reaction may be more readily standardized because the time of heating is not so critical as in the method of Thomas. Further, the time of heating is sufficiently long to allow equilibration between the boiling-water bath and the content of the test tubes, so that differences in wall thickness are not important. It is also unnecessary, since the colour is stable, to take readings within 8 min. of completion of the reaction. The reaction is thus easier to handle and more reproducible than the earlier method.

The present method is also preferable to that of Udenfriend & Cooper (1952) since it avoids extraction of the excess of nitrosonaphthol by ethylene dichloride. Recoveries also seem to be more complete by our method.

As far as specificity is concerned our method does not differ from those of Thomas (1944), Giral (1944) and Udenfriend & Cooper (1952). The only compound which could seriously interfere appears to be tyramine, which is commonly absent from protein hydrolysates.

SUMMARY

1. An improved method for the quantitative determination of tyrosine by the Gerngross–Voss–Herfeld (1933) reaction is described. Optimum experimental conditions were selected.

2. The solution of α-nitroso-β-naphthol was made in aqueous 0·1N-sodium hydroxide. The exclusion of ethanol from the reaction allowed stabilization of the colour.

3. Linearity of colour response was obtained with solutions containing between 1 and 16 μg. of tyrosine/ml.

4. The method can be used to estimate the tyrosine content of proteins.

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The Metabolism of the Trichloronitrobenzenes in the Rabbit

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In the rabbit the labile nitro group of pentachloronitrobenzene and of 2:3:4:6- and 2:3:5:6-tetrachloronitrobenzene is replaced by an acetylcysteyl group with the formation of a mercapturic acid (Betts, James & Thorpe, 1955*b*; Bray, Hybs, James & Thorpe, 1953), whereas in certain of the dichloronitrobenzenes mercapturic acid formation occurs by replacement of a labile chlorine atom (Bray, James & Thorpe, 1957). It was, therefore, of interest to examine the metabolic fate of the trichloronitrobenzenes with particular reference to mercapturic acid formation and its relationship to the lability of the chlorine atoms and nitro groups present. A preliminary account of the metabolism of three of these compounds has been given (Bray, James & Thorpe, 1955). In this paper a detailed account of the fate of the six isomers in the rabbit is presented.

MATERIALS

Melting points are uncorrected.

The trichloronitrobenzenes were prepared according to the method of Holleman & Haeften (1921). The melting points of the isomers were: 2:3:4-, 53°; 2:3:5-, 44–45°; 2:3:6-, 87–88°; 2:4:5-, 57°; 2:4:6-, 69°; 3:4:5-, 70–71°. The corresponding anilines were prepared by reduction of the nitro compounds with Sn and HCl in ethanol; melting points agreed with those given in the literature. 2:3:4-Trichloroaniline was acetylated to give 2:3:4-trichloroacetanilide, m.p. 131° [Beilstein & Kurbatow (1879) gave 120–122°] (Found: N, 5·8. Calc. for $C_8H_6ONCl_3$: N, 5·9%). 4-Bromo-3:5-dichloronitrobenzene, m.p. 88°, was prepared by the method of Flürscheim & Simon (1908).

2:4:5:2′:4′:5′- and 3:4:5:3′:4′:5′-*Hexachloroazoxybenzene*, m.p. 170° and 226° respectively, were prepared by refluxing in absolute ethanol for 0·5 hr. the corresponding trichlorophenylhydroxylamines, which were obtained by the method of Haworth & Lapworth (1921) (Found for 2:4:5-isomer Cl, 52·4. $C_{12}H_4ON_2Cl_6$ requires Cl, 52·6%).

2:4:6-*Trichloro*-3-*nitrophenol*, m.p. 69–70°, was prepared by the method of Daccomo (1885) (Found: N, 5·7. $C_6H_2O_3NCl_3$ requires N, 5·8%). Daccomo gave no analysis. Reduction with sodium dithionite gave 3-amino-2:4:6-trichlorophenol, m.p. 95° as found by Daccomo. (This compound, prepared by a different method, was characterized as the acetyl derivative by Jacobs, Heidelberger & Rolf, 1919.) 4-Amino-3:5-dichlorophenol, m.p. 154°, was prepared by the reduction with sodium dithionite of 3:5-dichloro-4-nitrophenol obtained by the method of Hodgson & Wignall (1927).

2:3:6:2′:3′:6′-*Hexachlorodiphenyl disulphide* was synthesized from 2:3:6-trichloroaniline by the method of Jondorf, Parke & Williams (1955). The yield was 1% of colourless

needles, m.p. 105° after recrystallization from aqueous ethanol (Found: C, 34·2; H, 1·0; Cl, 49·9; S, 15·2. $C_{12}H_4Cl_6S_2$ requires C, 33·9; H, 0·95; Cl, 50·1; S, 15·1%). 2:4:6:2′:4′:6′-Hexachlorodiphenyl disulphide, m.p. 173°, was prepared by the method of Jondorf *et al.* (1955).

Mercapturic acids were synthesized by the method of Parke & Williams (1951) as modified by Bray, James & Thorpe (1956). The difficulty of preparation of 2:3-dichloro-6-nitroaniline made an attempt at synthesis of *N*-acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-L-cysteine impracticable. *N-acetyl*-S-(2:4-*dichloro*-6-*nitrophenyl*)-L-*cysteine* was obtained from 2:4-dichloro-6-nitroaniline, m.p. 99°, prepared by the chlorination of *o*-nitroaniline with $KClO_3$ (cf. Holleman, 1904), N-*acetyl*-S-(4:5-*dichloro*-2-*nitrophenyl*)-L-*cysteine* from 4:5-dichloro-2-nitroaniline, m.p. 176°, prepared by the method of Beilstein & Kurbatow (1879), N-*acetyl*-S-(2:5-*dichloro*-4-*nitrophenyl*)-L-*cysteine* from 2:5-dichloro-4-nitroaniline, m.p. 153°, prepared by the method of Holleman & Haeften (1921), and N-*acetyl*-S-(2:6-*dichloro*-4-*nitrophenyl*)-L-*cysteine* from 2:6-dichloro-4-nitroaniline, m.p. 189–190°, prepared by the method of Holleman (1904). The properties of these acids are given in Table 1 and the solvents used for their recrystallization in Table 3. The 4-nitro isomers gave an intense yellow colour when heated with N-NaOH; the 2-nitro isomers gave only a feeble colour.

The product first obtained from 4:5-dichloro-2-nitroaniline did not analyse correctly for *N*-acetyl-*S*-(4:5-dichloro-2-nitrophenyl)-L-cysteine; the values for C and H were high and those for N, Cl and S were low. The same product was obtained biosynthetically from the urine of rabbits dosed with 2:4:5-trichloronitrobenzene. The identity of the products from the synthetic and biosynthetic routes made it difficult to believe that the compounds were not mainly the expected mercapturic acid. The properties were not changed by several recrystallizations from aqueous acetone. As evidence for the identity of the two preparations there was close agreement between the ultraviolet and infrared spectra, the optical rotations, the X-ray powder photographs and the behaviour on paper chromatograms. [The evidence of mixed m.p. was of doubtful value since there was no depression of m.p. of either preparation on admixture with *N*-acetyl-*S*-(2:5-dichloro-4-nitrophenyl)-L-cysteine.] Comparison of the infrared spectra with that of *N*-acetyl-*S*-(2:4-dichloro-6-nitrophenyl)-L-cysteine supported the results of the analyses for acetyl, which were in accord with a monoacetyl derivative. The discrepancy between the found and the expected elementary analyses was finally traced to the inclusion of some of the acetone used for recrystallization. By heating the compound for 10 hr. at 100° the loss in weight was 7·1%; $(C_{11}H_{10}O_5N_2Cl_2S)_2,C_3H_6O$ requires 7·6%. The unheated compound gave a positive nitroprusside test for acetone (Feigl, 1947) but the heated compound did not. Neither *N*-acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-L-cysteine nor *N*-acetyl-*S*-(2:5-dichloro-4-nitrophenyl)-L-cysteine after recrystallization from aqueous acetone gave a positive nitroprusside test. The infrared spectrum of the heated compound was not significantly different from that of the original compound, which for this measurement had been dried in high vacuum at room temp. to remove last traces of water. This treatment evidently removed much of the acetone although the original drying of the compounds for analysis at 65° at 15 mm. did not. When the infrared spectrum of the original compound was measured by the Nujol technique,

Table 1. *Properties of some* N-*acetyl*-S-*(dichloronitrophenyl)*-L-*cysteines*

Isomer	Source	Cryst. form	M.p.	$[\alpha]_D^{20}$ in ethanol		Absorption spectrum in 0·1 N-KOH			Inflexion	Analysis						
					(*c*)	$\lambda_{max.}$ (mμ)	$\epsilon_{max.}$	$\lambda_{min.}$ (mμ)	(mμ)	C (%)	H (%)	N (%)	Cl (%)	S (%)	$H_3C{\cdot}CO{\cdot}$ (%)	Equiv.
2:3-Dichloro-6-nitro	Urine	Yellow needles	163–164°	+58±5°	0·41	264	6 131	246	—	37·6	3·0	7·9	20·1	9·1	—	347
2:4-Dichloro-6-nitro	Urine	Yellow needles	159–160	+75±5	0·35	263	5 699	248	227	37·6	2·7	8·1	20·4	8·8	13·3	351
	Synthesis		159	+74±5	0·54	263	5 187	248	227	—	—	—	—	—	—	—
2:5-Dichloro-4-nitro	Synthesis	Buff elongated plates	176	+6±3	0·81	340	7 620	281	256	37·6	2·9	8·0	20·7	9·0	—	352
4:5-Dichloro-2-nitro	Synthesis	—	174	+67±5	0·30	255	18 290	231	—	37·6	2·8	7·3	20·5	9·6	—	327
						382	3 261	328								
Acetone complex of 4:5-dichloro-2-nitro*	Urine	Yellow needles	175	+64±5	0·44	255	18 400	231	—	39·1	3·4	7·3	18·3	8·1	11·4	370
						385	3 390	328								
	Synthesis		174	+67±5	0·41	255	20 200	231	—	38·7	3·8	7·3	18·2	8·0	12·2	390
						386	3 720	328								
2:6-Dichloro-4-nitro	Urine	Buff needles	180–182	+54±5	0·40	260	6 583	241	—	37·2	3·0	8·1	20·0	9·5	—	360
						335	5 140	298								
	Synthesis		178	+53±5	0·30	260	6 639	241	—	—	—	—	—	—	—	—
						335	5 148	298								
						$C_{11}H_{10}O_5N_2Cl_2S$ requires				37·4	2·8	7·9	20·1	9·1	12·2	353
						$(C_{11}H_{10}O_5N_2Cl_2S)_2,C_3H_6O$ requires				39·3	3·4	7·3	18·6	8·4	11·3	382

* See Materials section.

which avoided drying in high vacuum, two major differences were observed, a peak at 1700 cm.$^{-1}$ attributable to a ketonic C=O and enhanced intensity at 785 cm.$^{-1}$. The heated material did not show these features. It is reasonable to conclude, therefore, that the compounds which had been obtained after recrystallization from aqueous acetone (ethanol was not suitable) crystallized with 0·5 mol.prop. of acetone. The properties of the compounds are given in Table 1.

METHODS

Animals, diet and dosage. The rabbits and diet were as previously described (Bray, Ryman & Thorpe, 1947). The compounds were administered by stomach tube as suspensions in water at a dose level of 0·2, 0·4 or 0·8 g./kg. No toxic effects were observed. The urines were collected every 24 hr. and analysed usually for 3 days after dosing.

Determination of metabolites

Ethereal sulphate. The method of Folin (1905–06) and the turbidimetric method described by Bray & Thorpe (1954) were used.

Glucuronic acid. The method of Hanson, Mills & Williams (1944) as modified by Bray, Humphris, Thorpe, White & Wood (1952) was used. Copper-reducing material was determined as described by Bray, Neale & Thorpe (1946).

Mercapturic acid. The method of Stekol (1936) was modified as described by Betts *et al.* (1955*b*); the hydrolysis was effected with 2N-NaOH. The recoveries of *N*-acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-, *N*-acetyl-*S*-(2:4-dichloro-6-nitrophenyl)-, *N*-acetyl-*S*-(4:5-dichloro-2-nitrophenyl)- and *N*-acetyl-*S*-(2:6-dichloro-4-nitrophenyl)-L-cysteine added to urine were 60, 80, 33 and 39% (all ±5%) respectively. The last acid was also determined by the colorimetric method of Bray *et al.* (1956); the recovery of the acid added to urine was 95±2%.

Catechols. The method of Azouz, Parke & Williams (1953) was used with 4-chlorocatechol as standard.

Trichloroanilines. 2:3:4-, 2:3:5-, 2:4:5- and 3:4:5-Trichloroaniline were separated by steam-distillation of urine adjusted to pH 10 (free base from urine as collected and total base from urine after hydrolysis for 1 hr. with N-H_2SO_4 in a boiling-water bath) and were then determined by diazotization and coupling with *N*-(1-naphthyl)ethylenediamine (Bratton & Marshall, 1939). The recoveries of the bases added to urine were 100, 89, 94 and 100% (all ±5%) respectively. 2:4:5-Trichloroaniline was also determined (recovery 94%) by the Bratton & Marshall method applied directly to urine since these urines contained only very small amounts of aminophenols. 2:3:6- and 2:4:6-Trichloroaniline were determined by weighing the solid which separated from the steam-distillate and estimating the amount in the supernatant by the method of Bratton & Marshall (1939).

Examination of faeces

The 72 hr. faeces were examined for unabsorbed material present both as nitro compound and as the corresponding base. 2:3:6-, 2:4:6- and 2:4:5-Trichloronitrobenzene were determined polarographically (Bray *et al.* 1953). 2:3:4-, 2:3:5- and 3:4:5-Trichloronitrobenzene were separated by steam-distillation and determined after reduction to the corresponding base. All the trichloroanilines were determined in steam-distillates of the faeces.

Examination of urines

Isolation of metabolites. The urine was acidified (pH 1) and continuously extracted with ether. The extract was successively extracted with N-$NaHCO_3$ and 0·5N-NaOH and, after appropriate adjustment of pH, these aqueous extracts were extracted with ether; from these ether extracts (acid and phenol fractions), mercapturic acids and phenols respectively were separated; the trichloroanilines were isolated from the residual ether (base fraction). Alternatively, the bases were separated by steam-distillation as described above. Azoxy compounds, which occurred with the base, could be separated by their low solubility in ethanol. The acid fractions from urines of animals dosed with 2:3:6- and 2:4:6-trichloronitrobenzene were hydrolysed for 1 hr. with 2N-NaOH; the solution was then acidified and the thiophenol was separated by steam-distillation and oxidized with ethanolic iodine to the hexachlorodiphenyl disulphide.

The compounds listed in Table 3 were isolated from the expected acid, phenol, or base fraction unless stated otherwise. Melting points were not depressed by admixture with authentic samples.

Paper chromatography. Extracts A, B, C and D were prepared from the urines as described by Bray *et al.* (1956) and examined for bases, aminophenols and nitrophenols, and mercapturic acids, by the ascending method as described by Bray, Thorpe & White (1950). The R_F values of the reference compounds are given in Table 4.

RESULTS

Except with 3:4:5-trichloronitrobenzene, less than 1% of the dose was found in the faeces and this was present as trichloroaniline. About 4% of a dose of the 3:4:5-isomer was unabsorbed; part of this had not been reduced to the aniline. The average daily urinary excretions of normal metabolites by the rabbits used were of the same order as those found previously (e.g. Bray *et al.* 1953). The quantitative results obtained after administration of the trichloronitrobenzenes are summarized in Table 2 which shows that a satisfactory proportion of the dose has been accounted for with all except the 2:4:6-isomer, of which only 65% was traced. All except this isomer formed considerable amounts of mercapturic acid. The increase in the excretion of glucuronic acid, as measured by the naphthoresorcinol method, after feeding each isomer was always accompanied by an increase in the reducing value of the urine which corresponded to 60–100% of the glucuronic acid. The values for catechols excreted after dosage with 2:3:4- and 2:3:6-trichloronitrobenzene are imprecise since the correct reference compounds were not available as standards. Since the presence of these catechols was not confirmed by their detection or isolation, the amounts have not been included in the totals accounted for.

A more detailed examination of the excretion of the metabolites of 2:3:6- and 2:4:5-trichloronitrobenzene was made by analysis of successive urine

Table 2. *Excretion of metabolites of the trichloronitrobenzenes by the rabbit*

Results are expressed as percentages of the dose, given as means with ranges in parentheses; superior figures indicate the number of experiments. Urine was collected for 72 hr. except in experiments (indicated by superior m) where separate consecutive samples of urine, which were collected from rabbits given water at intervals (see Bray *et al.* 1951), were analysed until excretion of metabolites could no longer be detected.

Isomer	2:3:4-	2:3:5-	2:3:6-		2:4:5-			2:4:6-		3:4:5-
Dose (g.)	1·0	1·0	1·0	2·0	1·0	1·0	2·0	1·0	2·0	1·0
Metabolite										
Unabsorbed										
Trichloronitrobenzene	0	0	—	0	—	—	0	—	0	0·8
Trichloroaniline	0·6	0·2	—	Tr.	—	—	Tr.	—	Tr.	3
Mercapturic acid	41 (22–60)^{6}	21 (14–43)^{5}	10^{m} (7, 14)^{2}	8 (3–12)^{9}	30^{m}	—	15 (0–30)^{13}	0^{3}	<1 (0–5)^{8}	23* (12–42)^{8}
Glucosiduronic acid	28 (16–42)^{6}	36 (18–75)^{5}	67^{m} (59, 76)^{2}	67 (50–90)^{6}	41^{m}	42 (31, 53)^{2}	36 (18–51)^{10}	30 (25, 35)^{2}	33 (10–56)^{6}	26 (17–41)^{8}
Copper-reducing material (as glucuronic acid)	27 (14–45)^{6}	25 (17–45)^{6}	38^{m} (30, 45)^{2}	—	35^{m}	34 (27, 41)^{2}	—	18 (15, 21)^{2}	—	19 (11–30)^{6}
Ethereal sulphate	11 (8–16)^{6}	11 (8–13)^{3}	7^{m} (7, 7)^{2}	9 (1–23)^{6}	9^{m}	—	4 (0–11)^{10}	3 (0, 5)^{2}	2 (0–5)^{7}	13 (8–18)^{6}
Trichloroaniline, free	11 (5–21)^{8}	13 (7–24)^{7}	19^{m} (27, 10)^{2}	8	31^{m}	21	25	10	29	23 (15–28)^{8}
Trichloroaniline, combined	4	3	—	0		8	6	22		2
'Catechols'	4 (3, 5)^{2}	—	<2^{m}	—	—	—	—	—	—	—
Azoxy compound	—	—	—	—	—	1†	—	—	—	1†
Total accounted for (excluding 'catechols') and reducing material	96	84	103^{m}	92	111^{m}	106	86	65	65	92

* The colorimetric method gave 17 (14–20)^{6} for 1·0 g. dose and 18 (9–24)^{6} for 0·5 g. dose.
† By isolation.

samples as described by Bray, Thorpe & White (1951). The results of an experiment with 2:3:6-trichloronitrobenzene are shown in Fig. 1. There would appear to be considerable delay in absorption since no significant percentage of the dose is excreted until after 5 hr. (cf. Betts, Bray, Thorpe & White, 1955*a*). As soon as there is a sufficient excretion of metabolites to eliminate the possibility of large errors due to fluctuation in excretion of normal metabolites (after about 8 hr.) the ratio of glucuronic acid to reducing value is approximately constant. This suggests a labile glucosiduronic acid, and it is perhaps significant that the trichloroaminophenols detected were obtained from unhydrolysed acidified urine (see Table 4).

Metabolites isolated or detected

The metabolites isolated and the yields obtained are listed in Table 3. The properties of the mercapturic acids are given in Table 1. The metabolites detected by paper chromatography in extracts A, B, C and D of the urines are listed in Table 4. Although some of the aminophenols detected could not be compared with authentic synthetic specimens, structures can be attributed to them with fair certainty on the basis of chemical reactions and the fact that only two isomers are possible from the asymmetrical compounds and only one from the symmetrical 2:4:6- and 3:4:5-trichloronitrobenzenes. The chemical tests used were the bright-yellow diazo oxide colour at the nitrous acid stage of the Bratton & Marshall (1939) test, indicating an *o*-aminophenol, and the indophenol test (Thorpe, Williams & Shelswell, 1941), indicating a *p*-aminophenol. Only the following metabolites require comment.

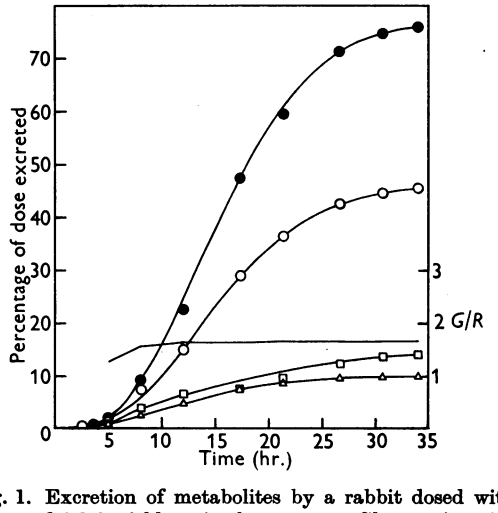

Fig. 1. Excretion of metabolites by a rabbit dosed with 1 g. of 2:3:6-trichloronitrobenzene. ●, Glucuronic acid; ○, reducing material calculated as glucuronic acid; □, mercapturic acid; △, trichloroaniline. The curve without experimental points shows the values (amount of glucuronic acid/amount of reducing material) (*G*/*R*).

From 2:3:4-*trichloronitrobenzene*. The amount of aminophenol isolated was insufficient for an elementary analysis. It gave a positive Folin & Ciocalteu reaction and an intense yellow colour with nitrous acid. It is, therefore, probable that this was the *o*-aminophenol, 2-amino-3:4:5-trichlorophenol. There was no evidence for the presence of 5-amino-2:3:4-trichlorophenol.

From 2:3:5-*trichloronitrobenzene*. The aminophenol was isolated as 2:3:5-*trichloro*-6-*hydroxyacetanilide* (Found: N, 5·3. $C_8H_6O_2NCl_3$ requires N, 5·5%). The crystals gave a positive Folin & Ciocalteu reaction and, after hydrolysis with 2N-HCl, a bright yellow colour with nitrous acid. This identification is supported by the detection of the other possible isomer, the *p*-aminophenol, by paper chromatography (Table 4).

From 2:3:6-*trichloronitrobenzene*. 2:3:6-Trichloroaniline was obtained as a precipitate when the urine was acidified (pH 1). This material did not contain sulphur. No mercapturic acid was isolated from the syrupy acid fraction, but paper chromatography revealed a sulphur-containing zone corresponding to R_F 0·95 in solvent *A*. No nitro compounds (yellow spots) were detected in this zone. In another experiment alkaline hydrolysis of the acid fraction and oxidation of the product (see Methods) yielded 2:3:6:2′:3′:6′-hexachlorodiphenyl disulphide. The phenol fraction yielded 4-amino-2:3:5-trichlorophenol (Found: C, 33·5; H, 1·8; N, 6·6. $C_7H_4ONCl_3$ requires C, 33·9; H, 1·7; N, 6·6%). It gave an indophenol test for *p*-aminophenols. No other aminophenol was detected. The nitrophenol detected in extract B was 2:3:5-trichloro-4-nitrophenol, for when the phenol was eluted from the chromatogram and reduced it gave only 4-amino-2:3:5-trichlorophenol.

From 2:4:5-*trichloronitrobenzene*. The base fraction contained, in addition to trichloroaniline, 2:4:5:2′:4′:5′-*hexachloroazoxybenzene* (Found: C, 35·7; H, 1·3; N, 6·9. $C_{12}H_4ON_2Cl_6$ requires C, 35·6; H, 1·0; N, 6·9%). No aminophenols were isolated from either unhydrolysed or hydrolysed urine but one *o*-, one *m*- and one *p*-aminophenol were detected by paper chromatography. The *p*-aminophenol was identified as 4-amino-2:5-dichlorophenol by comparison with a synthetic specimen (Bray *et al.* 1957). The colour with diazotized *p*-nitroaniline was pale green. The *o*- and *m*-aminophenols were presumably 2-amino-3:5:6- and 5-amino-2:3:6-trichlorophenol (the only possible aminotrichlorophenols), since the R_F values were different from those of 2-amino-4:5- and of 5-amino-2:4-dichlorophenol (Bray *et al.* 1957). The nitrophenol detected

Table 3. *Metabolites isolated from urine of rabbits given trichloronitrobenzenes or related compounds*

The dose was 1 g./rabbit unless stated otherwise. Melting points and solvents given in parentheses are for the derivative isolated. Abbreviations for solvents used for recrystallization: A, acetone; E, ethanol; L, light petroleum (b.p. 90–100°); P, light petroleum (b.p. 40–60°); W, water.

Compound administered	No. of rabbits	Compound isolated	M.p.	Solvent	Yield (% of dose)
2:3:4-Trichloronitrobenzene	4	2:3:4-Trichloroaniline*	(131°)	(L)	1
	4	2-Amino-3:4:5-trichlorophenol†	154–158°	WA	0·1
	4	*N*-Acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-L-cysteine†	163–164	WA	4
2:3:5-Trichloronitrobenzene	3	2:3:5-Trichloroaniline	71–72	P	3
	3	2-Amino-3:4:6-trichlorophenol*	(158–162)	(WE)	0·5
	3	*N*-Acetyl-*S*-(2:4-dichloro-6-nitrophenyl)-L-cysteine	159–160	WE	3
2:3:6-Trichloronitrobenzene	6‡	2:3:6-Trichloroaniline	63	E	7
	4	4-Amino-2:3:5-trichlorophenol	141–143	WE	4
	2	2:3:6:2′:3′:6′-Hexachlorodiphenyl disulphide	102	WE	0·1
2:4:5-Trichloronitrobenzene	6‡	2:4:5-Trichloroaniline	94–96	WE	14
	3	2:4:5:2′:4′:5′-Hexachloroazoxybenzene	170	E	1
	6	*N*-Acetyl-*S*-(4:5-dichloro-2-nitrophenyl)-L-cysteine§	(175)	(WA)	8
2:4:6-Trichloronitrobenzene	1	2:4:6-Trichloroaniline	78	WE	11
	4	2:4:6:2′:4′:6′-Hexachlorodiphenyl disulphide	169–171	E	0·05
3:4:5-Trichloronitrobenzene	6	3:4:5-Trichloroaniline	96–98	WE	7
	6	3:4:5:3′:4′:5′-Hexachloroazoxybenzene	226	E	1
	6	6-Amino-2:3:4-trichlorophenol‖	(203)	(WA)	1·3
	6	*N*-Acetyl-*S*-(2:6-dichloro-4-nitrophenyl)-L-cysteine	180–182	WE	2
2:4:6-Trichloroaniline	6	2:4:6-Trichloroaniline	78	WE	44
	6	4-Amino-3:5-dichlorophenol	151–153	W	0·7
4-Bromo-3:5-dichloronitrobenzene	3	*N*-Acetyl-*S*-(2:6-dichloro-4-nitrophenyl)-L-cysteine	180	WE	0·5

* As monoacetyl derivative.
§ As acetone complex.
† Authentic sample not available for mixed m.p.
‖ As diacetyl derivative.
‡ Dose 2 g./rabbit.

was probably 2:3:6-trichloronitro-5-nitrophenol since after reduction it gave the phenol identified as 5-amino-2:3:6-trichlorophenol above.

From 2:4:6-trichloronitrobenzene. 2:4:6-Trichloroaniline was obtained both by acid precipitation and by steam-distillation. Paper chromatography of the syrupy acid fraction indicated the presence of a sulphur-containing metabolite with an R_F value 0·35 in solvent *A* and of 2:4:6-trichloro-3-nitrophenol. The syrup was hydrolysed with 2N-NaOH and the resulting thiophenol was converted into 2:4:6:2′:4′:6′-hexachlorodiphenyl disulphide. The phenol fraction yielded crystals, m.p. 150° after recrystallization from light petroleum (b.p. 90–100°). Insufficient was obtained for a mixed m.p. but it was identified by paper chromatography as

Table 4. *R_F values and detection of metabolites of trichloronitrobenzenes and 2:4:6-trichloroaniline*

Solvent mixtures and times of run on Whatman no. 4 paper: *A*, *n*-butanol–3N-$(NH_4)_2CO_3$–3N-NH_3 soln. (4:3:3 by vol.; Corner & Young, 1954) (2 hr.); *B*, light petroleum (b.p. 90°)–benzene–98% formic acid (3:1:2 by vol.) (1 hr.). Detecting reagents: *a*, 2N-HCl, 0·1% $NaNO_2$, 0·5% ammonium sulphamate and 0·1% *N*-(1-naphthyl)ethylenediamine dihydrochloride (Bratton & Marshall, 1939); *b*, 20% (w/v) Na_2CO_3. Colours of spots: B, blue; M, magenta; O, orange; P, purple; R, red; Y, yellow; d, deep; p, pale; vp, very pale. The compounds in parentheses or brackets were not synthesized. The arguments for the suggested constitutions are given in the Results section.

Metabolites were sought in extracts A, B, C and D (see Methods). Compounds found in extracts of hydrolysed urine (C and D) are probably excreted in combined form. +, Present; (+), present in traces; −, absent.

	R_F values in solvent mixture		Colour with detecting reagent		Detected in extract			
	A	*B*	*a*	*b*	A	B	C	D
Derivatives of 2:3:4-trichloronitrobenzene								
2:3:4-Trichloroaniline	0·92	0·95	M	—	+	+	−	+
(2-Amino-3:4:5-trichlorophenol)	0·81	0·24	vpR*	—	−	+	+	−
[*N*-Acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-L-cysteine]	0·60	0·0	—	pY	−	+	−	−
Derivatives of 2:3:5-trichloronitrobenzene								
2:3:5-Trichloroaniline	0·92	0·97	M	—	+	+	+	+
(2-Amino-3:4:6-trichlorophenol)	0·75	0·74	vpR*	—	−	+	+	(+)
(4-Amino-2:3:6-trichlorophenol)	0·50	0·20	P	—	+	+	+	+
N-Acetyl-*S*-(2:4-dichloro-6-nitrophenyl)-L-cysteine	0·65	0·0	—	pY	−	+	−	−
Derivatives of 2:3:6-trichloronitrobenzene								
2:3:6-Trichloroaniline	0·96	0·98	pOR	—	+	−	−	−
(4-Amino-2:3:5-trichlorophenol)	0·72	0·52	M	—	+	+	−	+
(2:3:5-Trichloro-4-nitrophenol)	0·60	0·60	—	Y	−	(+)	−	−
Derivatives of 2:4:5-trichloronitrobenzene								
2:4:5-Trichloroaniline	0·90	0·94	M	—	+	+	−	+
(2-Amino-3:5:6-trichlorophenol)	0·80	0·91	vpR*	—	−	+	−	−
(5-Amino-2:3:6-trichlorophenol)	0·85	0·5†	M	—	−	+	−	−
4-Amino-2:5-dichlorophenol	0·77	0·0	B	—	(+)	(+)	+	+
(2:3:6-Trichloro-5-nitrophenol)	0·78	0·30	—	Y	(+)	−	−	−
N-Acetyl-*S*-(4:5-dichloro-2-nitrophenyl)-L-cysteine	0·65	0·0	—	Y	−	+	−	−
N-Acetyl-*S*-(2:5-dichloro-4-nitrophenyl)-L-cysteine	0·76	0·0	—	dY	−	−	−	−
Derivatives of 2:4:6-trichloronitrobenzene								
2:4:6-Trichloroaniline	0·94	0·98	pOR	—	+	(+)	−	+
(2:4:6-Trichloroaniline, conjugate)	0·60	0·0	pOR	—	−	+	−	−
3-Amino-2:4:6-trichlorophenol	0·53	0·81	M	—	−	(+)	−	−
4-Amino-3:5-dichlorophenol	0·81	0·15	M	—	(+)	−	−	+
2:4:6-Trichloro-3-nitrophenol	0·68	0·91	—	Y	−	(+)	−	−
3:5-Dichloro-4-nitrophenol	0·81	0·39	—	Y	−	−	−	−
Derivatives of 3:4:5-trichloronitrobenzene								
3:4:5-Trichloroaniline	0·92	0·92	M	—	+	+	(+)	+
(6-Amino-2:3:4-trichlorophenol)	0·74	—	vpR*	—	+	+	−	+
N-Acetyl-*S*-(2:6-dichloro-4-nitrophenyl)-L-cysteine	0·71	0·0	—	dY	−	+	−	−
Derivatives of 2:4:6-trichloroaniline								
2:4:6-Trichloroaniline	0·94	0·98	pOR	—	+	−	−	+
(2:4:6-Trichloroaniline conjugate)	0·60	0·0	pOR	—	−	+	−	−
3-Amino-2:4:6-trichlorophenol	0·53	0·81	M	—	−	−	−	(+)
4-Amino-3:5-dichlorophenol	0·81	0·15	M	—	(+)	+	−	+

* Bright yellow with nitrous acid. † Approximate; spot very elongated.

4-amino-3:5-dichlorophenol. (This compound was detected in urine extracts A and D; see Table 4. It was also isolated from the urine of rabbits given 2:4:6-trichloroaniline; see below.)

From 2:4:6-trichloroaniline. Steam-distillation of urine adjusted to pH 10 yielded 2:4:6-trichloroaniline corresponding to 8% of the dose. The urine was then acidified and immediately a further amount of the base (corresponding to 36% of the dose) separated. The phenol fraction from urine, which had been hydrolysed by boiling with 5N-H_2SO_4 for 1 hr., gave 4-amino-3:5-dichlorophenol.

From 3:4:5-trichloronitrobenzene. From the base fraction *3:4:5:3′:4′:5′-hexachloroazoxybenzene* was separated (Found: C, 35·4; H, 1·3; Cl, 51·2. $C_{12}H_4ON_2Cl_6$ requires C, 35·6; H, 1·0; Cl, 52·6%). The syrupy phenol fraction was acetylated to give colourless needles of *ON-diacetyl-6-amino-2:3:4-trichlorophenol* (Found: N, 4·7. $C_{10}H_8O_3NCl_3$ requires N, 4·7%). Although the crystals gave a positive Folin & Ciocalteu test the colour was much more intense after hydrolysis. The hydrolysed material gave an intense yellow colour with nitrous acid.

DISCUSSION

The trichloronitrobenzenes are almost completely absorbed in the rabbit; a significant amount of unabsorbed material was found only with the 3:4:5-isomer. The compounds all undergo mercapturic acid formation, reduction and hydroxylation, although within the group there is a wide variation in the relative extents to which these three metabolic pathways are used. The probable origins of the metabolites detected are summarized in Fig. 2.

Mercapturic acid formation

In the trichloronitrobenzenes certain of the chlorine atoms are labile. Their positions in the benzene ring and the rate coefficients for their liberation on treatment with sodium methoxide were determined by Holleman & Haeften (1921). These workers also showed that the nitro group of the 2:3:6- and 2:4:6-isomers was liberated as nitrite ion on treatment with sodium methoxide; this lability was confirmed by Betts *et al.* (1955*b*), although the nitro group in these two compounds was considerably less labile than that in 2:3:4:6-tetrachloronitrobenzene. The nitro group of the 2:3:5-isomer is also labile (Table 5). The present investigation shows that, in effect, 2:3:4-, 2:3:5-, 2:4:5- and 3:4:5-trichloronitrobenzene form mercapturic acids by acetylcysteyldechlorination and 2:3:6- and 2:4:6-trichloronitrobenzene undergo acetylcysteyldenitration. The constitution of the acids formed from the 2:3:5- and 3:4:5-isomers is proved by their identity with synthetic specimens of *N*-acetyl-*S*-(2:4-dichloro-6-nitrophenyl)- and *N*-acetyl-*S*-(2:4-dichloro-4-nitrophenyl)-L-cysteine respectively. Further confirmation of the structure of the mercapturic acid formed from 3:4:5-trichloronitrobenzene is provided by the isolation of *N*-acetyl-*S*-(2:6-dichloro-4-nitrophenyl)-L-cysteine from the urine of rabbits given 4-bromo-3:5-dichloronitrobenzene.

In the absence of a synthetic specimen of *N*-acetyl-*S*-(2:3-dichloro-6-nitrophenyl)-L-cysteine the constitution of the mercapturic acid isolated from the urine of rabbits dosed with 2:3:4-trichloronitrobenzene could not be proved by direct comparison. Two observations, however, support the attribution of this structure to the biosynthetic material. Four nitromercapturic acids, in which the acetylcysteyl group is *para* to the nitro group, all have absorption spectra with a peak in the region of 340 mμ, whereas five nitromercapturic acids, in which the acetylcysteyl group is *ortho* to the nitro group, do not show a peak in this region (Table 1; Bray *et al.* 1956, 1957). The mercapturic acid from 2:3:4-trichloronitrobenzene showed no peak at 340 mμ. All the four *p*-nitromercapturic acids give an intense yellow colour when heated in N-NaOH, whereas the five *o*-nitromercapturic acids yield only a feeble colour under these conditions (see this paper and Bray *et al.* 1956, 1957). The mercapturic acid from 2:3:4-trichloronitrobenzene did not give an intense yellow colour when heated with N-NaOH. It was therefore concluded that the acid was formed, in effect, by acetylcysteyldechlorination in the expected position 2, and not in position 4. Substitution of the chlorine in position 3 is most unlikely, not only because the chlorine in the *meta* position to the nitro group is the most stable (see Bunnett & Zahler, 1951) but also because in eight other chloronitrobenzenes, which form mercapturic acids by acetylcysteyldechlorination and which have chlorine in the *meta* position, either an *ortho* or a *para* chlorine is substituted by the acetylcysteyl group (Table 5; Bray *et al.* 1957; unpublished results for 2:3-dichloronitrobenzene and 2:3:4:5-tetrachloronitrobenzene). *m*-Chloronitrobenzene does not form a mercapturic acid (Bray *et al.* 1956).

According to Holleman & Haeften (1921) the most labile chlorine atom in 2:4:5-trichloronitrobenzene is in position 4, although the preparation of 4:5-dichloro-2-nitroaniline from 2:4:5-trichloronitrobenzene by treatment with ethanolic ammonia solution at 200° (Beilstein & Kurbatow, 1879) suggests that the chlorine atom in position 2 is the most readily replaceable. The mercapturic acid isolated from the urine of rabbits dosed with 2:4:5-trichloronitrobenzene was compared with synthetic specimens of both *N*-acetyl-*S*-(4:5-dichloro-2-nitrophenyl)- and *N*-acetyl-*S*-(2:5-dichloro-4-nitrophenyl)-L-cysteine. The ultraviolet-

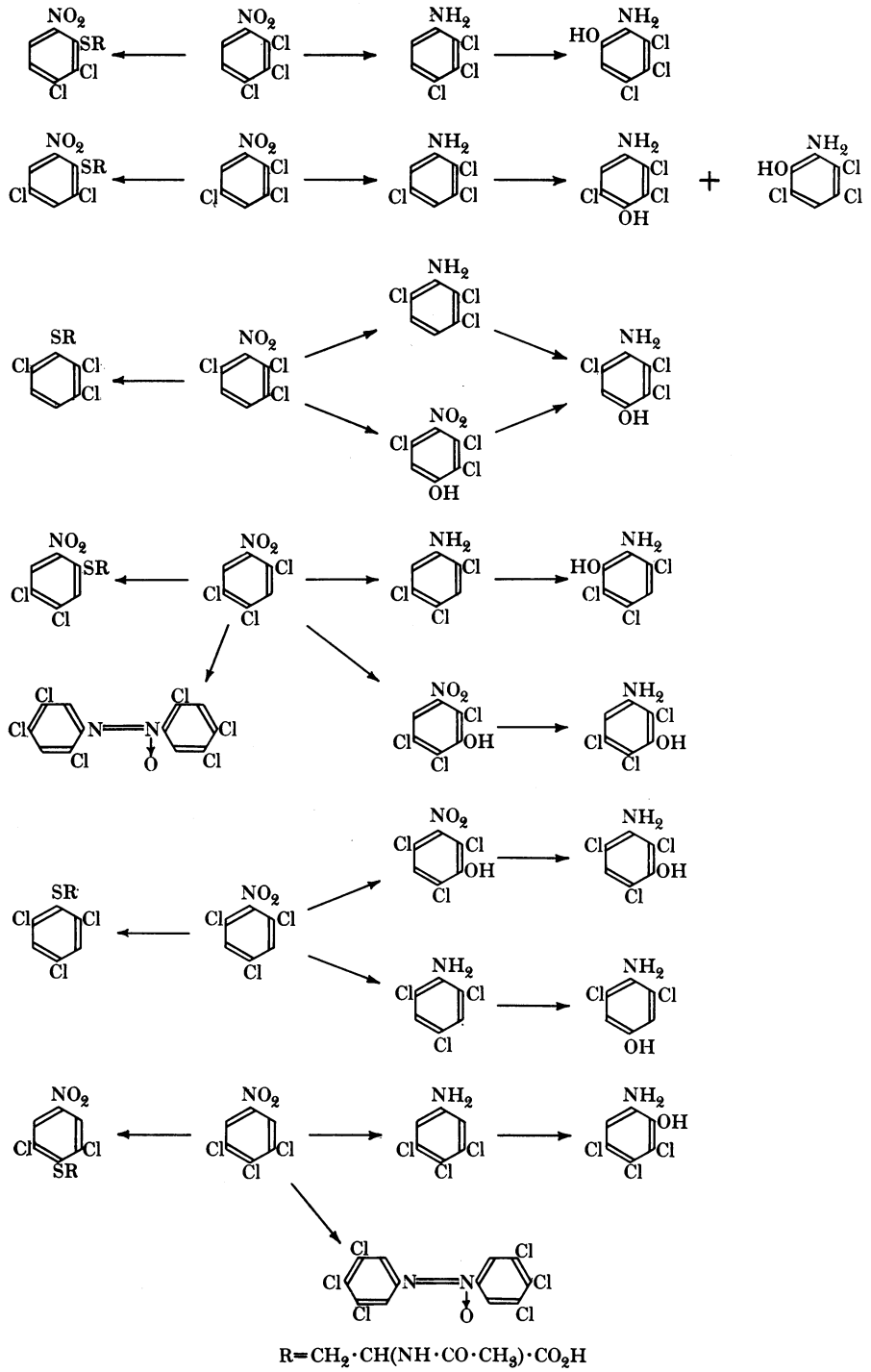

Fig. 2. Probable metabolic pathways of the six trichloronitrobenzenes in the rabbit. 4-Amino-2:5-dichlorophenol was identified as a metabolite of 2:4:5-trichloronitrobenzene, but is not shown in the scheme as there is no evidence to show whether the nitro compound or 2:4:5-trichloroaniline is dechlorinated.

and infrared-absorption spectra and optical rotations left no doubt that the biosynthetic material was the *o*-nitro isomer and quite different from the *p*-nitro isomer. Thus whereas 2:3:4-, 2:3:5- and 3:4:5-trichloronitrobenzene form mercapturic acids, in effect, by acetylcysteyldechlorination at the chlorine atom found to be the most labile by Holleman & Haeften (1921), in 2:4:5-trichloronitrobenzene acetylcysteyldechlorination occurs in position 2 and not in the expected position 4.

It is reasonable to conclude that 2:3:6- and 2:4:6-trichloronitrobenzene, in which even the most labile chlorine atoms have a relatively low lability (Table 5), form mercapturic acids by acetylcysteyldenitration, since 2:3:6:2′:3′:6′- and 2:4:6:2′:4′:6′-hexachlorodiphenyl disulphides respectively were obtained from the urines of dosed animals. The amount of mercapturic acid formed by the 2:4:6-isomer was very small. Paper chromatography failed to reveal a mercapturic acid containing a nitro group in either of the urines; it is, therefore, unlikely that acetylcysteyldechlorination occurs with these two isomers. Although the nitro group in 2:3:5-trichloronitrobenzene is more labile than that in 2:3:6-trichloronitrobenzene, the chlorine in position 2 is evidently so much more readily displaced that acetylcysteyldechlorination occurs to the exclusion of acetylcysteyldenitration. The only compounds for which evidence of acetylcysteyldenitration has been observed are nitrobenzenes which are substituted with chlorine in both *ortho* positions, i.e. pentachloro- and 2:3:4:6-tetrachloronitrobenzene (Betts *et al.* 1955*b*), 2:3:5:6-tetrachloronitrobenzene (Bray *et al.* 1953), 2:3:6- and 2:4:6-trichloronitrobenzene. 2:6-Dichloronitrobenzene does not form a mercapturic acid (unpublished work).

Reduction

All the isomers undergo reduction to the corresponding trichloroaniline. The extent is least (8% of the dose) with 2:3:6-trichloronitrobenzene and all the 2:3:6-trichloroaniline is excreted in the free form. Only small amounts of the 2:3:4-, 2:3:5-, 2:4:5- and 3:4:5-trichloroanilines are excreted in combined form. 2:4:6-Trichloronitrobenzene, however, resembles 2:3:4:6-tetrachloronitrobenzene (Betts *et al.* 1955*b*) in that nearly one-third of a dose is reduced to the corresponding base, of which about two-thirds is excreted as an acid-labile conjugate. When 2:4:6-trichloroaniline was administered, 44% of the dose was excreted as the base but about 80% of this was in the form of the acid-labile conjugate. Azoxy compounds were excreted after administration of 2:4:5- and 3:4:5-trichloronitrobenzene and their origin may be similar to that of 3:4:3′:4′-tetrachloroazoxybenzene formed from 3:4-dichloronitrobenzene (Bray *et al.* 1957).

Hydroxylation

If a trichloroaniline is hydroxylated, it would be expected that the hydroxyl group would occupy positions *ortho* or *para* to the amino group, since this group is the dominant directing group. The *m*-aminotrichlorophenols excreted by rabbits dosed with 2:4:5- and 2:4:6-trichloronitrobenzene are more likely, therefore, to have been formed by reduction of the corresponding nitrophenol rather than by hydroxylation of the aminophenol. This is supported by the detection of these two nitrophenols as metabolites. All the theoretically possible aminotrichlorophenols were detected with the exception of the *m*-aminophenols from 2:3:4- and 2:3:6-trichloronitrobenzene. These two may

Table 5. *Mercapturic acid formation in relation to labile groups in trichloronitrobenzenes and 4-bromo-3:5-dichloronitrobenzene*

The positions of the labile chlorine atoms and the values for their mobilities (rate coefficients for hydrolysis with methanolic sodium methoxide at 35°) are those given by Holleman & Haeften (1921). The lability of the nitro group is based on reaction with ethanolic NaOH for 15 min. at 100° (Betts *et al.* 1955*b*). In columns 3 and 4, m_{Cl} represents the mobility of labile chlorine and m_{NO_2} that of the nitro group.

Compound	Position of labile halogen	$10^5\ m_{Cl}$ (l. mole^{-1} min.$^{-1}$)	m_{NO_2} (Percentage of N liberated as NO_2^-)	Mercapturic acid formed by rabbit: Amount (% of dose)	Mercapturic acid formed by rabbit: Group replaced by acetylcysteyl and position
2:3:4-Trichloronitrobenzene	2	832	0	41	Cl, 2
2:3:5-Trichloronitrobenzene	2	263	14	21	Cl, 2
2:3:6-Trichloronitrobenzene	6	16	8*	10	NO_2, 1
2:4:5-Trichloronitrobenzene	4	2310†	0*	30	Cl, 2
2:4:6-Trichloronitrobenzene	2	37	4*	1	NO_2, 1
3:4:5-Trichloronitrobenzene	4	2460	0	23	Cl, 4
4-Bromo-3:5-dichloronitrobenzene	4	—	—	0·5‡	Br, 4

* From Betts *et al.* 1955*b*.

† This is for the mobility of chlorine in position 4, not for the chlorine displaced in the formation of the mercapturic acid.

‡ By isolation. On the basis of the amount isolated from 3:4:5-trichloronitrobenzene this would represent an excretion of about 6% of the dose as mercapturic acid.

have escaped detection if only traces were excreted, since many aminophenols are unstable and small amounts may be destroyed during isolation, particularly from hydrolysed urine.

The identification of 4-amino-3:5-dichlorophenol as the major phenolic metabolite of 2:4:6-trichloronitrobenzene was unexpected. It is probable that this metabolite was formed by hydroxyldechlorination of 2:4:6-trichloroaniline because (*a*) no 3:5-dichloro-4-nitrophenol was detected, (*b*) the dichloroaminophenol was also isolated from the urine of rabbits dosed with 2:4:6-trichloroaniline, (*c*) the dechlorination takes place at position 4 and not at position 2, where according to Holleman & Haeften (1921) the chlorine of 2:4:6-trichloronitrobenzene is most labile, and (*d*) the lability of even the chlorine in position 2 is so small that mercapturic acid is formed by acetylcysteyldenitration rather than by acetylcysteyldechlorination. With 2:4:6-trichloroaniline, hydroxylation in an *ortho* or *para* position to the amino group is impossible without elimination of a chlorine atom; this may account for the fact that the aminodichlorophenol was the main phenolic metabolite from this isomer. There is not sufficient evidence to indicate the origin of the small amount of 4-amino-2:5-dichlorophenol formed from 2:4:5-trichloronitrobenzene.

The observation that hydroxyldechlorination can occur makes it necessary to give the reasons for the identification of the phenolic metabolites of the other trichloronitrobenzenes as aminotrichlorophenols. Only an *o*-aminophenol was detected from 2:3:4-trichloronitrobenzene; the R_F value was different from that of 6-amino-2:3-dichlorophenol (Bray *et al.* 1957), the only possible *o*-aminodichlorophenol. Elementary analysis showed that the major phenolic metabolite of 2:3:5-trichloronitrobenzene was a trichloro compound; the minor metabolite was a *p*-aminophenol, which must be the trichloro compound since no *p*-aminodichlorophenol can be formed by hydroxyldechlorination of 2:3:5-trichloronitrobenzene. The only phenolic metabolite of the 2:3:6-isomer was a *p*-aminophenol; this analysed correctly for a trichloro compound and no *p*-aminodichlorophenol is possible. The argument for the aminophenols from 2:4:5-trichloronitrobenzene has been given in the Results section. The only aminophenol from 2:4:6-trichloronitrobenzene besides 4-amino-3:5-dichlorophenol was identified as 3-amino-2:4:6-trichlorophenol by comparison with a synthetic specimen. This is the only possible aminotrichlorophenol, and the only other possible aminodichlorophenol is an *o*-aminophenol. The only phenolic metabolite of 3:4:5-trichloronitrobenzene was an *o*-aminophenol, which analysed correctly for a trichloro compound; only *m*- and *p*-dichlorophenols are possible by hydroxyldechlorination. It is thus reasonable to conclude that all the aminophenols detected, except 4-amino-3:5-dichlorophenol and 4-amino-2:5-dichlorophenol, were trichlorophenols.

SUMMARY

1. The metabolism of the six isomeric trichloronitrobenzenes has been studied in the rabbit.

2. Considerable amounts of mercapturic acids are formed by all the isomers except 2:4:6-trichloronitrobenzene, of which less than 1 % of the dose is converted into mercapturic acid.

3. The mercapturic acids from 2:3:4-, 2:3:5-, 2:4:5- and 3:4:5-trichloronitrobenzenes are formed, in effect, by acetylcysteyldechlorination and were isolated from urine.

4. The mercapturic acids from 2:3:6- and 2:4:6-trichlorobenzene are formed, in effect, by acetylcysteyldenitration, and their presence in urine has been shown by isolation of the corresponding hexachlorodiphenyl disulphides.

5. All the trichloronitrobenzenes are reduced to the corresponding anilines.

6. Hexachloroazoxybenzenes have been isolated from the urine of rabbits dosed with the 2:4:5- and 3:4:5-isomers.

7. All the trichloronitrobenzenes yield aminotrichlorophenols. Aminotrichlorophenols have been isolated from urines of rabbits given 2:3:4-, 2:3:5-, 2:3:6- and 3:4:5-trichloronitrobenzene. The excretion of a trichloronitrophenol was detected after administration of the 2:3:6-, 2:4:5- and 2:4:6-isomers.

8. 4-Amino-3:5-dichlorophenol is a metabolite of 2:4:6-trichloronitrobenzene and 2:4:6-trichloroaniline. It is probably formed by hydroxyldechlorination of the trichloroaniline. 4-Amino-2:5-dichlorophenol is a minor metabolite of 2:4:5-trichloronitrobenzene.

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Labelling of Phospholipid Phosphorus in Rat-Brain Dispersions

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In surviving slices of cat brain the *in vitro* incorporation of inorganic phosphate labelled with ^{32}P into phospholipid depends upon conditions optimum for oxidative phosphorylation (Strickland, 1954). Dinitrophenol, glutamate, glutamine, K^+ ion and NH_4^+ ion, in concentrations that do not decrease the oxygen consumption, greatly decrease phospholipid labelling (Strickland, 1954; Findlay, Magee & Rossiter, 1954). In addition, anaerobiosis, or the presence of respiratory inhibitors such as cyanide or azide, almost completely inhibits the labelling of lipid in brain, liver or kidney slices (Taurog, Chaikoff & Perlman, 1942; Schachner, Fries & Chaikoff, 1942; Strickland, 1954).

Schachner *et al.* (1942) and Strickland (1954) showed that if brain tissue was homogenized before incubation, the labelling of the phospholipid was reduced to very low levels. However, the respiratory activity of brain dispersions is low, unless suitable 'reinforcing' agents are added to the medium (Banga, Ochoa & Peters, 1939; Racker & Krimsky, 1945; Reiner, 1947).

When inorganic ^{32}P was added to an actively respiring dispersion of rat brain 'reinforced' with glucose, hexose diphosphate, adenosine triphosphate, fumarate, diphosphopyridine nucleotide, nicotinamide, cytochrome *c* and Mg^{2+} ions, good labelling of phospholipid was obtained. An unexpected finding, however, was that the phospholipids were more strongly labelled when oxygen was excluded from the system. This was surprising, since Dawson (1953) demonstrated good phospholipid labelling in 'reinforced' dispersions of guinea-pig brain in the presence of oxygen, but a greatly decreased labelling under anaerobic conditions.

An investigation of the apparent discrepancy between our observations and those of Dawson (1953) revealed that in rat-brain dispersions there are two systems capable of supporting the labelling of phospholipids: (*a*) a glycolytic system, demonstrated in dispersions of brain prepared in water, and (*b*) an oxidative system, demonstrated in mitochondria isolated from isotonic dispersions. Best labelling is obtained under optimum conditions for glycolytic or oxidative phosphorylation respectively. In the present paper the properties of the glycolytic system are described. The

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