20}H_{12}O_4Cl_2$: Cl, 18·3%.) The yield was 0·1 g. or 2·5% of the dose.

RESULTS

The quantitative results are summarized in Table 3. The major metabolites of both *o*- and *p*-dichlorobenzenes (70% for *o*- and 60% for *p*- in 6 days) are *O*-conjugates containing glucuronic and sulphuric acids. These conjugates reach a maximum on the first day after dosing with *o*-dichlorobenzene and on the second day with the *para* isomer, possibly owing to slower absorption of the latter. The *para* isomer, however, could not be detected in the faeces eliminated during the 6 days after dosing. The excretion of detectable metabolites appears to be complete with the *ortho* isomer in 6 days after dosing but with the *para* isomer the excretion of metabolites is still appreciable after this time.

The *O*-conjugates of *o*-dichlorobenzene yielded on acid hydrolysis 2:3- and 3:4-dichlorophenols and 3:4- and 4:5-dichlorocatechols. 3:4-Dichlorophenol is the major phenol (at least 30% of the dose) and reaches its peak excretion on the first day after dosing, whereas the 2:3-phenol (about 9% of the dose) reaches its peak on the second day (this was shown to occur in six different animals). Thereafter both phenols are excreted in approximately equal amounts. The catechols produced are minor metabolites (4% of the dose) and their peak excretion occurs on the first day after dosing and they cease to be measurable on the third day. Mercapturic acid excretion also reaches a peak on the first day after dosing and ceases to be measurable on the fifth day; the mercapturic acid is a minor metabolite (5% of the dose).

In the case of *p*-dichlorobenzene, only glucuronides and ethereal sulphates are excreted. There is no catechol or mercapturic acid excretion. Two phenols are produced namely 2:5-dichlorophenol which is the major one (35% of the dose) and 2:5-dichloroquinol which may account for about 6% of the dose (rough estimate from paper chromatograms). The glucuronide, ethereal sulphate and 2:5-dichlorophenol excretion reach their maximum on the second day after dosing.

The results in this paper are discussed, together with those of *m*-dichlorobenzene, in the succeeding paper (Parke & Williams, 1955).

SUMMARY

1. *o*-Dichlorobenzene, when fed to rabbits (0·5 g./kg.), is mainly oxidized to 3:4-dichlorophenol, which is excreted conjugated with glucuronic and sulphuric acids. Conjugates of 2:3-dichlorophenol, 4:5-dichlorocatechol and 3:4-dichlorocatechol are also excreted as minor metabolites.

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 sulphonated than *m*-dichlorobenzene. The 'impure *m*-DCB' (25 ml.) was heated on the water-bath with conc. H_2SO_4 (25 ml.) for 8 hr. with constant shaking (during this period equal volumes of chlorobenzene and conc. H_2SO_4 formed a single phase). The mixture was poured into water and the hydrocarbon layer, washed with water, dried over anhydrous Na_2SO_4 and distilled. The 'pure *m*-DCB' had b.p. 172–173°, d_4^{20} 1·288 and n_D^{22} 1·544 (literature values, b.p. 172°, d_4^{20} 1·288 and n_D^{21} 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. $C_{13}H_{10}O_3Cl_2S$ 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