# Studies in Detoxication

## 55. THE METABOLISM OF ALKYLBENZENES. (*a*) GLUCURONIC ACID EXCRETION FOLLOWING THE ADMINISTRATION OF ALKYLBENZENES. (*b*) ELIMINATION OF TOLUENE IN THE EXPIRED AIR OF RABBITS

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Little is known about the metabolism in the animal body of monoalkylbenzenes other than toluene and ethylbenzene (cf. Williams, 1947). The information available suggests that the *n*-alkyl side chains undergo $\omega$-oxidation followed by $\beta$-oxidation of the resulting phenyl fatty acid, with the production of benzoic acid or phenylacetic acid, according to whether the alkyl chain carries an odd or even number of carbon atoms, respectively (cf. *n*-propyl-, *n*-butyl-, *n*-pentyl-, and *n*-hexyl-benzenes, Thierfelder & Klenk, 1924). However, there is evidence in the literature which suggests that $\omega$-oxidation may not be the only and perhaps not the main route of biological oxidation of some of these compounds. Both Neubauer (1901) and Thierfelder & Daiber (1923) showed that ethylbenzene yielded appreciable amounts of phenylmethylcarbinol in rabbits, as well as benzoic acid and small amounts of mandelic acid. Thus, it appeared that ethylbenzene underwent $(\omega-1)$, or penultimate, oxidation rather than $\omega$-oxidation, which should yield phenylacetic acid. However, ethylbenzene could be regarded as a special case, since it contains an active methylene group in the $(\omega-1)$-position. However, recent studies on the oxidation of alkyl side chains in barbiturates have confirmed that oxidation may also occur in positions other than the $\omega$, for it has been shown (Maynert & Dawson, 1952; Maynert, 1952*a*–*c*) that considerable amounts of the longer side chain in 5-ethyl-5-(1′-methylbutyl)-, 5-butyl-5-ethyl-, and 5-ethyl-5-hexyl-barbituric acids are oxidized in dogs in the penultimate or $(\omega-1)$ position. 5-Butyl-5-ethylbarbituric acid, for example, is partly oxidized to 5-(3′-hydroxybutyl)-5-ethylbarbituric acid.

The alkyl chain in the alkylbenzenes could therefore be oxidized in the $\omega$-position to produce fatty acids, and in some other position to produce alcohols. Such fatty acids could then be excreted as reducing glucuronides (in the latest systematic terminology (Rules of Carbohydrate Nomenclature, *J. chem. Soc.*, 1952, p. 5108), glucosiduronic acids) and the alcohols as non-reducing glucuronides. These possibilities are shown below (where $G=C_6H_9O_6$) for the case of *n*-propylbenzene.

$$\mathrm{Ph.CH_2.CH_2.CH_3}\begin{cases}\nearrow \mathrm{Ph.CH_2.CH_2.COOH\rightarrow Ph.COOH\rightarrow Ph.CO.OG}\\ \searrow \begin{cases}\mathrm{Ph.CH_2.CH(OH).CH_3\rightarrow Ph.CH_2.CH(OG).CH_3}\\ \mathrm{Ph.CH(OH).CH_2.CH_3\rightarrow Ph.CH(OG).CH_2.CH_3}\end{cases}\end{cases}$$

Thus the examination of the glucuronic acid output after feeding with these hydrocarbons could be used to detect the existence of forms of oxidation other than that of the $\omega$ carbon atom. This paper deals in part with the excretion of glucuronic acid by rabbits receiving doses of methyl-, ethyl-, propyl-, *iso*-propyl-, butyl-, *sec.*-butyl-, *tert.*-butyl-, *sec.*-pentyl-, and *tert.*-pentyl-benzenes. It will be shown that all these compounds, except toluene and cumene (*iso*-propylbenzene), cause the excretion of considerable amounts of non-reducing glucuronides. With cumene, large amounts of reducing glucuronides were excreted, whereas with toluene no glucuronic acid at all was excreted. The details of the metabolism of individual alkylbenzenes will be described in later papers.

The metabolic fate of toluene would seem to be largely known. In the dog, 80–90 % is excreted as hippuric acid (Knoop & Gehrke, 1925). In the rabbit up to 70 % of an injected dose is oxidized to benzoic acid (Epstein & Braunstein, 1931). Recently Bray, Thorpe & White (1951) have accounted for 40–64 % of oral doses of toluene in rabbits, and they also showed that it does not stimulate glucuronic acid excretion. Similarly, Baumann & Herter (1877) found that toluene does not increase ethereal sulphate output. Our present results confirm these findings concerning the glucuronic acid and ethereal sulphate excretions. It thus appears that some 70–80 % of an oral dose or of injected toluene can be accounted for as benzoic acid (including hippuric acid). In the present paper, we shall show that the rest of the toluene (some 18 %) is eliminated as such in the expired air of the animal. In human beings exposed for 5 hr. to toluene vapour of con-

centration 0·3–1·2 mg./l., Srbová & Teisinger (1952) found 16·3% of the absorbed toluene to be eliminated in the expired air.

## EXPERIMENTAL

### *Glucuronic acid excretion caused by alkylbenzenes*

*Materials.* All alkylbenzenes were purchased and were purified by distillation (cf. Table 1 for b.p.'s). They were administered with water by stomach tube to rabbits kept on an unvarying diet.

*Analytical methods.* Glucuronic acid was determined using a modification of the naphthoresorcinol method (cf. Hanson, Mills & Williams, 1944) given by Paul (1951), who used 15 N-$H_2SO_4$ instead of HCl, and ethyl acetate instead of the pentanol of the original method. Ethereal sulphates were determined essentially according to Sperber (1948). The results are given in Table 1.

*Qualitative tests.* All urines were tested for reducing power with Fehling's and Benedict's reagents. Positive reactions were considered indicative of the presence of reducing glucuronides. Rothera's nitroprusside test was used to detect the presence of methyl ketones, and Brady's reagent (2:4-dinitrophenylhydrazine in dil. HCl) for aldehydes and ketones. To test for methylcarbinols ($CH_3.CH(OH).R$) or methyl ketones ($CH_3.CO.R$), the iodoform reaction of Fuson & Tullock (1934) was used. Since neither Rothera's test nor Brady's reagent gave positive results with any of the urines, ketones were considered to be absent, and thus the iodoform reaction could be used as a test for $CH_3.CH(OH)$— groups. This test was not carried out directly upon the urine but upon concentrated ether extracts of hydrolysed urines. The test, details of which are quoted by Hickinbottom (1948), was tried out on methylphenylcarbinol, acetophenone, benzylmethylcarbinol, benzyl methyl ketone, phenethylmethylcarbinol and phenethyl methyl ketone. It worked well with the first four, but was not satisfactory with the two last compounds: the odour of iodoform was discernible, but no crystals were obtained.

It was also observed that some alkylbenzenes yielded urines containing glucuronides very labile to dilute acid. Such urines also gave a naphthoresorcinol reaction for glucuronic acid very rapidly. This was first observed with urine obtained after feeding *iso*propylbenzene (cumene) and from which the labile glucuronide was isolated and shown to be dimethylphenylcarbinyl glucuronide (Robinson, Smith & Williams, unpublished data). It readily decomposes in dilute acid to α-methylstyrene and urines containing this glucuronide become cloudy on warming with dilute acid, owing to the separation of oily drops of α-methylstyrene. Thus, warming the urines with dilute acid could be used as a test for the presence of tertiary dialkylphenylcarbinols. All urines were tested in this way and the results are given in Table 1.

### *Experiments with toluene*

*Spectrophotometric determination of toluene.* The spectrum of toluene in ethanol solution shows fine structure (cf. Braude, 1945). For the determination of toluene in these experiments, the peak used was at 262 mμ. (where $\epsilon_{max.}$ was 288, using a Unicam spectrophotometer, model S.P. 500 with slit width 1 mm.).

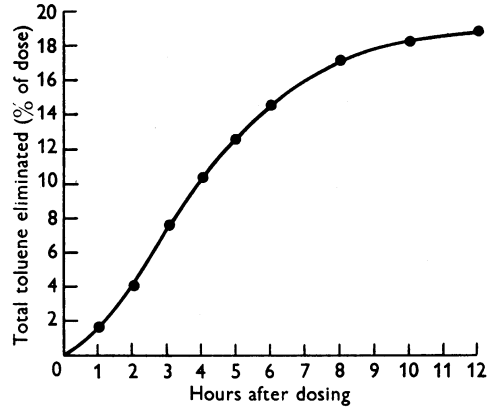


Fig. 1. The elimination of unchanged toluene in the expired air of a rabbit which had received 1·45 ml. of toluene orally.

The animal experiments were carried out in the tank described by Parke & Williams (1950; cf. Azouz, Parke & Williams, 1952). The absorption train consisted, in order, of a bottle containing anhydrous $CaCl_2$, two bottles containing $Mg(ClO_4)_2$ (Anhydrone), and two bottles containing glass beads and 12 ml. absolute ethanol at −70°, to absorb toluene. The first ethanol bottle was renewed periodically throughout an experiment. The toluene concentration in the ethanol was determined spectrophotometrically by measuring the whole absorption curve of toluene from 260 to 300 mμ. The recovery of toluene from the tank was checked by exposing known amounts of toluene (b.p. 110°) in it. Thus, when 266 mg. were exposed, 257·6 mg. were recovered in the first bottle and 10·2 mg. in the second (total: 267·8 mg. 100·6%) after drawing a current of air through the tank for 7·75 hr. In two experiments with rabbits which had received 1·5 and 1·45 ml. of toluene (350 mg./kg. body wt.) orally, the recoveries were 17·4% of the dose in 14·5 hr. and 18·8% in 12 hr., respectively. The first experiment was continued for 35 hr., but less than 1% of the dose was eliminated in the expired air during the period 14·5–35 hr. after dosing. In this experiment, also, a bottle containing 150 ml. of Brady's reagent (2:4-dinitrophenylhydrazine in dil. HCl) was placed between the absorption train and the tank, but no hydrazone was formed, showing that no benzaldehyde was expired. The rate of elimination of toluene in expired air is shown graphically in Fig. 1.

Toluene did not alter the glucuronic acid or the ethereal sulphate output of rabbits (see Table 1), nor was any benzaldehyde detected in the urine.

## RESULTS AND DISCUSSION

Table 1 shows that all the alkylbenzenes studied, except toluene, give rise to the excretion of appreciable amounts of extra glucuronic acid. The qualitative tests carried out on the urines suggest the type of compound which is excreted. In Table 1, the alkylbenzenes are divided into four groups, according to the output of extra glucuronic acid and the results of the qualitative tests.

Table 1. *Glucuronic acid excretion and some properties of urines obtained after feeding some alkylbenzenes to rabbits*

(Figures in parentheses indicate the b.p. See text for details of qualitative tests; ' + ' means positive result, ' – ' negative result.)

| Hydrocarbon fed | Formula | Dose (mg./kg.) | Glucuronic acid excretion (% of dose) | | Ethereal sulphate excretion (average % of dose) | Reaction of urine with Fehling's or Benedict's reagent | Iodoform test | Test for oil formation |
|---|---|---|---|---|---|---|---|---|
| | | | Individual expts. | Average value | | | | |
| Toluene (110°) | $Ph.CH_3$ | 347 | 0, 0, 0 | 0 | 0 | None | – | – |
| Ethylbenzene (136°) | $Ph.CH_2.CH_3$ | 433 | 34, 33, 28 | 32 | 0 | None | + | – |
| Propylbenzene (158°) | $Ph.[CH_2]_2.CH_3$ | 450 | 43, 29, 18 | 30 | 2 | None | + | – |
| Butylbenzene (180°) | $Ph.[CH_2]_3.CH_3$ | 500 | 58, 40, 32 | 43 | 5 | None | – | – |
| *iso*Propylbenzene (cumene) (152°) | $Ph.CH(CH_3)_2$ | 450 | 69, 68 | 68 | 1 | Reduction | – | + |
| *sec.*-Butylbenzene (173°) | $Ph.CH(CH_3).CH_2.CH_3$ | 500 | 80, 60, 57 | 66 | 11 | None | + | + |
| *sec.*-Pentylbenzene (188°) | $Ph.CH(CH_3).CH_2.CH_2.CH_3$ | 550 | 61, 57, 37 | 52 | 0 | None | + | + |
| *tert.*-Butylbenzene (167°) | $Ph.C(CH_3)_3$ | 500 | 75, 58 | 66 | 3 | Slight reduction | – | – |
| *tert.*-Pentylbenzene (189°) | $Ph.C(CH_3)_2.CH_2.CH_3$ | 550 | 53, 45, 45 | 48 | 0 | Reduction | – | – |

Toluene, the only compound in the first group, is mainly oxidized to benzoic acid. There is, however, no excretion of benzoylglucuronide (1-benzoylglucosiduronic acid), a reducing ester glucuronide, despite the fact that when benzoic acid itself is fed to rabbits it is partly excreted as this glucuronide (Smith & Williams, 1950; Bray *et al.* 1951).

The second group consists of the *n*-alkylbenzenes. Here the glucuronic acid conjugation is about 30–40 % of the dose and the urines are non-reducing This suggests that these compounds, like ethylbenzene, are partly converted into hydroxyalkylbenzenes, which are excreted as non-reducing glucuronides. The urine extracts from ethyl- and propyl-benzenes also give a positive result with the iodoform test, suggesting the presence of the methylcarbinol group. By analogy with the metabolism of ethylbenzene (see succeeding paper, Smith, Smithies & Williams, 1954), it seems likely that methylbenzylcarbinol ($Ph.CH_2.CH(OH).CH_3$) is formed from propylbenzene, which could also form ethylphenylcarbinol ($Ph.CH(OH).CH_2.CH_3$). The metabolism of butylbenzene produced over 40 % of non-reducing glucuronides which might be conjugates of phenylpropylcarbinol ($Ph.CH(OH).CH_2.CH_2.CH_3$) or benzylethylcarbinol ($Ph.CH_2.CH(OH).CH_2.CH_3$). The negative result of the iodoform test in this experiment would suggest that *n*-butylbenzene is not oxidized to methylphenethylcarbinol ($Ph.CH_2.CH_2.CH(OH).CH_3$). However, since this carbinol gives only a very feeble result with the iodoform test (see Experimental section), this deduction may not be valid.

In the third group there are three compounds, producing glucuronide outputs of about 50–70 % and acid-labile glucuronides. These three compounds are branched at the α-carbon atom and have the general formula $Ph.CH(CH_3).R$ (R = Me, Et, Pr). Other work in this laboratory has proved that *iso*propylbenzene (R = Me) yields dimethylphenylcarbinol ($Ph.C(OH)(CH_3)_2$) in the rabbit and that the glucuronide of this substance is acid labile, yielding α-methylstyrene ($Ph.C(CH_3):CH_2$). Since the urines from *sec.*-butylbenzene (R = Et) and *sec.*-pentylbenzene (R = Pr) yield oily droplets on gentle warming with dilute acid, as with *iso*propylbenzene (see Table 1), it is reasonable to suppose that these two alkylbenzenes are also oxidized *in vivo* to tertiary carbinols, i.e. $Ph.COH(CH_3).R$ (where R = Et and Pr). The urine from *iso*propylbenzene is also reducing to Fehling's solution, thus suggesting the excretion of an alkali-labile ester glucuronide. Other work in this laboratory has proved this to be the glucuronide of hydratropic acid ($Ph.CH(CH_3).CO_2H$) thus showing the occurrence of ω-oxidation. The urines from *sec.*-butyl- and *sec.*-pentyl-benzenes are non-reducing, thus suggesting the absence of ester glucuronides, although any carboxylic acids

formed might be excreted unconjugated as in the case of benzoic acid arising from toluene. The above urines also gave positive results with iodoform tests, suggesting the possible formation of $Ph.CH(CH_3).CH(OH).CH_3$ and $Ph.CH(CH_3).CH_2.CH(OH).CH_3$.

Finally, in the fourth group, the two tertiary compounds give relatively high glucuronic acid conjugations. In *tert.*-butylbenzene, the only expected reaction is the oxidation of one or more of the methyl groups. The urine obtained after feeding with this compound was only feebly reducing, suggesting that the ester glucuronide, $PhC:(CH_3)_2.CO_2G$, is produced only in small amounts. The high glucuronic acid conjugation, however, could be accounted for by the formation of conjugated $\beta\beta$-dimethyl-$\beta$-phenylethanol ($Ph.C(CH_3)_2.CH_2OH$). With *tert.*-pentylbenzene, the reducing urine suggests the excretion of an ester glucuronide either of $Ph.C(CH_3)(C_2H_5).CO_2H$ or of $Ph.C(CH_3)_2.CH_2.CO_2H$.

## SUMMARY

1. The glucuronic acid conjugation of nine alkylbenzenes has been studied in rabbits.

2. These alkylbenzenes can be divided into four groups according to the amount and nature of the conjugated glucuronic acid excreted after their administration.

3. These groups are (*a*) toluene, (*b*) ethyl-, *n*-propyl-, and *n*-butyl-benzene, (*c*) *iso*propyl-, *sec.*-butyl-, and *sec.*-pentyl-benzene, and (*d*) *tert.*-butyl- and *tert.*-pentyl-benzene.

4. About 18% of an oral dose of 350 mg./kg. body weight of toluene is eliminated unchanged in the expired air.

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# Studies in Detoxication

## 56. THE METABOLISM OF ALKYLBENZENES. STEREOCHEMICAL ASPECTS OF THE BIOLOGICAL HYDROXYLATION OF ETHYLBENZENE TO METHYLPHENYLCARBINOL

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The oxidation of ethylbenzene to methylphenylcarbinol in rabbits was first observed by Neubauer (1901). This observation was confirmed by Thierfelder & Daiber (1923), who also showed that acetophenone was reduced in the rabbit to the same carbinol. Since the formation of methylphenylcarbinol involves the production of an asymmetric carbon atom, the stereochemical implications of the oxidation of ethylbenzene become important. Attempts to deal with this aspect were made by Thierfelder & Daiber (1923) and Thierfelder & Klenk (1924*a*), and they suggested that ethylbenzene and acetophenone were metabolized to the same stereoisomer of methylphenylcarbinol. We shall show, however, that ethylbenzene is hydroxylated in the rabbit to both stereoisomers of methylphenylcarbinol, whereas acetophenone is reduced to only one form, namely (−)-methylphenylcarbinol. A preliminary account of this work has been published (Smith, Smithies & Williams, 1953).