Studies in Detoxication

80. THE METABOLISM OF GLYCOLS*

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The glycols, particularly ethylene, propylene and hexylene glycols, are important industrial chemicals (see Curme & Johnston, 1953). Although many toxicological studies have been made on these compounds (see Browning, 1953), few of them have been investigated from a metabolic point of view. In this paper a general survey of the metabolic reactions of 22 glycols has been carried out. Some were of industrial importance, and others had been tested for anaesthetic properties (see Buttle & Bower, 1958).

The metabolic reactions of the glycols are likely to depend upon the occurrence of primary, secondary or tertiary alcoholic groups, and upon the spatial relationships of these groups, i.e. whether they are vicinal or separated. A possible reaction of all glycols is conjugation with glucuronic acid. Other expected metabolites are hydroxymonocarboxylic acids, dicarboxylic acids and, in some cases, ketones. It has been reported that ethylene glycol does not increase the glucuronic acid excretion of rabbits (Miura, 1911) even after large doses (4 ml./kg.) (Fellows, Luduena & Hanzlik, 1947). A small glucuronic acid conjugation in rabbits has been reported for propane-1:2-diol (Neubauer, 1901; Miura, 1911; Fellows *et al.* 1947). Glucuronides are formed from butane-2:3-diol (Westerfeld & Berg, 1943) and 2:3-dimethylbutane-2:3-diol (pinacol) (Thierfelder & Mering, 1885) in rabbits, and from the anticonvulsant 2:2-diethylpropane-1:3-diol, in man (Berger & Ludwig, 1950).

EXPERIMENTAL

Animals. Chinchilla rabbits (2-3 kg. wt.) were kept on a constant diet of 60 g. of rat cubes (diet 41; Associated London Flour Millers) and 100 ml. of water per day. Glycols were administered by stomach tube with water.

Analytical method. Urine was analysed daily for glucuronic acid by the naphtharesorcinol method of Paul (see Mead, Smith & Williams, 1958).

Materials. Ethane-1:2-diol, propane-1:2- and -1:3-diol, butane-1:3-, -1:4- and -2:3-diol, pentane-1:5-diol, 2-methylpentane-1:4-diol, 3-methylpentane-1:5-diol, hexane-1:6-diol and 2:3-dimethylbutane-2:3-diol were purchased and purified. Butane-1:2-diol was synthesized (Matignon,

Moireu & Dodé, 1935). Heptane-1:3-diol, 2-ethyl-, 2:2-dimethyl- and 2:2-diethyl-propane-1:3-diol, 2-methyl-2-*n*-propylpropane-1:3-diol, 2-methyl- and 2-ethyl-pentane-1:3-diol, 2-ethylhexane-1:3-diol, 2-ethylheptane-1:3-diol and 2-methylpentane-2:4-diol were presented by Professor G. A. H. Buttle and by Mr E. E. Connolly of The Distillers Co. Ltd. (Chemical Division), Hull (see also Buttle & Bower, 1958). Boiling points and melting points were checked with the literature.

Metabolites of ωω'-diols

Isolation of metabolites. The glycols diluted with water were administered orally to rabbits at a dose level of 1.0-1.5 g./kg. Urines were collected from the animals usually for 1-2 days after dosing and were acidified to a pH of about 2 by the addition of conc. HCl. They were then continuously extracted with ether for 16 hr. The ether extracts were evaporated and the residues dissolved in 2*N*-Na₂CO₃ (20 ml.). The alkaline solutions thus obtained were extracted with ether to remove non-acidic material, but only with 3-methylpentane-1:5-diol was any unchanged diol isolated from this fraction. The ether-extracted alkaline solutions were acidified with 2*N*-HCl and the acids present taken up with ether. On evaporation of this ethereal solution partly crystalline gummy residues of dicarboxylic acids were obtained, from which the acids were isolated by recrystallization or as suitable derivatives (see below and Table 1).

Propane-1:3-diol. A total of 16 g. of this diol was fed to four rabbits. Urines were collected for 3 days after dosing, but neither the unchanged diol nor malonic acid was isolated.

Butane-1:4-diol. From the urine of four rabbits given a total of 9 g. of this diol there was isolated 0.81 g. (7% of dose) of succinic acid, m.p. and mixed m.p. 189°, after recrystallization from water. After treatment with thionyl chloride, followed by aniline, the acid yielded succinilide, m.p. and mixed m.p. 228°. No unchanged diol was found in the urine.

Pentane-1:5-diol. Four rabbits were given a total of 8.5 g. of this diol. The unchanged glycol was not excreted. The Na₂CO₃ solution of the extract from this urine was acidified with 2*N*-HCl, boiled to expel CO₂ and then neutralized with NH₃ soln. A saturated aqueous solution of CuSO₄ (10 ml.) was added and the mixture, which had become acid, was neutralized with NH₃ soln. A precipitate (0.7 g.) of copper salts separated and was filtered and washed with water, ethanol and finally ether. The precipitate was dissolved in 2*N*-HCl (20 ml.) and the solution extracted with ether. Evaporation of the ether left a gum (0.4 g.), which, on treatment with phenacyl bromide (1 g.)

* Part 79: Elliott, Parke & Williams (1959).

and recrystallization of the product from ethanol, gave phenacyl glutarate, m.p. and mixed m.p. 104° (yield 136 mg. or 0.5% of dose).

3-Methylpentane-1:5-diol. A total of 10 g. of the glycol (1.5 g./kg.) was fed to two rabbits. The ether extract of the acidified urine was extracted with 2N-Na₂CO₃ (3 × 20 ml.) to remove acids, and was then evaporated to dryness to leave a pale-brown liquid (1.45 g.). This was distilled (b.p. 224–225°) and the distillate was identified as 3-methylpentane-1:5-diol, n_D^{25} , 1.453 [Wojcik & Adkins (1933) give n_D^{25} , 1.452]. Treatment of the diol with 3:5-dinitrobenzoyl chloride gave the bis-3:5-dinitrobenzoate of 3-methylpentane-1:5-diol, m.p. and mixed m.p. 121° from aqueous ethanol (cf. Dische & Rittenberg, 1954). The Na₂CO₃ extract was now acidified and extracted with ether. Evaporation of the extract gave a partially crystalline solid (3.5 g.; 30% of the dose) which was dried over P₂O₅ *in vacuo* and then triturated with benzene to yield crystals (1.9 g.) which were identified as 3-methylglutaric acid, m.p. and mixed m.p. 87° after recrystallization from benzene. The acid was further identified as its anhydride (m.p. and mixed m.p. 45°) after refluxing with acetic anhydride and distilling (Darbishire & Thorpe, 1905), and as 3-methylglutaric monoanilide, m.p. and mixed m.p. 120°, after heating the anhydride with aniline in benzene solution.

Hexane-1:6-diol. The diol (2.8 g.) was fed to a rabbit. None of the unchanged diol was isolated from the 2-day urine. The carboxylic acid fraction of the urine in Na₂CO₃ was acidified with an excess of conc. HCl and kept overnight at 0°. A crystalline deposit (320 mg.; 9% of the dose) separated, which was purified by dissolution in 2N-Na₂CO₃ (charcoal) and precipitation with HCl. The crystals were identified as adipic acid, m.p. and mixed m.p. 152°, from which the *p*-nitrobenzyl ester, m.p. and mixed m.p. 106°, was prepared.

2-Substituted propane-1:3-diols

Crystalline metabolic products could not be isolated from the urine of rabbits receiving 2-ethyl- or 2:2-diethylpropane-1:3-diol, although Berger & Ludwig (1950) have shown that 2:2-diethylhydracrylic acid is a metabolite of the 2:2-diethyldiol in man.

2:2-Dimethylpropane-1:3-diol. This diol is highly conjugated (62%) with glucuronic acid in the rabbit (see Table 1). The glucuronide and its triacetyl methyl ester were readily obtained as gums by the lead acetate procedure (cf. Kamil, Smith & Williams, 1951), but these gums could not be crystallized.

The 24 hr. urine of four rabbits given a total of 8 g. of the diol was brought to pH 10.0 with NH₃ soln. and then continuously extracted with ether for 6 hr. Evaporation of the extract and sublimation of the residue yielded unchanged 2:2-dimethylpropane-1:3-diol, m.p. and mixed m.p. 127° (yield, 56 mg. or 0.7% of the dose). The residual urine was brought to pH 4.0 with 2N-HCl and again continuously extracted with ether for 6 hr. Evaporation of the extract gave an acidic gum which partly crystallized on keeping at 0° for a week. Repeated crystallization of this material from ethanol-ether mixtures gave white needles (150 mg.) of hydroxypivalic acid (3-hydroxy-2:2-dimethylpropionic acid), m.p. and mixed m.p. 126° (Found: equiv. 121. Calc. for C₅H₁₀O₃; equiv. 118). The authentic acid was prepared according to Wessely (1901).

2-Methyl-2-n-propylpropane-1:3-diol. Four rabbits were given a total of 32 g. of this diol, and the subsequent 24 hr. urine was continuously extracted with ether as described above, first after making alkaline with NH₃ soln. and again after making the residual urine acid. Neither of these extractions yielded crystalline products. The residual urine after the second extraction was therefore saturated with (NH₄)₂SO₄ and then extracted by shaking with a mixture of ethanol and ether (1:2). Evaporation of the solvents yielded a glucuronide gum (9.4 g.) which did not crystallize. The gum was therefore methylated with diazomethane and then acetylated with pyridine and acetic anhydride as described by Kamil *et al.* (1951), but again the triacetyl methyl ester was obtained as a gum. This gum was taken up in CHCl₃ and the solution washed three times with 2N-HCl and then thrice with saturated aq. NaHCO₃ solution. The washed solution, after drying over anhydrous Na₂SO₄, was evaporated to yield a gum (2.3 g.), which crystallized with considerable difficulty to yield 170 mg. of a compound, m.p. 116° and $[\alpha]_D^{20}$ –25° in CHCl₃ (c, 1), which analysed as methyl (2-acetoxyethyl-2-methyl-*n*-pentyl tri-O-acetylglucosid)uronate (Found: C, 53.95; H, 6.9; CH₃CO, 35.9. C₂₅H₃₄O₁₂ requires C, 54.0; H, 6.8; CH₃CO, 35.2%).

The glucuronide gum (0.5 g.), which had been prepared by ethanol-ether extraction as described above from the 24 hr. urine of a rabbit dosed with 5 g. of the diol, was heated under reflux for 2 hr. with 5N-HCl (10 ml.). The resulting solution was extracted with ether, and the extract was dried over anhydrous Na₂SO₄ and then evaporated. The dark semi-crystalline residue was treated with a solution of phosgene in toluene according to Ludwig & Piech (1951) to form a carbamate, and the product was recrystallized from hot water. There was obtained 2-methyl-2-n-propylpropane-1:3-diol dicarbamate (meprobamate), m.p. and mixed m.p. 105°.

Other diols

Unsuccessful attempts were made to isolate crystalline glucuronides or other metabolites with propane-1:2-diol (of which 300 g. was fed in one experiment), 2-methyl- and 2-ethylpentane-1:3-diol, 2-methylpentane-1:4- and -2:4-diol, and 2:3-dimethylbutane-2:3-diol (pinacol). Most of these diols contain one or two asymmetric carbon atoms and therefore more than one glucuronide could be formed; this would tend to make the isolation of crystalline glucuronides difficult.

Butane-2:3-diol. This compound could be expected to give rise to at least two glucuronides of the diol itself, to acetoin and its glucuronides and to diacetyl. Neither diacetyl nor acetoin was detected (by spectra and colour tests; see Feigl, 1956) in the expired air or urine of rabbits dosed with 1.2–1.5 g. of this compound. However, a glucuronide was isolated from the urine of four rabbits which had been dosed with 2 g. each of the diol. The glucuronide gum was prepared by the lead acetate method and was methylated and acetylated in the usual manner. The triacetyl methyl ester was obtained in poor yield from ethanol as white needles of m.p. 151° and $[\alpha]_D^{20}$ –18° in CHCl₃ (c, 1). It analysed as methyl (2-hydroxybutyl-3-tri-O-acetylglucosid)uronate (Found: C, 49.9; H, 6.2; CH₃CO, 32.4. C₁₇H₂₈O₁₁ requires C, 50.2; H, 6.45; CH₃CO, 31.8%).

Table 1. *Glucuronic acid conjugation and metabolites of glycols in rabbits*

The results for glucuronic acid conjugation are given as means for three animals with ranges in parentheses.

Glycol fed	Dose (m-moles/ kg.)	Glucuronic acid conjugation (% of dose)	Urinary metabolites characterized
1. Glycol type: $(\text{CH}_2)_n \cdot (\text{CH}_2 \cdot \text{OH})_2$			
Ethane-1:2-diol	4	0 (0)	CO_2 in expired air, traces of oxalate in urine
Propane-1:3-diol	4	1 (0-2)	None found
Butane-1:4-diol	4	1 (0-2)	Succinic acid
Pentane-1:5-diol	2	0 (0)	Glutaric acid
3-Methylpentane-1:5-diol	2	7 (3-11)	3-Methylglutaric acid
Hexane-1:6-diol	2	7 (4-9)	Adipic acid
2. Glycol type: $\text{CRR}' \cdot (\text{CH}_2 \cdot \text{OH})_2$			
2-Ethylpropane-1:3-diol ($\text{R} = \text{H}$)	2	23 (18-28)	—
2:2-Dimethylpropane-1:3-diol	2	62 (53-67)	3-Hydroxy-2:2-dimethylpropionic acid
2:2-Diethylpropane-1:3-diol	2	35 (11-66)	—
2-Methyl-2-propylpropane-1:3-diol	2	33 (23-49)	Glucuronide of the glycol
3. Glycol type: $\text{R} \cdot \text{CH}(\text{OH}) \cdot \text{CHR}' \cdot \text{CH}_2 \cdot \text{OH}$			
Butane-1:3-diol ($\text{R}' = \text{H}$)	4	1 (0-2)	None found
2-Methylpentane-1:3-diol	2	22 (21-23)	None isolated
2-Ethylpentane-1:3-diol	2	48 (46-52)	—
2-Ethylhexane-1:3-diol	2	7 (4-11)	None isolated
Heptane-1:3-diol ($\text{R}' = \text{H}$)	2	9 (7-13)	—
2-Ethylheptane-1:3-diol	2	9 (8-10)	—
4. Glycol type: $\text{R} \cdot (\text{CH} \cdot \text{OH})_2 \cdot \text{R}'$			
Propane-1:2-diol ($\text{R} = \text{H}$)	4	1 (0-3)	None found
Butane-1:2-diol ($\text{R} = \text{H}$)	2	12 (7-17)	None found
Butane-2:3-diol ($\text{R} = \text{R}' = \text{CH}_3$)	4	22 (20-26)	Glucuronide of the glycol
5. Miscellaneous glycols			
2:3-Dimethylbutane-2:3-diol	2	27 (25-29)	Non-crystalline glucuronides not characterized
2-Methylpentane-2:4-diol	1	67 (49-93) ⁵	
2-Methylpentane-1:4-diol	1.5	37 (32-43) ⁴	

RESULTS AND DISCUSSION

The results of our experiments are summarized in Table 1. The first group of glycols have the general formula $\text{CH}_2(\text{OH}) \cdot [\text{CH}_2]_n \cdot \text{CH}_2 \cdot \text{OH}$ and do not undergo glucuronic acid conjugation unless they contain six carbon atoms (i.e. 3-methylpentane-1:5-diol and hexane-1:6-diol), and even then the conjugation is less than 10% of the dose. The metabolic fate of ethane-1:2-diol has been briefly reported by Gessner & Williams (1959). The main metabolite of the ^{14}C -labelled compound in rats and rabbits is carbon dioxide. Oxidation to oxalic acid is a very minor reaction of the glycol, but rats and cats produce more oxalate than do rabbits and guinea pigs. Ethane-1:2-diol is oxidized in liver slices to glycolaldehyde and is then converted into carbon dioxide (Gessner, 1958). No malonic acid was detected in the urine of rabbits receiving propane-1:3-diol even with doses of 4 g./kg. Three oxidation products of propane-1:3-diol are possible, β -hydroxypropionic aldehyde, β -hydroxypropionic acid (hydracrylic acid) and malonic acid. Malonic acid can apparently be metabolized through the

tricarboxylic acid cycle (Lee & Lifson, 1951) and recently malonyl-coenzyme A has been found to be an intermediate in fatty acid synthesis (Brady, 1958; Gibson, Titchener & Wakel, 1958). Hydracrylic acid has also been reported to be metabolized in animal tissues (Green, Dewan & Leloir, 1937). It appears probable that propane-1:3-diol is oxidized completely to carbon dioxide in the body.

Butane-1:4-diol appears to be metabolized via succinic acid, since we were able to isolate this acid (yield, 7% of the dose) from the urine of rabbits given 0.75 g. of the diol/kg. Succinic acid is readily metabolized unless administered in large doses (Weitzel, 1947a, b; Emmrich, Neumann & Emmrich-Glaser, 1941). Similarly, pentane-1:5-diol appears to be metabolized via glutaric acid, since we were able to isolate small amounts of glutaric acid (0.5% of the dose of 0.75 g./kg.) from the urine after feeding with the glycol. Glutaric acid is known to be readily metabolized to carbon dioxide (Hobbs & Koeppe, 1958; Rothstein & Miller, 1952). As much as 30% of the dose (1.5 g./kg.) of 3-methylpentane-1:5-diol was isolated from the urine as 3-methylglutaric acid. It appears that the

3-methyl group hinders the further oxidation of the dicarboxylic acid. Our results suggest that the main outlines of the metabolism of these $\omega\omega'$ -diols are:

The second group of glycols in Table 1 are 2-substituted propane-1:3-diols, which are diprimary alcohols. These compounds are of interest for a number of them possess anticonvulsant and tranquilizing properties. In this group, however, glucuronic acid conjugation is appreciable, and dicarboxylic acids (i.e. substituted malonic acids) were not found as metabolites. Berger & Ludwig (1950) isolated 2:2-diethyl-3-hydroxypropionic acid as a metabolite of 2:2-diethylpropane-1:3-diol in man, but found no diethylmalonic acid. We have isolated 3-hydroxy-2:2-dimethylpropionic acid (hydroxypivalic or 2:2-dimethylhydracrylic acid) as a metabolite of 2:2-dimethylpropane-1:3-diol, and we also obtained the glucuronide of 2-methyl-2-propylpropane-1:3-diol. These results suggest that the main metabolic routes of this type of glycol are:

The third group of glycols in Table 1 are derivatives of propane-1:3-diol with one primary and one secondary alcohol group. Butane-1:3-diol is a compound of low toxicity. No metabolite of it is known and none was found in this work; it is probable that it is completely oxidized in the body via β -hydroxybutyric acid, which should be its primary oxidation product. 2-Methylpentane-1:3-diol and 2-ethylhexane-1:3-diol were also examined in some detail, but no recognizable metabolic product was isolated. The other glycols in this group were available only in small quantities and detailed examination of their metabolites was not possible. At present we have no clear idea of the metabolic fate of this type of glycol.

The fourth group of glycols are derivatives of propane-1:2-diol. No urinary metabolites of propane-1:2-diol or of butane-1:2-diol were isolated. Propane-1:2-diol is relatively non-toxic (its LD_{50} for rats is about 26 g./kg.; Smyth, Seaton & Fischer, 1941) and it is probably oxidized to lactic acid, through which it can enter normal metabolic processes; it is, in fact, converted into glycogen in rats (Rudney, 1950). Butane-2:3-diol is not oxidized to butane-2:3-dione (diacetyl) in dogs (Westerfeld & Berg, 1943) and we have found no diacetyl in the urine or expired air of rabbits dosed with the glycol. Neuberg & Gottschalk (1925)

reported that it formed a glucuronide in rabbits. We have isolated this glucuronide as a triacetyl methyl ester and from elementary analysis it appears to be a monoglucuronide. Since butane-2:3-diol contains two asymmetric carbon atoms, monoglucuronides corresponding to the *dextro*-, *laevo*- and *meso*-glycols are possible.

The three glycols 2:3-dimethylbutane-2:3-diol (pinacol), 2-methyl-pentane-2:4-diol and 2-methyl-pentane-1:4-diol are conjugated with glucuronic acid to an appreciable extent. None of these glucuronides was isolated in a crystalline form.

SUMMARY

1. The fate of oral doses of 22 glycols has been investigated in the rabbit.
2. Glycols of the general formula,

where $n = 0-6$, do not form appreciable amounts of conjugated glucuronic acid. Ethane-1:2-diol is mainly oxidized to carbon dioxide. Butane-1:4-diol, pentane-1:5-diol and hexane-1:6-diol yield small amounts of the corresponding dicarboxylic acids in the urine, but appear to be mainly destroyed *in vivo*. 3-Methylpentane-1:5-diol yields large amounts of 3-methylglutaric acid in the urine.

3. Glycols of the general formula

form appreciable amounts of conjugated glucuronic acid. These appear to be glucuronides of the unchanged diols, for the monoglucuronide of 2-methyl-2-*n*-propylpropane-1:3-diol was isolated. These glycols do not form dicarboxylic acids *in vivo*, but monocarboxylic acids. 3-Hydroxy-2:2-dimethylpropionic acid was isolated as a metabolite of 2:2-dimethylpropane-1:3-diol.

4. No definite information about the other glycols in Table 1 was obtained, except that some are highly conjugated with glucuronic acid (2-methyl- and 2-ethyl-pentane-1:3-diol, butane-2:3-diol, 2:3-dimethylbutane-2:3-diol, and 2-methyl-pentane-1:4- and -2:4-diol). The monoglucuronide of butane-2:3-diol was isolated.

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Studies in Detoxication

81. THE METABOLISM OF HALOGENOBENZENES: (a) PENTA- AND HEXA-CHLOROBENZENES. (b) FURTHER OBSERVATIONS ON 1:3:5-TRICHLOROBENZENE

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The metabolic fate of mono-, di-, tri- and tetra-chlorobenzene has already been reported (Smith, Spencer & Williams, 1950; Azouz, Parke & Williams, 1955; Parke & Williams, 1955; Jondorf, Parke & Williams, 1955, 1958). These studies suggested that the more chlorine the halogenated benzene contained, the less readily was it metabolized. To complete the series of chlorinated benzenes, an investigation of the fate of penta- and hexa-chlorobenzene in rabbits has been carried out. Pentachlorobenzene is only slightly altered *in vivo*, and hexachlorobenzene seems to be metabolically inert. Studies of these highly chlorinated benzenes have been carried out in connexion with work in this Laboratory on the metabolism of the highly chlorinated insecticides, dieldrin and aldrin, which also appear to be metabolically inert. Pentachlorobenzene does not have any important practical application, but hexachlorobenzene has limited use as a fungicidal agent for seeds.

Jondorf *et al.* (1955) were unable to account for the major portion of the dose of 1:3:5-trichloro-

benzene given to rabbits. This compound has been re-investigated and it is now clear that it is not readily metabolized.

METHODS AND MATERIALS

Melting points are corrected.

Materials. 1:3:5-Trichlorobenzene was prepared from 2:4:6-trichloroaniline and carefully purified; it had m.p. 69° (m.p. values from 61° to 64° are quoted in the literature). Pentachlorobenzene, m.p. 88° (Holleman, 1920) and hexachlorobenzene, m.p. 230° (Silberrad, 1922) were prepared and carefully purified, the samples used being judged to be free of less-chlorinated benzenes by absorption spectra (see Table 1, and tables of spectra quoted by Jondorf *et al.* 1958). *p*-Chlorophenol, m.p. 42°, and its toluene-*p*-sulphonate, m.p. 71°, and pentachlorophenol, m.p. 191°, and its benzoate, m.p. 160°, were prepared as reference compounds.

Animals. Chinchilla doe rabbits, kept throughout on a diet of 80 g. of rat cubes (diet 41; Associated London Flour Millers) and 100 ml. of water/day, were used. The chlorinated benzenes were administered either by stomach tube as aqueous suspensions or subcutaneously as 10% (w/v) solutions in arachis oil. Urine was collected daily.