The Metabolism of Pentachloronitrobenzene and 2:3:4:6-Tetrachloronitrobenzene and the Formation of Mercapturic Acids in the Rabbit

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In the rabbit 2:3:5:6-tetrachloronitrobenzene is metabolized by reduction of the nitro group, hydroxylation and mercapturic acid formation (Bray, Hybs, James & Thorpe, 1953). The last reaction is of particular interest since it is accompanied by loss of a nitro group directly attached to the benzene ring. Two further examples of this type of reaction were encountered when pentachloronitrobenzene and 2:3:4:6-tetrachloronitrobenzene were given to rabbits (Betts, James & Thorpe, 1953). A more detailed study of the metabolism of these two compounds is now reported. The stability of the nitro group in pentachloro-, the tetrachloro- and three trichloro-nitrobenzenes has been examined in relation to formation of mercapturic acids from the highly chlorinated nitrobenzenes.

METHODS

Melting points are uncorrected.

Materials. Pentachloronitrobenzene, m.p. 146°, was supplied by Bayer Agriculture Ltd. and Hickson and Welch Ltd. and 2:3:5:6-tetrachloronitrobenzene, m.p. 99°, by Bayer Agriculture Ltd. 2:3:4:6-Tetrachloronitrobenzene, m.p. 41°, was prepared from 2:4:6-trichloroaniline, which was converted by a Sandmeyer reaction into 1:2:3:5-tetrachlorobenzene; this was nitrated by refluxing for 0·5 hr. with HNO_3 (sp.gr. 1·52) to give 2:3:4:6-tetrachloronitrobenzene. (Details were kindly supplied by Dr M. Bird.) The 2:3:6-, 2:4:5- and 2:4:6-trichloronitrobenzenes, m.p. 89°, 57° and 69° respectively, were made by the methods of Holleman & Haeften (1921). Pentachloroaniline, m.p. 232°, and 2:3:4:6-tetrachloroaniline, m.p. 88°, were obtained by reduction of the corresponding nitro compounds with Sn and HCl in ethanol. Commercial technical-grade pentachlorophenol was recrystallized from carbon tetrachloride several times to m.p. 186–188°.

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. Pentachloronitrobenzene and pentachlorophenol caused considerable anorexia, but with pentachloroaniline and 2:3:4:6-tetrachloronitrobenzene this effect was only slight.

Determination of metabolites

Ethereal sulphate. The method of Folin (1905–6) was used, and also the turbidimetric method described by Bray & Thorpe (1954).

Glucuronic acid. A modification (Bray, Humphris, Thorpe, White & Wood, 1952) of the naphthoresorcinol method of Hanson, Mills & Williams (1944) was used. Reducing material was determined as described by Bray, Neale & Thorpe (1946).

Mercapturic acid. The iodometric-titration method of Stekol (1936) was modified by carrying out the hydrolysis in an atmosphere of N_2 for 1 hr. with N-NaOH for the mercapturic acid from 2:3:5:6-tetrachloronitrobenzene but 2N-NaOH for the others. Recoveries of *N*-acetyl-*S*-(2:3:5:6-tetrachlorophenyl)-L-cysteine and *N*-acetyl-*S*-(2:3:4:6-tetrachlorophenyl)-L-cysteine added to urine were 98±5 and 77·5±5% respectively, but only 25% of added *N*-acetyl-*S*-pentachlorophenyl-L-cysteine was recovered by this procedure. When pure pentachlorophenylmercapturic acid was hydrolysed by 2N-NaOH and the resulting thiophenol oxidized by iodine in acid solution only 29% of the theoretical amount of iodine was taken up. That this is due to incomplete oxidation of pentachlorothiophenol under these conditions was shown by titration of the pure thiophenol, when only 28% of the theoretical amount of iodine was consumed. The factor of 4 was therefore used in calculating the amount of mercapturic acid formed from pentachloronitrobenzene, but values obtained in this way cannot be regarded as precise. There was no significant difference between the iodine uptake of unhydrolysed normal urine and that of unhydrolysed urines containing mercapturic acids formed from the polychloronitrobenzenes. An alternative method used in some experiments with pentachloronitrobenzene was steam distillation of the alkali-hydrolysed urine; the pentachlorothiophenol collected from the distillate was weighed.

Nitrite. The method of Stieglitz & Palmer (1934) was used. The colours were read on a Spekker photoelectric absorptiometer with a Chance glass filter O.G. 1 (max. transmission at 530 mμ.). A calibration curve was constructed for each batch of determinations.

Polychloroanilines. Pentachloroaniline and 2:3:4:6-tetrachloroaniline were determined by weighing after isolation from the urine by steam distillation. (The solubility correction for these compounds was negligible.) The colours given by these compounds on diazotizing and coupling with *N*-(1-naphthyl)ethylenediamine were too feeble for use in a quantitative method. The values described as 'total polychloroaniline' in Table 1 were obtained by steam distillation of urines which had been treated with 0·2 vol. of conc. HCl.

Pentachloronitrobenzene. The 72 hr. faeces were ground with water and steam-distilled; the pentachloronitrobenzene was collected from the distillate and weighed. The recovery of the compound added to normal faeces was about 90%.

RESULTS

Quantitative experiments

The average daily excretions of normal metabolites were similar to those found previously (e.g. Bray *et al.* 1953).

The quantitative results from dosed animals are recorded in Table 1. The urines were usually collected over 72 hr. and analysed daily for metabolites. The excretion of glucuronic acid usually increased after feeding pentachloronitrobenzene but, in contrast to the findings with other metabolites, rarely returned to the base-line level in 2 or 3 days. The amount of glucuronic acid excreted was in some cases greater than that which could have been formed from that part of the dose not otherwise accounted for; in two experiments the excretion of glucuronic acid was roughly parallel with the increase in excretion of reducing material (in unhydrolysed urine); in one animal there was no significant increase in glucuronic acid excretion. The increased excretion could not therefore be attributed wholly to a phenolic glucosiduronic acid from pentachloronitrobenzene.

After 2:3:4:6-tetrachloronitrobenzene had been given the increased excretion of glucuronic acid ceased after 2 or 3 days and, in those experiments in which both determinations were made, corresponded quantitatively with the increased excretion of reducing material (in unhydrolysed urine). The amounts of glucuronic acid excreted under these conditions were not greater than could be accounted for by the formation of a glucosiduronic acid from that part of the dose not otherwise accounted for. The nature of this glucuronic acid is discussed below.

The value for the average percentage of the dose excreted as ethereal sulphate after feeding pentachloronitrobenzene (2 g.) is somewhat raised by the response of one animal, which gave no evidence of mercapturic acid formation but which excreted additional ethereal sulphate corresponding to 10% of the dose. In the other six animals the increases in excretion of ethereal sulphate were very small. At the 3 g. dose level the increase in ethereal sulphate was negligible. Great reliance is not placed on values for the increases in ethereal sulphate, since in every case pentachloronitrobenzene caused pronounced anorexia. The values in Table 1 have, perforce, been calculated by using as base-line the normal excretion of sulphate before dosing and on a normal food intake. On reduced food intake it is probable that the true base-line values would be lower and, consequently, the extent of increased excretion might be greater than that recorded.

Table 1. *Metabolites of some polychloronitrobenzenes excreted in urine by rabbits*

Results are expressed as percentage of the dose, ranges are given in parentheses and the number of experiments as superior figures. Urine was collected for 72 hr. with all compounds except 2:3:4:6-tetrachloronitrobenzene (48 hr.).

Substituted nitrobenzene	Dose (g.)	Unabsorbed	Mercapturic acid	Glucosiduronic acid	Ethereal sulphate	Total polychloroaniline	Total accounted for
Pentachloro-	1	46 (27–64)^{3}	5* (3–10)^{17}	—	—	14 (7–30)^{21}	65
	2	62 (49–80)^{3}	14† (0–30)^{9}	Determination not reliable, see text	3 (0–10)^{3}	12 (4–18)^{19}	91
	3	59 (40–82)^{3}	4* (2–13)^{13}	—	0^{3}	14 (7–26)^{18}	77
2:3:5:6-Tetrachloro-	2	61‡	14 (8–28)^{11}	12‡	1‡	9‡	97
2:3:4:6-Tetrachloro-	1	0	37 (14–62)^{8}	26§ (10–60)^{13}	0	24 (12–26)^{10}	61
	2	0	32 (9–65)^{11}	27 (4–61)^{8}	0	31‖ (25, 34)^{2}	63

* By isolation of thiophenol after alkaline hydrolysis of urine.
† For reliability of these iodometric values see text.
‡ Bray *et al.* (1953) for 1·5 g. dose.
§ In view of the uncertainty whether this represents the excretion of glucosiduronic acid or free glucuronic acid (see Discussion) this amount has not been included in the total accounted for.
‖ By isolation from pooled urine of three and six rabbits.

Isolation of metabolites of pentachloronitrobenzene

Pentachloronitrobenzene from faeces. The solid which separated from the steam distillate of 72 hr. faeces gave a very feeble diazo reaction, and after several recrystallizations from aqueous ethanol gave pentachloronitrobenzene, m.p. 141–143°, unchanged by admixture with an authentic sample. m.p. 146°. Yields are given in Table 1.

Pentachloroaniline from urine, The pentachloroaniline formed by rabbits from pentachloronitrobenzene is probably present in urine partly as a complex. Steam distillation of the neutral or slightly alkaline urine gave only part of this base, m.p. 232° unchanged by admixture with an authentic sample, after recrystallization from aqueous acetone. The remainder of the base separated and collected on the condenser (fraction A) when the urine was boiled under reflux with 0·2 vol. of conc. HCl. The base was not held as a stable chemical compound, since if the urine was acidified to pH 1 and then set aside for 2 days all the base present passed over on steam distillation. The complex did not appear to be an acetyl derivative, since exhaustive extraction with ether of the urine after the free base had been removed by steam distillation gave an extract which did not yield the base after treating with HCl. In a typical experiment the urine from twelve rabbits, each of which had received 2 g. of pentachloronitrobenzene, was collected for 3 days. Direct steam distillation afforded 0·64 g. of pentachloroaniline and the HCl treatment gave a further 1·65 g. (Total yield 10·6 % of the dose.)

N - *Acetyl* - S - *pentachlorophenyl* - L - *cysteine*. The urine from nine rabbits which had each received 2 g. of pentachloronitrobenzene was collected for 48 hr. and acidified (HCl) to pH 1. The flocculent precipitate, which was mainly a mixture of mercapturic acid and pentachloroaniline, was collected by centrifuging and washed with water. It was extracted with 0·1 N-NaOH and the insoluble base was removed by centrifuging. The crude mercapturic acid was precipitated by acidifying the supernatant and was filtered, dried and extracted from inorganic material with ethanol. The addition of water to the concentrated ethanolic extract gave mercapturic acid still containing a small amount of pentachloroaniline. The acid was again dissolved in 0·1 N-NaOH, precipitated from the solution by HCl, and repeatedly recrystallized from aqueous ethanol to give small white needles of N-*acetyl*-S-*pentachlorophenyl*-L-*cysteine* (55 mg.), m.p. 233°, $[\alpha]_D^{23°}$ $+29·2° \pm 2°$ in ethanol (*c*, 0·22). (Found: C, 32·1; H, 2·1; N, 3·5; Cl, 42·5; S, 7·5 %; equiv. 418. $C_{11}H_8O_3NCl_5S$ requires C, 32·1; H, 2·0; N, 3·4; Cl, 43·1; S, 7·8 %; equiv. 412.) The mercapturic acid (42 mg.) in 10 ml. of 2 N-NaOH was boiled under reflux in a current of N_2. The solution was cooled and acidified. The precipitated thiophenol was recrystallized from acetone to give needles (7·5 mg.), m.p. 232–234°. A second crop (10 mg.), m.p. 231–233°, was obtained on the addition of water to the mother liquor (total yield 62·5 %). *Pentachlorothiophenol* gave a positive Folin–Ciocalteu reaction. (Found: C, 25·8; H, 0·5; Cl, 62·8; S, 10·7. C_6HCl_5S requires C, 25·5; H, 0·4; Cl, 62·8; S, 11·3 %.)

Phenolic metabolite. A syrupy glucosiduronic acid fraction was separated by the usual lead-salt procedure (Bray *et al.* 1947) from the 48 hr. urine of six rabbits, each dosed with 2 g. of pentachloronitrobenzene. The syrup, which did not crystallize, was refluxed for 2 hr. with N-HCl, when a small amount of crystalline material collected on the condenser. Recrystallization from aqueous ethanol yielded 2 mg., m.p. 180°, mixed m.p. 180–186° with pentachlorophenol of m.p. 186–188°. The crystals gave an orange colour when treated with fuming HNO_3 as described by Deichmann & Schafer (1942). (Pentachlorophenol gives only a very feeble Folin–Ciocalteu reaction.) More drastic hydrolysis of the glucosiduronic acid fraction with 5 N-HCl caused extensive decomposition. Other experiments failed to reveal pentachlorophenol, although 10 mg. of this phenol added to the same volume of urine were readily detected. Attempts to separate the phenol by chromatography on a cellulose column were also unsuccessful. It was concluded that only very small amounts of pentachlorophenol were excreted. Pentachlorophenol could not be identified in the pentachloroaniline fraction A.

Experiments with pentachloroaniline

Not more than 75 % of pentachloroaniline given to rabbits (dose 1 or 2 g.) was absorbed, and no evidence could be obtained for the excretion of a mercapturic acid in urine. The precipitate obtained on acidification of urine was crude pentachloroaniline and gave no significant sulphur test. The iodine consumption of the urine after alkaline hydrolysis was no greater than that of normal urine. There was no increase in the excretion of ethereal sulphate. An increase in glucuronic acid excretion was observed in two animals, but in one it was accompanied by increased excretion of reducing material greater than that corresponding to the glucuronic acid. The raised excretion of both glucuronic acid and reducing material persisted for more than 4 days. In another animal the excretion of glucuronic acid after dosage was not significantly above normal. No pentachlorophenol could be detected in the urines.

Experiments with pentachlorophenol

The 72 hr. urine of a rabbit which had received 1 g. of pentachlorophenol was acidified to pH 1 with HCl. The precipitate which formed on standing was collected by centrifuging and dissolved in acetone. On addition of water a precipitate was obtained which gave negative sulphur and nitrogen tests and, after recrystallization from aqueous acetone, yielded 310 mg. of pentachlorophenol, m.p. 184–186° unchanged by admixture with an authentic sample. Steam distillation of the residual urine gave a further 365 mg. Since by

steam distillation the average recovery of pentachlorophenol added to urine was 72%, these yields accounted for 83% of the dose. In another experiment with the 72 hr. urine of three rabbits each dosed with 0·7 g. of pentachlorophenol, 76% was recovered by steam distillation of the urine adjusted to pH 1. This amount was not significantly raised by hydrolysis of the urine with 5N-HCl before steam distillation, confirming the statement of Deichmann, Machle, Kitzmiller & Thomas (1942) that pentachlorophenol is not excreted in a conjugated form.

Isolation of metabolites of 2:3:4:6-tetrachloronitrobenzene

Examination of faeces. 2:3:4:6-Tetrachloronitrobenzene could not be detected in the 72 hr. faeces of rabbits which had received 2 g. of the compound, although 50 mg. of the compound added to faeces was quantitatively recovered. Tetrachloroaniline was not detected.

2:3:4:6-*Tetrachloroaniline from urine.* Direct steam distillation of the urine at pH 7 gave about one-third of the tetrachloroaniline obtained by steam distillation of acidified urine. The base was obtained as colourless needles, m.p. 88°, unchanged by admixture with an authentic sample.

N-*Acetyl*-S-(2:3:4:6-*tetrachlorophenyl*)-L-*cysteine.* The urine was acidified with HCl to pH 1. The precipitate, treated like the corresponding precipitate from the urine of rabbits dosed with pentachloronitrobenzene, gave small colourless needles of N-*acetyl*-S-(2:3:4:6-*tetrachlorophenyl*)-L-*cysteine*, m.p. 193°, $[\alpha]_D^{25°} + 22° \pm 2°$ in ethanol (*c*, 0·96). (Found: C, 35·4; H, 2·5; N, 3·7; Cl, 37·7; S, 8·3%; equiv. 386. $C_{11}H_9O_3NCl_4S$ requires C, 35·0; H, 2·4; N, 3·7; Cl, 37·6; S, 8·5%; equiv. 377.) In a typical experiment acidification of the 48 hr. urine of five rabbits, each of which had been given 2 g. of 2:3:4:6-tetrachloronitrobenzene, gave a precipitate from which 845 mg. of tetrachloroaniline and 327 mg. of the mercapturic acid were isolated. Ether extraction of the residual urine yielded a further 285 mg. of the base.

Hydrolysis of the mercapturic acid by the method used for the pentachlorophenyl mercapturic acid gave colourless needles (yield 52%) of 2:3:4:6-*tetrachlorothiophenol*, m.p. 102–104°, giving an intense colour with the Folin–Ciocalteu reagent. (Found: C, 29·0; H, 1·0; S, 12·8. $C_6H_2Cl_4S$ requires C, 29·0; H, 0·8; S, 12·9%.)

Phenolic metabolite. A syrupy glucosiduronic acid fraction was obtained by the usual lead-salt procedure from the urine of five rabbits each given 1 g. of tetrachloronitrobenzene. This did not crystallize, and was hydrolysed by boiling with an equal vol. of 10N-H_2SO_4 for 1 hr. No phenolic metabolite was isolated, but the hydrolysed material gave a cherry-red diazo reaction quite different from the orange colour given by tetrachloroaniline. The cherry-red diazo colour was also obtained with acid- or alkali-hydrolysed urine from which, before hydrolysis, tetrachloroaniline had been removed by exhaustive steam distillation at pH 2. These findings are compatible with the presence of an aminophenol.

Decomposition of chloronitrobenzenes with ethanolic NaOH

The effect of treating some polychloronitrobenzenes with ethanolic NaOH is shown in Table 2. With 2:4:5-trichloro- and 2:3:4:5-tetrachloro-nitrobenzene an intense yellow colour developed on treatment with alkali, and no nitrite was detected.

Table 2. *Decomposition of some polychloronitrobenzenes by ethanolic sodium hydroxide*

The chloronitrobenzene (0·068 mM) was either refluxed for 15 min. or incubated at 37° for the stated time with ethanol (12·5 ml.) and 4N-NaOH (12·5 ml.). The solution or a sample was then quickly cooled by tenfold dilution with ice-cold water and nitrite determined on a portion of this solution which had been cleared by centrifuging. The values for mercapturic acid formation for the trichloronitrobenzenes are the averages of determinations of iodine uptake after alkaline hydrolysis of urine. (The superior figure indicates the number of experiments.) The constitution of these mercapturic acids has not been established.

	Percentage of N liberated as nitrite						Mercapturic acid formation in rabbit (% of absorbed dose)
	After 15 min. boiling		At 37°				
Compound		Average	2 hr.	6 hr.	12 hr.	20 hr.	
Pentachloronitrobenzene	39, 42, 48	43	37	41	41	41	37
2:3:5:6-Tetrachloronitrobenzene	83, 82, 82	82	25	65	85	91	36
2:3:4:6-Tetrachloronitrobenzene	20, 19, 19, 23	20	4	11	17	17	37
2:3:4:5-Tetrachloronitrobenzene	0, 0, 0, 0	0	0	0	0	0	0*
2:3:6-Trichloronitrobenzene	9, 6	8	0	0	<1	<1	8^9
2:4:6-Trichloronitrobenzene	3, 4	4	0	0	0	0	0^8
2:4:5-Trichloronitrobenzene	0, 0	0	0	0	0	0	5^{13}

* This value, given by Bray *et al.* (1953), is probably true for mercapturic acid formed by acetylcysteyldenitration, but further experiments with paper chromatograms have shown that a mercapturic acid containing a nitro group is formed, probably by acetylcysteyldechlorination.

DISCUSSION

The quantitative results show that 2:3:4:6-tetrachloronitrobenzene is completely absorbed, whereas about 60% of pentachloronitrobenzene is unabsorbed. Neither compound appeared to undergo significant reduction to the corresponding aniline in the alimentary canal before absorption but, in the body, reduction of the nitro group of both compounds occurred and the corresponding aniline was isolated from the urine. No unchanged nitro compound was detected in urine. The correct interpretation of the quantitative results is not yet clear. For reasons already given the increased excretion of glucuronic acid (i.e. material measured by the naphthoresorcinol method) after doses of pentachloronitrobenzene cannot be wholly attributed to a glucosiduronic acid of a phenol formed from pentachloronitrobenzene. For the formation of such a glucosiduronic acid, a nitro group or chlorine atom must be eliminated, but examination of the excretion products failed to yield more than a trace of a phenolic metabolite (probably pentachlorophenol). Furthermore, if pentachlorophenol were formed it is unlikely that it would be extensively conjugated, since it has been shown (Deichmann *et al.* 1942), and we have confirmed, that, when preformed pentachlorophenol is administered to the rabbit, 70–80% is recovered from the urine and there is no evidence that any of the phenol is excreted in a stable conjugated form. It would seem that at least part of the increased excretion of glucuronic acid after administration of pentachloronitrobenzene must be due to a toxic effect such as has been observed with certain other compounds (cf. Bray, Hybs & Thorpe, 1951).

After dosage with 2:3:4:6-tetrachloronitrobenzene the increased excretion of glucuronic acid was quantitatively compatible with the formation of a glucosiduronic acid derived from the compound administered. This glucuronic acid could be considered as being excreted in four possible forms: (1) as a stable *O*-glucosiduronic acid, (2) as a labile *O*-glucosiduronic acid, (3) as an *N*-glucosiduronic acid, and (4) as free glucuronic acid.

(1) If an *O*-glucosiduronic acid were formed from 2:3:4:6-tetrachloronitrobenzene it is reasonable to assume that it would be formed from a phenol—either 2:3:4:6-tetrachloronitrophenol or 5-amino-2:3:4:6-tetrachlorophenol. No phenolic metabolite, however, could be isolated, although a diazo reaction, distinct from that of tetrachloroaniline and attributable to an aminophenol, was observed. Glucosiduronic acids of phenols are usually stable to alkali and do not reduce alkaline cupric reagents without previous hydrolysis. In three experiments, however, in which both naphthoresorcinol and copper reducing methods (on unhydrolysed urine) were used, the results corresponded quantitatively for each 24 hr. urine. This suggests that if an *O*-glucosiduronic acid is formed it is not of the usual stable ether-type.

(2) This correspondence of naphthoresorcinol and reducing values could result from the existence of a labile glucosiduronic acid. If a labile *O*-glucosiduronic acid were formed it would seem more likely that it would be formed from the nitrophenol, in which all substituents are electron-attracting, than from the aminophenol (cf. Nath & Rydon, 1954).

(3) The formation of an *N*-glucosiduronic acid of the type formed from 2-naphthylamine (Boyland & Manson, 1955) would provide an explanation, not only for the correspondence between naphthoresorcinol and reducing values, but also for the excretion of 2:3:4:6-tetrachloroaniline in combined form. A search for chromatographic evidence for *O*- and *N*-glucosiduronic acids was not satisfactory, since tetrachloroaniline gives a diazo colour which is too feeble for reliable quantitative determination. Experiments with the glucosiduronic acid fractions of urines of rabbits dosed with 2:3:4:6-tetrachloronitrobenzene certainly showed two zones on a paper chromatogram from which, after hydrolysis, cherry-red and orange diazo colours respectively were obtained, the former presumably being due to an *O*-glucosiduronic acid of aminotetrachlorophenol. The identification of the second zone with an *N*-glucosiduronic acid and its distinction from adherent free tetrachloroaniline would, however, require a quantitative equivalence between the glucuronic acid and the base which could not be estimated with the insensitive colour reaction available for determination of the base. Furthermore, if the increased excretion of glucuronic acid was mainly due to an *N*-glucosiduronic acid, it would be expected that there would be some quantitative agreement between the excretion of combined tetrachloroaniline and the increases in excretion of glucuronic acid and reducing material in respect of both the amount and the time of excretion. The results in Table 3, however, suggest that the excretion of glucuronic acid is unrelated to that of the combined tetrachloroaniline.

(4) The excretion of free glucuronic acid would fit the observed facts better than any of the foregoing explanations and would provide another example of what has been described previously as a toxic effect (cf. Bray *et al.* 1951). In such conditions, however, the excretion of glucuronic acid is usually quantitatively unrelated to that of the metabolites of the compound administered and extends beyond the time of their excretion. This was not observed after dosage with 2:3:4:6-tetrachloronitrobenzene. From the existing evidence it cannot be decided whether the increased excretion

of glucuronic acid is due to the excretion of free glucuronic acid or of an alkali-labile glucosiduronic acid, or both.

The anorexia produced by dosing with pentachloro- and 2:3:4:6-tetrachloro-nitrobenzene makes it difficult to decide whether there is any significant excretion of metabolites of these compounds conjugated with sulphuric acid. It seems possible that normally a very small amount may be formed from pentachloronitrobenzene, since in one animal which did not excrete a detectable amount of mercapturic acid there was a significant increase (corresponding to 10% of the dose) in excretion of ethereal sulphate.

The lability of the nitro group in some chlorinated nitrobenzenes was reported by Berckmans & Holleman (1925) and Holleman & Haeften (1921). In the present work the ease of elimination of the nitro group from some polychloronitrobenzenes has been compared by measuring the formation of nitrite on treatment with ethanolic sodium hydroxide. These results are compared in Table 2 with the extent to which the compounds form mercapturic acids in the rabbit. There is fair agreement between the results obtained *in vitro* at 37° and on boiling; pure mercapturic acids have been isolated from the urines of rabbits that were given those compounds from which the nitro group

Table 3. *Excretion of glucuronic acid and tetrachloroaniline by rabbit no.* 417

The rabbit was given 1 g. of 2:3:4:6-tetrachloronitrobenzene. There was no increased excretion of glucuronic acid or excretion of tetrachloroaniline after the second day. Experiments with two other rabbits gave similar results.

Metabolite	Percentage of dose excreted: 1st day	2nd day	Total
Free tetrachloroaniline	5	0	5
Combined tetrachloroaniline	12	0	12
Increased excretion of glucuronic acid			
Naphthoresorcinol method	19	14	33
Copper reducing method	18	13	31

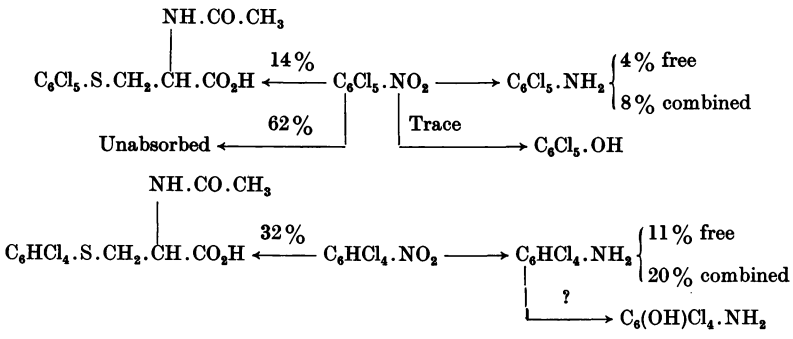

Fig. 1. The metabolism of pentachloronitrobenzene and of 2:3:4:6-tetrachloronitrobenzene. The percentages indicate the average percentage of a 2 g. dose which is converted into the respective metabolite.

On the basis of the information available, the probable metabolic paths followed by penta- and 2:3:4:6-tetra-chloronitrobenzene in the rabbit could be represented by Fig. 1. Pentachlorophenol has been shown as formed directly from pentachloronitrobenzene rather than from pentachloroaniline, because preformed pentachloroaniline is not converted into pentachlorophenol and the replacement of an amino group by hydroxyl in other compounds of this type has not been observed (Bray *et al.* 1953). It has been assumed, by analogy with the aminophenol formed from 2:3:5:6-tetrachloronitrobenzene (Bray *et al.* 1953), that 3-amino-2:4:5:6-tetrachlorophenol is formed from 2:3:4:6-tetrachloroaniline.

is readily eliminated (penta-, 2:3:4:6-tetra- and 2:3:5:6-tetra-chloronitrobenzenes). Preliminary examination of the urines of rabbits given 2:3:6-, 2:4:5- or 2:4:6-trichloronitrobenzene has indicated that mercapturic acids are formed from the 2:3:6- and 2:4:5- but not from the 2:4:6-isomer. That from the 2:3:6-isomer has not been isolated, but the 2:4:5-isomer gave a mercapturic acid containing a nitro group (unpublished work).

The fact that pentachloronitrobenzene is converted into *N*-acetyl-*S*-pentachlorophenyl-L-cysteine proves that the *N*-acetylcysteyl radical enters the position previously occupied by the nitro group. In the decomposition of pentachloronitrobenzene with sodium methoxide the nitro group is replaced

by a methoxyl group (Berckmans & Holleman, 1925), and it is of interest that the bacterial decomposition of certain nitrophenols results in the replacement of the nitro by the hydroxyl group (Simpson & Evans, 1953). It might therefore be expected that, when elimination of the nitro group occurs in the rabbit, this group would be replaced by an hydroxyl group and pentachlorophenol would be formed. Although there appears to be evidence that a very small amount of pentachlorophenol is formed as a metabolite of pentachloronitrobenzene, preformed pentachlorophenol is not converted into a mercapturic acid by the rabbit. It is also unlikely that the nitro group is reduced to amino and that this reacts to give a mercapturic acid, since pentachloroaniline does not form a mercapturic acid. Bray *et al.* (1953) showed that, whereas *N*-acetyl-*S*-(2:3:5:6-tetrachlorophenyl)-L-cysteine was formed from 2:3:5:6-tetrachloronitrobenzene in the rabbit, none was formed from 2:3:5:6-tetrachloroaniline. The available evidence suggests therefore that, in the formation of mercapturic acids from the polychloronitrobenzenes under discussion, the nitro group is directly replaced by a cysteyl or *N*-acetylcysteyl radical, and that this reaction is, in effect, an acetylcysteyldenitration which occurs in those compounds in which the disposition of chlorine atoms is such that the nitro group is relatively labile.

An examination of the possibility that some other chloronitrobenzenes are converted into mercapturic acids in the rabbit is in progress and evidence has been obtained that mercapturic acids are formed from certain dichloronitrobenzenes (Bray, James & Thorpe, 1955), although in most of these cases the reaction appears to be not an acetylcysteyldenitration but an acetylcysteyldechlorination.

SUMMARY

1. The metabolism of penta- and 2:3:4:6-tetrachloronitrobenzene in the rabbit has been studied.

2. An average of 62% of a 2 g. dose of pentachloronitrobenzene is unabsorbed and excreted in faeces. The average percentages excreted in urine as pentachloroaniline and *N*-acetyl-*S*-pentachlorophenylcysteine were 12 and 14 respectively. Both metabolites have been isolated.

3. The average percentages of a 2 g. dose of 2:3:4:6-tetrachloronitrobenzene excreted in urine as 2:3:4:6-tetrachloroaniline and *N*-acetyl-*S*-(2:3:4:6-tetrachlorophenyl)cysteine were 31 and 32 respectively. Both metabolites have been isolated. The significance of the increased excretion of glucuronic acid caused by this compound is discussed.

4. The formation of the mercapturic acid from pentachloronitrobenzene involves replacement of the nitro group by an acetylcysteyl group. The mercapturic acid from 2:3:4:6-tetrachloronitrobenzene is probably formed in a similar way.

5. The stability of the nitro group of some polychloronitrobenzenes towards ethanolic sodium hydroxide has been compared with the ease of the formation of mercapturic acid *in vivo*.

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