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Studies in Detoxication

46. THE METABOLISM OF ALIPHATIC ALCOHOLS. THE GLUCURONIC ACID CONJUGATION OF ACYCLIC ALIPHATIC ALCOHOLS

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(*Received* 7 *May* 1952)

A large number of aliphatic alcohols are used industrially as solvents and as starting materials for the manufacture of other chemicals such as esters and ethers. Those most commonly used are the monohydric and dihydric alcohols, and glycerol. According to von Oettingen (1943) those containing more than three hydroxyl groups are of no industrial toxicological importance. Aliphatic alcohols, other than ethanol, also occur in small amounts in wines and liquors, but their nature and amounts are not accurately known. In analysis, these alcohols are lumped together as 'higher alcohols' or fusel oil. Rum, brandy and whisky may contain 100–400 mg./100 ml. of higher alcohols, the amounts varying according to the 'mash' used, the yeast culture employed and the conditions of fermentation. *n*-Propanol, *iso*propanol, *n*-butanol, *iso*butanol, *n*-, active and *iso*-amyl alcohols have been reported in fusel oil (Kirk & Othmer, 1947). In addition to these alcohols, heptyl alcohol has been reported in a 25-year-old brandy (Ordinneau, 1886; cf. Herstein & Jacobs, 1948), and Hewitt (1928) states that alcohols as high as octyl and nonyl occur in some wines and spirits. The bouquet or flavour of wines is in part due to the presence of esters of these alcohols. The amyl alcohols appear to arise by fermentation of leucine and isoleucine (Herstein & Jacobs, 1948). Appreciable quantities of higher alcohols are therefore being constantly consumed by human beings. Methanol up to 0·36 % may occur in the cheap brandies (marc brandy); this usually arises as the result of the fermentation of pectin.

Most of the known data on the toxicity and metabolism of the aliphatic alcohols have been assembled by von Oettingen (1943), and it is clear from his publication that our knowledge of the metabolism of these compounds is but fragmentary. In general, however, it is known that aliphatic alcohols are oxidized *in vivo*, the primary alcohols being initially oxidized to aldehydes and the secondary to ketones (see Williams, 1947). Some of the more volatile alcohols are undoubtedly eliminated to some extent in the unchanged state by the lungs. The tertiary alcohols, *tert.*-butyl and amyl are known to be partly excreted in the urine as glucuronides. Our knowledge of the fate of methanol and ethanol is fairly extensive, and a valuable study of the metabolism of seven out of the eight possible isomers of amyl alcohol has been made by Haggard, Miller & Greenberg (1945). The latter workers showed quite clearly that the rate of the metabolism of the amyl alcohols in the rat was in the order primary > secondary > tertiary.

In most of the studies on alcohols few workers have paid any attention to whether these alcohols give rise to conjugated glucuronic acids. Neubauer (1901), from qualitative observations on dogs and rabbits, concluded that a number of alcohols were excreted as glucuronides, including ethanol but not methanol. Deichmann & Thomas (1943), however,

found neither methanol (2·3 ml./kg.) nor ethanol (3·5 ml./kg.) to affect the glucuronic acid output of the rabbits. Thierfelder & Mering (1885) showed, by isolation of the glucuronides, that *tert.*-butyl and amyl alcohols were conjugated by rabbits.

It is also possible that aliphatic alcohols conjugate with sulphuric acid. According to Pringsheim (1908) ethanol is excreted to a slight extent as an ethereal sulphate. However, Anderton, Smith & Williams (1948) found no increase in the ethereal sulphate output of rabbits receiving ethanol, and Thierfelder & Mering (1885) have reported similarly for *tert.*-butyl alcohol. In the present work three alcohols, *tert.*-amyl alcohol, pentan-2-ol and pentan-3-ol, were tested for their effect on the ethereal sulphate output of rabbits, but in no case was ethereal sulphate formation detected.

The object of the present work was to find out to what extent various monohydric aliphatic alcohols conjugate with glucuronic acid in the rabbit, to relate the findings to the structure and other properties of the alcohol and to make any relevant observation concerning other paths of metabolism of the alcohol.

EXPERIMENTAL

Alcohols

Most of the alcohols used were purchased and were of laboratory reagent quality. All alcohols were redistilled and boiling points checked before use. The *n*-butyl, *sec.*-butyl and *iso*butyl alcohols were purified through their respective hydrogen phthalates and *tert.*-butyl alcohol through its *p*-nitrobenzoate before finally distilling. *iso*Butylmethylcarbinol, 2-ethylbutan-1-ol, *n*-hexyl alcohol, di*iso*propylcarbinol and 2-ethylhexan-1-ol were British Drug Houses Ltd. technical grade alcohols and were fractionated before use. All boiling points agreed with those given by Huntress & Mulliken (1946) and, where necessary, solid derivatives were prepared and melting points checked.

Heptan-2-ol was prepared by reduction of methyl *n*-amyl ketone (see Blatt, 1943). All alcohols used containing an asymmetric carbon atom were DL forms (2-ethylbutanol, 2-ethylhexanol, butan-2-ol, pentan-2-ol, hexan-2-ol, 4-methylpentan-2-ol, heptan-2-ol, heptan-3-ol and octan-2-ol), and this was checked polarimetrically.

Quantitative estimations

Large chinchilla rabbits of about 3 kg. weight were used and kept on a constant diet of 75 g. rat cubes/day. The animals were kept on this diet for at least 1 week before administration of an alcohol in order to obtain as steady a value as possible for the normal output of glucuronic acid. The excretion of glucuronic acid was determined daily by the method of Hanson, Mills & Williams (1944). The alcohols were administered with water by stomach tube and the glucuronic acid output for each alcohol was determined simultaneously on three animals. The dose of alcohol in most cases was 25 m-moles/rabbit. The results are given in Tables 1–4. In most instances the excretion of extra glucuronic acid after feeding these alcohols was complete within 24 hr. Only in the cases of *iso*propanol, *n*-decanol, di*iso*propylcarbinol, *tert.*-amyl alcohol, 2-ethylhexanol and 3-heptanol did extra glucuronic acid appear in the urine on the second day after dosing. Ethereal sulphates were determined by the method of Sperber (1948).

Qualitative experiments

Melting points are uncorrected.

Isolation of the glucuronides. Only in a few cases was it possible to isolate the free glucuronides of the aliphatic alcohols. They usually formed non-crystalline water-soluble gums. In some instances, however, crystalline salts with *p*-toluidine and benzylamine were obtained and occasionally a crystalline potassium salt was isolated. In almost every case, however, the glucuronide could be obtained as a crystalline triacetyl methyl ester. All these aliphatic esters were highly soluble in ethanol except the ethyl derivative.

In most cases the crude glucuronides were isolated from the urine by extraction with ether or mixtures of ether and ethanol, but in some instances isolation through the basic lead salt, as described by Kamil, Smith & Williams (1951) for the substituted phenylglucuronides was successful. Many aliphatic glucuronides are, however, not completely precipitated by basic lead acetate even when the latter is made highly alkaline with NH_4OH. These glucuronides could be readily extracted either by continuous extraction of the urine with ether or by extraction in a separating funnel with ether/ethanol mixtures. The urine of rabbits fed

Table 1. *The glucuronic acid conjugation of normal aliphatic alcohols in the rabbit*

Alcohol		Dose	Extra glucuronic acid excreted	
Name (normal)	Formula	(m-moles/3 kg.)	(% of dose)	Average
Methyl	CH_3OH	75	0, 0, 0	0
Ethyl	C_2H_5OH	50	0·58, 0·32, 0·56	0·5
n-Propyl	C_3H_7OH	40	0·8, 1·1, 0·8	0·9
n-Butyl	C_4H_9OH	16	1·1, 1·0, 3·4	1·8
n-Amyl	$C_5H_{11}OH$	25	6·5, 7·4, 6·1	6·7
n-Hexyl	$C_6H_{13}OH$	25	10·0, 8·4, 12·4	10·3
n-Heptyl	$C_7H_{15}OH$	25	5·5, 4·7, 5·3	5·3
n-Octyl	$C_8H_{17}OH$	25	12·5, 7·8, 8·2	9·5
n-Nonyl	$C_9H_{19}OH$	25	4·6, 3·3, 4·5	4·1
n-Decyl*	$C_{10}H_{21}OH$	25	3·5, 3·6*	3·5
n-Octadecyl*	$C_{18}H_{37}OH$	25	5·1, 10·1*	—

* Absorption of the alcohol incomplete and irregular, and the alcohol could be isolated in quantity from the faeces.

Table 2. *The glucuronic acid conjugation of arborescent primary alcohols in the rabbit*

Alcohol*	Formula	Extra glucuronic acid excreted: % of dose	Extra glucuronic acid excreted: Average
Branching at β-carbon			
*iso*Butanol (β:β-dimethylethanol)	$(CH_3)_2CH.CH_2OH$	2·7, 6·3, 4·1	4·4
2-Methylbutan-1-ol (β-methyl-β-ethylethanol)	$C_2H_5.CH(CH_3)CH_2OH$	9·1, 10·4, 9·2	9·6
2-Ethylbutan-1-ol (β:β-diethylethanol)	$(C_2H_5)_2CH.CH_2OH$	49, 36, 34	40†
2-Ethylhexan-1-ol (β-ethyl-β-*n*-butylethanol)	$n\text{-}C_4H_7.CH(C_2H_5)CH_2OH$	89·6, 88·1, 83·0	86·9†
Branching at γ-carbon			
3-Methylbutan-1-ol (primary *iso*amyl)	$(CH_3)_2CH.CH_2CH_2OH$	11·0, 7·7, 8·4	9·0

* Dose: 25 m-moles/3 kg. rabbit.
† Mainly reducing glucuronide of corresponding fatty acid (see text).

Table 3. *The glucuronic acid conjugation of some acyclic aliphatic alcohols in the rabbit*

Alcohol*	Formula	Extra glucuronic acid excreted: % of dose	Extra glucuronic acid excreted: Average
Propan-2-ol (*iso*propanol)	$CH_3.CHOH.CH_3$	8·9, 13·5, 8·2	10·2
Butan-2-ol (*sec.*-butanol)	$C_2H_5.CHOH.CH_3$	15·3, 16·4, 11·7	14·4
Pentan-2-ol (methyl-*n*-propylcarbinol)	$n\text{-}C_3H_7.CHOH.CH_3$	47·3, 45·4, 41·7	44·8
Pentan-3-ol (diethylcarbinol)	$C_2H_5.CHOH.C_2H_5$	40·1, 42·1, 35·3	39·2
Hexan-2-ol (methyl-*n*-butylcarbinol)	$n\text{-}C_4H_9CHOH.CH_3$	48·3, 60·9, 53·8	54·3
4-Methylpentan-2-ol (methyl*iso*butylcarbinol)	$iso\text{-}C_4H_9.CHOH.CH_3$	36·5, 37·3, 27·8	33·7
Heptan-2-ol (methyl-*n*-amylcarbinol)	$n\text{-}C_5H_{11}.CHOH.CH_3$	57·7, 57·5, 48·8	54·6
Heptan-3-ol (ethyl-*n*-butylcarbinol)	$n\text{-}C_4H_9.CHOH.C_2H_5$	58·6, 63·9, 63·2	61·9
Heptan-4-ol (di-*n*-propylcarbinol)	$n\text{-}C_3H_7.CHOH.n\text{-}C_3H_7$	69·9, 69·5, 62·9	67·4
2:4-Dimethylpentan-3-ol (di*iso*propylcarbinol)	$iso\text{-}C_3H_7.CHOH.iso\text{-}C_3H_7$	60·4, 64·6, 75·2	66·4
Octan-2-ol (methyl-*n*-hexylcarbinol)	$n\text{-}C_6H_{13}.CHOH.CH_3$	14·5, 16·6 —	15·5

* Dose: 25 m-moles/3 kg. of rabbit, except in the case of *iso*propanol where the dose was 50 m-moles/kg.

Table 4. *The glucuronic acid conjugation of acyclic aliphatic tertiary alcohols in the rabbit*

Alcohol	Formula	Dose (m-moles/3 kg.)	Extra glucuronic acid excreted: % of dose	Extra glucuronic acid excreted: Average
Trimethylcarbinol (*tert.*-butyl)	$(CH_3)_3COH$	12	27·2, 23·1, 22·9	24·4
Dimethylethylcarbinol (*tert.*-amyl)	$C_2H_5(CH_3)_2COH$	15	58·5, 54·3, 60·8	57·5
Dimethyl-*n*-propylcarbinol (*tert.*-hexyl)	$C_3H_7(CH_3)_2COH$	25	61·5, 50·2, 58·9	56·7

Table 5. *The glucuronic acid conjugation of primary, secondary and tertiary alcohols*

Alcohol	Conjugation (as % of dose)			
	Primary		Secondary	Tertiary
Propyl		0·9	10·2	—
Butyl	Normal	1·8	14·4	24·4
	iso	4·4		
Amyl	Normal	6·7	Pentan-2-ol 44·8	57·5
	iso	9·0	Pentan-3-ol 39·2	
	2-Methylbutanol	9·6		
Hexyl	Normal	10·3	Hexan-2-ol 54·3	56·7

with the alcohols was collected for 24 hr., saturated with solid $(NH_4)_2SO_4$ and acidified to pH 1–2 with conc. HCl. If extraction is carried out in a separating funnel the acidified urine is shaken with 0·5–1 vol. of ether, or 1:2, 1:3 or 1:4 ethanol/ether mixtures according to the solubility of the glucuronide in these solvents as indicated by preliminary tests. The extraction is repeated at least three times. If the extraction is carried out in a continuous extractor, it is made with ether and allowed to proceed for 16 hr. The extract is then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure at 40–50°.

The crude glucuronide gums obtained by these methods, after attempts were made to crystallize them or form crystalline salts, were methylated in methanol with ethereal diazomethane and then acetylated with pyridine and acetic anhydride to convert them to triacetyl methyl esters (cf. Kamil *et al.* 1951). The crude triacetyl methyl esters were recrystallized from aqueous ethanol and finally from *n*-hexane.

The optical rotations of the triacetyl methyl esters are tabulated (see Table 6).

Normal primary alcohols

It is clear from Table 1 that the conjugation of these alcohols is very low. Attempts, however, were made to isolate the glucuronides of methyl, ethyl, *n*-amyl, *n*-hexyl and *n*-octyl alcohols. Success was only achieved with the methyl, ethyl and *n*-hexyl derivatives. The isolation of methyl and ethyl glucuronides and observations on the metabolism of methanol and ethanol will be published later. To obtain the glucuronide gum of *n*-hexyl alcohol, the 24 hr. urine of three rabbits which had each received 3 ml. of *n*-hexyl alcohol by stomach tube was extracted as described above with 1:4 ethanol/ether. From the gum there was prepared *triacetyl β-n-hexyl-*D*-glucuronide methyl ester* which slowly crystallized during 2 months from aqueous ethanol as long, white, silky needles, m.p. 70–71°. (Found: C, 55·1; H, 7·3. $C_{19}H_{30}O_{10}$ requires C, 54·5; H, 7·2%.) (Yield, 0·35 g.)

Arborescent primary alcohols

iso*Butanol.* Gray, Adams & Hauptmann (1950) have suggested from studies using [*carboxy*-^{14}C] and [*methyl*-^{14}C]-*iso*butyric acid that, in rats, *iso*butyric acid is decarboxylated and may give rise to acetone. *iso*Butanol could therefore give rise to *iso*butyraldehyde and *iso*butyric acid by oxidation, and possibly acetone via *iso*butyric acid. A rabbit was therefore given 6 ml. of *iso*butanol by stomach tube and placed in the tank described by Parke & Williams (1950). The expired air was drawn through Brady's reagent (2:4-dinitrophenylhydrazine in HCl) for 6 hr. No hydrazone was formed and it was concluded that no volatile aldehyde or ketone was eliminated via the lungs. The urine from rabbits receiving *iso*butanol was non-reducing and contained no aldehyde or ketone when tested with Brady's reagent. The glucuronic acid conjugation of this alcohol was low but significant (see Table 2).

DL-2-*Methylbutanol and* iso*amyl alcohol.* These gave urines which did not reduce Benedict's reagent but gave positive naphthoresorcinol tests. The urines from both alcohols did not contain aldehydes or ketones. Both these alcohols yielded about 9–10% of non-reducing glucuronide as determined by the naphthoresorcinol method (see Table 2). In the case of *iso*amyl alcohol, the glucuronide was isolated. The 24 hr. urines from six rabbits which had received a total of 14 g. of *iso*amyl alcohol were worked up by the basic lead acetate method and yielded 0·5 g. of glucuronide gum. This yielded 0·1 g. of *triacetyl β-iso*amyl-D-*glucuronide methyl ester* which formed colourless needles, m.p. 96°, after recrystallization from acetic acid and finally *n*-hexane. (Found: C, 54·0; H, 6·7. $C_{18}H_{28}O_{10}$ requires C, 53·5; H, 7·0%.)

Our studies on 2-ethylbutanol and 2-ethylhexanol are described in the succeeding paper (Kamil, Smith & Williams, 1953).

Secondary alcohols

iso*Propanol.* Neubauer (1901) has referred to a severe case of diabetes studied by Mayer (1899) in which there was a considerable acetone excretion and a large quantity of conjugated glucuronic acid; Neubauer suggested that this glucuronide may have been *iso*propylglucuronide. *iso*-Propanol has also been found in the blood, milk and rumen contents of cows suffering from acetonaemia, and Robertson, Thin & Sterling (1950) suggest that it may be a precursor or a metabolite of acetone. The conversion of *iso*propanol to acetone *in vivo* is well known (cf. Neymark, 1938). Neubauer (1901) has shown by qualitative tests that *iso*propanol increases the output of glucuronic acid in dogs and rabbits. In the present work the conjugation of *iso*propanol with glucuronic acid has been confirmed and the glucuronide isolated.

The expired air of a rabbit which had received 6 ml. of *iso*propanol was drawn through Brady's reagent for 6 hr. after feeding the alcohol. There were isolated 94 mg. (0·5% of dose) of acetone 2:4-dinitrophenylhydrazone, m.p. 124° and mixed m.p. 122° after recrystallization. (Found: N, 23·1%. Calc. for $C_9H_{10}N_4O_4$: N, 23·5%.)

The glucuronide of *iso*propanol was isolated from the 24 hr. urine of seven rabbits which had each received 5 ml. of the alcohol. The acidified $(NH_4)_2SO_4$-saturated urine was extracted with 1:2 ethanol/ether and the extract gave a

gum on evaporation which eventually yielded 0·7 g. of crude *triacetyl β-isopropyl-D-glucuronide methyl ester*. This formed colourless needles, m.p. 140°, after recrystallization from aqueous ethanol and finally *n*-hexane. (Found: C, 51·5; H, 6·4. $C_{16}H_{24}O_{10}$ requires C, 51·1; H, 6·4%.)

DL-*Butan-2-ol.* No studies appear to have been made on this alcohol. In rabbits it appears to form a conjugated glucuronide to the extent of about 14–15% (Table 3). It is also oxidized to ethyl methyl ketone for, in an experiment where the expired air of a rabbit, which had been fed 10 ml. of the alcohol, was drawn through Brady's reagent, we isolated 207 mg. (0·75% of the dose) of ethyl methyl ketone 2:4-dinitrophenylhydrazone, m.p. and mixed m.p. 110°. (Found: C, 47·2; H, 4·6; N, 22·6%. Calc. for $C_{10}H_{12}N_4O_4$: C, 47·6; H, 4·8; N, 22·2%.)

sec.-*Butylglucuronide.* This was isolated from a 24 hr. urine by the basic lead acetate method after feeding three rabbits with 8 ml. each of the alcohol. The glucuronide was a gum (3 g.) which yielded crystalline *triacetyl β-sec.-butyl-D-glucuronide methyl ester* as needles, m.p. 107°, from aqueous ethanol and then *n*-hexane. (Found: C, 52·6; H, 6·7. $C_{17}H_{28}O_{10}$ requires C, 52·3; H, 6·7%.)

Secondary amyl alcohols

Haggard *et al.* (1945) have shown that the secondary pentanols are converted in rats into the corresponding ketones, 38–54% of the dose appearing as ketones in the expired air and urine. Small amounts of the alcohols (0·5–11% of the dose) are also eliminated unchanged by these two routes. We have now found that in rabbits some 40% of pentan-2- and -3-ols are excreted as glucuronides (Table 3). Rabbits which received 0·73 g./kg. of these alcohols fell asleep for several hours. Glucuronides, but no ketones, were isolated from their urines.

Pentan-2-ol. The glucuronide of this alcohol was isolated by the basic lead acetate method. Three rabbits, which had collectively received 6·7 g. of the alcohol, yielded 4·55 g. of purified glucuronide gum in 24 hr. Half of this gum yielded 1·72 g. of *triacetyl β-pentyl-2-D-glucuronide methyl ester*, m.p. 90°. (Found: C, 53·6; H, 7·0. $C_{18}H_{28}O_{10}$ requires C, 53·5; H, 7·0%.) The rest of the dried gum (2 g.) in dry ether with *p*-toluidine (2 g. in ether) yielded the crystalline *p*-toluidine salt of *pentyl-2-glucuronide*, m.p. 132° after recrystallization from ethanol and $[\alpha]_D$ – 30·3° in water (*c*, 1). (Found: C, 57·8; H, 7·8; N, 3·9. $C_{18}H_{29}O_7N$ requires C, 58·2; H, 7·9; N, 3·8%.)

This alcohol did not increase the ethereal sulphate output of the rabbits.

Pentan-3-ol. In this case the glucuronide gum was isolated by extraction of the acidified $(NH_4)_2SO_4$-saturated urine with 1:3 ethanol/ether, and 6·7 g. of the alcohol yielded 7·31 g. of glucuronide gum. From 5·3 g. of the gum there were obtained 4·95 g. of *triacetyl β-pentyl-3-D-glucuronide methyl ester*, m.p. 76°. (Found: C, 53·8; H, 6·9%.)

This alcohol did not increase the ethereal sulphate output of rabbits.

Secondary hexyl alcohols

Hexan-2-ol. The urine was non-reducing to Benedict solution and gave a positive Rothera test and a faint turbidity with Brady's reagent overnight. Thus a small amount of a methyl ketone was being excreted. The naphthoresorcinol test was intense and the glucuronide was extracted (continuous) from the acidified urine with ether. The glucuronide gum (5 g.) yielded *triacetyl β-hexyl-2-D-glucuronide methyl ester* as needles, m.p. 80–85°, from *n*-hexane. (Found: C, 54·6; H, 7·4. $C_{19}H_{30}O_{10}$ requires C, 54·5; H, 7·2%.)

4-*Methylpentan-2-ol.* The urine from rabbits receiving this alcohol gave a non-reducing urine giving a faint Rothera test and a faint turbidity with Brady's reagent. A small amount of a methyl ketone (presumably *iso*butyl methyl ketone) was thus appearing in the urine. The 24 hr. urine from four rabbits which had each received 3 ml. yielded by extraction with 1:4 ethanol/ether a glucuronide gum which was characterized as *triacetyl β-4-methylpentyl-2-D-glucuronide methyl ester*, m.p. 119° (yield, 5·4 g.). (Found: C, 53·7; H, 7·4%.)

Secondary heptyl alcohols

Secondary heptyl alcohols. Four of these alcohols were obtained and all appear to be highly conjugated in rabbits (approx. 60%, see Table 3).

Heptan-2-ol. The glucuronide in this case was isolated by continuous ether extraction. On treatment of an ethereal solution of the gum (2 g.) with *p*-toluidine (2 g.) in ether the *p-toluidine salt* of *β-heptyl-2-D-glucuronide* was obtained as colourless leaflets from ethanol and had m.p. 130° and $[\alpha]_D$ – 39·7° in water (*c*, 1). (Found: C, 59·6; H, 8·2; N, 3·5. $C_{20}H_{33}O_7N$ requires C, 60·1; H, 8·3; N, 3·5%.) The *triacetyl β-heptyl-2-glucuronide methyl ester* formed fine needles, m.p. 80–90°. (Found: C, 54·9; H, 7·3. $C_{20}H_{32}O_{10}$ requires C, 55·6; H, 7·5%.) On hydrolysis of the triacetyl methyl ester (0·75 g.) by refluxing for 7 hr. with N-HCl (50 ml.), heptan-2-ol was liberated and identified as the 3:5-dinitrobenzoate, m.p. and mixed m.p. 49° (authentic heptan-2-ol 3:5-dinitrobenzoate has m.p. 49·4°; see Huntress & Mulliken (1946)).

The expired air of a rabbit which had received 5 ml. of heptan-2-ol was drawn through Brady's reagent. After 1·5 hr. from dosing the reagent became cloudy, and ketone continued to be exhaled up to 5 hr. The precipitate of heptan-2-one 2:4-dinitrophenylhydrazone (0·4 g.) was recrystallized from 70% ethanol and had m.p. 69° not depressed by authentic material. Allen (1930) gives the m.p. of heptan-2-one (*n*-amyl methyl ketone) 2:4-dinitrophenylhydrazone as 89°. Highly purified synthetic heptan-2-one 2:4-dinitrophenylhydrazone was prepared independently for us by Dr G. King who reports the m.p. as 74·5°. (Found: C, 53·3; H, 6·3; N, 19·2. Calc. for $C_{13}H_{18}O_4N_2$: C, 53·1; H, 6·2; N, 19·0%.) This highly purified material did not depress the m.p. of our urinary material and it appears that the m.p. quoted by Allen (1930) is too high.

Heptan-2-one. The output of glucuronide in three rabbits each receiving 0·95 g./kg. orally of *n*-amyl methyl ketone was found to be 39·9, 41·2 and 42·5% (average 41%) of the dose, respectively. Thus a considerable amount of the ketone was reduced to the alcohol, which was then excreted conjugated. The glucuronide was isolated from these urines as a gum by ether extraction and proved to be heptyl-2-glucuronide by the preparation from it of the *p*-toluidine salt of heptyl-2-glucuronide, m.p. and mixed m.p. 130° and $[\alpha]_D^{20}$ – 41·1° in water (*c*, 1). (Found: C, 60·1; H, 8·6; N, 3·3. Calc. for $C_{21}H_{33}O_7N$: C, 60·1; H, 8·3; N, 3·5%.) Also the triacetyl β-heptyl-2-glucuronide methyl ester (m.p. and mixed m.p. 80–90° and $[\alpha]_D$ – 30·6° in $CHCl_3$ (*c*, 1)) was made. (Found: C, 55·8; H, 7·5. Calc. for $C_{20}H_{32}O_{10}$: C, 55·6; H, 7·5%.)

The urine also contained small amounts of the unchanged ketone since it gave a weak Rothera test and a faint precipitate with Brady's reagent.

Heptan-3-ol. The glucuronide gum (8 g.) from the urine of three rabbits which had collectively received 9 g. of heptan-3-ol was obtained by continuous extraction with ether for 32 hr. *Triacetyl β-heptyl-3-D-glucuronide methyl ester* formed long fine needles, m.p. 79–80°. (Found: C, 55·5; H, 7·4%.)

On hydrolysis this ester yielded heptan-3-ol, which was identified as the 3:5-*dinitrobenzoate*, m.p. and mixed m.p. 51°. (Found: C, 54·8; H, 6·0; N, 9·4. $C_{14}H_{18}O_6N_2$ requires C, 54·2; H, 5·9; N, 9·0%.) The 3:5-dinitrobenzoate of heptan-3-ol appears to be a new compound and it was therefore synthesized from heptan-3-ol, pyridine and 3:5-dinitrobenzoyl chloride. The authentic material formed pale yellow plates from aqueous methanol, m.p. 53°. (Found: C, 54·5; H, 5·9; N, 9·0%.) Its m.p. was depressed by the isomeric 3:5-dinitrobenzoate of heptan-2-ol, m.p. 51°.

Heptan-4-ol (*di*-n-*propylcarbinol*). The glucuronide gum (4·5 g.) was obtained from the urine of three rabbits which had each received 3 g. of heptan-4-ol, by continuous extraction with ether for 3 hr. This gum was soluble in $CHCl_3$. *Triacetyl β-heptyl-4-D-glucuronide methyl ester* formed very fine colourless needles from aqueous methanol, m.p. 103°. (Found: C, 55·0; H, 7·7%.)

2:4-*Dimethylpentan-3-ol* (*di*isopropylcarbinol). The urine from three rabbits which had each received 3 g. of the alcohol was extracted for 1 week with ether. The glucuronide gum (7 g.) partly crystallized. It was completely crystallized by dissolution in a little ethanol, adding an excess of benzene and allowing the solution to evaporate slowly. *β-2:4-Dimethylpentyl-3-D-glucuronide* formed colourless needles from ethyl acetate, m.p. 150° and $[\alpha]_D^{25°} - 54° \pm 2°$ in water (*c*, 1). (Found: C, 53·3; H, 8·0. $C_{13}H_{24}O_7$ requires C, 53·4; H, 8·3%.) It was very soluble in water and ethanol, soluble in ether and moderately soluble in ethyl acetate. It formed a *potassium salt* with K_2CO_3 and this, on recrystallization from 95% ethanol, formed colourless needles of the *dihydrate* of $[\alpha]_D^{20°} - 43·3° \pm 1·5°$ in water (*c*, 1). (Found: C, 43·0; H, 7·5; K, 11·3. $C_{13}H_{23}O_7K.2H_2O$ requires C, 42·6; H, 7·4; K, 10·7%.) The *benzylamine salt* of the glucuronide was formed on treating the glucuronide gum (0·3 g.) with benzylamine (0·1 g.). The mixture set solid and was recrystallized from 95% ethanol. This salt (0·23 g.) formed needles, m.p. 195° and $[\alpha]_D - 39·4° \pm 0·5°$ in water (*c*, 1) and was very soluble. (Found: N, 3·3. $C_{20}H_{33}O_7N$ requires N, 3·5%.) *Triacetyl β-2:4-dimethylpentyl-3-D-glucuronide methyl ester* formed colourless needles, m.p. 154°. (Found: C, 55·6; H, 7·5%.)

sec.-*Octyl alcohol* (*octan-2-ol*). This alcohol was a diuretic and yielded a urine which did not reduce Benedict solution but which gave a Rothera test. The ketone (presumably octan-2-one) only occurred in small amounts in the urine and was not isolated. The conjugation of this alcohol with glucuronic acid was low (see Table 3). The glucuronide was isolated as a gum, but neither this nor its triacetyl methyl ester could be induced to crystallize.

Tertiary alcohols

The three tertiary alcohols studied show high conjugation with glucuronic acid, and the glucuronides were readily isolated from the urine.

tert.-*Butyl alcohol.* Six rabbits were each given 8 ml. of the alcohol, and the glucuronide gum (7·2 g.) was isolated from the 24 hr. urine by the basic lead acetate method. On methylation and acetylation the gum yielded *triacetyl β-1:1-dimethylethyl-D-glucuronide methyl ester* (3·76 g. after recrystallization from ethanol/*n*-hexane mixtures), m.p. 168°. (Found: C, 52·3; H, 6·8. $C_{17}H_{26}O_{10}$ requires C, 52·3; H, 6·7%.) No aldehyde or ketone was detected in the expired air of a rabbit which had received 6 ml. of this alcohol.

tert.-*Amyl alcohol.* The glucuronide gum was isolated from the acidified $(NH_4)_2SO_4$-saturated urine by repeated extraction with equal volumes of ethanol/ether (1:4), after feeding 8·25 g. of the alcohol to three rabbits (3 ml. each). On methylation and acetylation of half of the glucuronide gum there was obtained *triacetyl β-1:1-dimethylpropyl-D-glucuronide methyl ester* (2·3 g. pure), m.p. 111°, after recrystallization from ethanol/water. (Found: C, 53·9; H, 7·1. $C_{18}H_{28}O_{10}$ requires C, 53·5; H, 7·0%.)

Estimations of the ethereal sulphate output were also carried out with this alcohol, but no change from normal was noted.

1:1-*Dimethylbutan-1-ol* (tert.-*hexyl alcohol*). The glucuronide of this alcohol was isolated as the basic lead salt formed with basic lead acetate at pH 9. It was obtained as a water-soluble gum from which was prepared *triacetyl β-1:1-dimethylbutyl-D-glucuronide methyl ester*, m.p. 104°. (Found: C, 54·8; H, 7·2. $C_{19}H_{30}O_{10}$ requires C, 54·5; H, 7·2%.)

DISCUSSION

It appears that aliphatic alcohols are metabolized and eliminated from the body along three paths, namely (1) by oxidation and elimination of the products (acids, aldehydes, ketones and carbon dioxide) in the urine and expired air, (2) by conjugation with glucuronic acid and elimination of the glucuronides in the urine and (3) by elimination of the unchanged alcohol in the expired air or urine. Which of these routes constitutes a major pathway for any particular alcohol will depend on a number of factors. Physical factors such as volatility and dose of alcohol are involved to some extent, but the present work suggests that much depends on chemical factors, such as the number of carbon atoms in the alcohol, the nature of the alcoholic hydroxyl group and the extent of branching of the hydrocarbon chain.

It seems that, on the whole, the lower alcohols, e.g. methanol (Lund, 1948), ethanol (Bartlett & Barnet, 1949), amyl alcohol (Haggard *et al.* 1945) are extensively metabolized before being eliminated, since only small amounts are eliminated unchanged at doses of about 1 g./kg. The two processes involved are oxidation and conjugation, and the results reported here suggest that the portion of the alcohol not oxidized is conjugated to form water-soluble glucuronides. Examination of Tables 1–5 shows that the general order of conjugation of primary, secondary and tertiary alcohols is in the order tertiary > secondary > primary, and, since Haggard *et al.* (1945) have shown that oxidation is in the reverse order to this, it follows that the portion of alcohol not oxidized is probably conjugated.

Normal primary alcohols. These alcohols are in general readily oxidized in the body, via aldehydes, to the corresponding acids and to carbon dioxide (e.g. Stotz, 1943, on ethanol; Orskov, 1950, on propanol; Haggard *et al.* 1945, on amyl alcohols). The lower alcohols, methanol and ethanol, only give appreciable amounts of glucuronides when given in large doses (Kamil, Smith & Williams, 1952), but as the chain length increases the conjugation is more apparent, although still less than 10%. This conjugation has been proved by isolation of the glucuronides of methanol, ethanol and *n*-hexanol.

Arborescent primary alcohols. These are discussed in the succeeding paper (Kamil *et al.* 1953).

Secondary alcohols. The main metabolic route of the secondary alcohols may be written

$$RR'CHOH \begin{cases} \nearrow RR'CO \\ \searrow RR'CHOC_6H_9O_6 \end{cases}.$$

In the three cases examined, *iso*propanol, *sec.*-butanol and heptan-2-ol, ketones were found in the expired air, and all the alcohols are conjugated to some extent with glucuronic acid, the amount increasing with molecular weight up to 2-heptanol. Since our observations did not extend beyond 2-octanol we cannot say if this is an exception or whether we have a peak of conjugation at seven carbon atoms.

The reaction $RR'CHOH \rightarrow RR'CO$ appears readily reversible *in vivo*. Heptan-2-one gave rise to heptyl-2-glucuronide (42% of the dose by quantitative estimations), and this was found to be identical with the glucuronide obtained on feeding DL-heptan-2-ol. No evidence was found of any asymmetrical reduction of the ketone, but the optical activity of heptan-2-ol is so small ($[\alpha]_D$ about $\pm 11°$) that it would have been difficult to detect any resolutions in our preparations.

Tertiary alcohols. Oxidation here could only occur by oxidizing the hydrocarbon groups or disrupting the molecule and with this increased difficulty of oxidation the conjugations (Table 4) are high.

Optical rotations of the triacetyl methyl esters

The rotations of these derivatives in chloroform are quoted in Table 6 and have values ranging from $-30°$ to $-40·9°$, which are not very different from the synthetic triacetyl-β-methyl-D-glucuronide methyl ester (Goebel & Babers, 1935). No attempt was made to resolve glucuronides produced from those alcohols having an asymmetric carbon atom, which could have been mixtures of different proportions of two diastereoisomers. Since, however, the optical forms of the alcohols have low rotations, not more than $\pm 10°$, the rotations of these diastereoisomeric glucuronides are not likely to be very different. This can be seen from Table 6. The optically inactive alcohols are *iso*propanol, pentan-3-ol, heptan-4-ol and 2:4-dimethylpentan-3-ol, and the specific rotations of the triacetyl methyl esters are, respectively, $-37·8°$, $-33·3°$, $-37·7°$ and $-40·9°$. All derivatives of the optically active alcohols are not far from this range, the lowest being the glucuronide of heptan-2-ol, $-31·6°$, and the highest that of 2-methylpentan-2-ol, $-47·3°$.

Table 6. *The optical rotations of* 1% *solutions in chloroform of the triacetyl methyl esters of aliphatic β-glucuronides*

β-Glucuronide	M.p. (°)	$[\alpha]_D^{20°}$ (c, 1)	$[M]_D/100$
Primary alcohols:			
Methyl*	148 (149–150)†	−30·3 (−28·9)†	−105
Ethyl*	144	−32·8	−118
n-Hexyl	70–71	−31·8	−133
*iso*Amyl	96	−30	−121
Secondary alcohols:			
*iso*Propyl	140	−37·8	−132
Butyl-2-	107	−37·0	−144
Pentyl-2-	90	−44·2	−179
Pentyl-3-	76	−33·3	−135
Hexyl-2-	80–85	−37·2	−155
4-Methylpentyl-2-	119	−47·3	−198
Heptyl-2-	78	−31·6	−137
Heptyl-3-	79–80	−35·7	−154
Heptyl-4-	103	−37·7	−163
2:4-Dimethylpentyl-3-	154	−40·9	−177
Tertiary alcohols:			
tert.-Butyl	168	−29·2	−114
tert.-Amyl	111	−36·6	−148
1:1-Dimethylbutyl (*tert.*-hexyl)	104	−27·4	−115

* Kamil *et al.* (1952 and unpublished observations).
† Recorded by Goebel & Babers (1935) for the synthetic compound.

SUMMARY

1. A study has been made of the glucuronic acid conjugation in the rabbit of thirty aliphatic alcohols. All were found to conjugate to some extent.

2. The conjugation of normal primary alcohols from methyl to decyl was found to be low, being less than 10% of the dose, but its occurrence was proved by isolation.

3. The conjugation of secondary alcohols increased with increasing number of carbon atoms in the molecule from 10% with *iso*propanol to 60–70% for the secondary heptyl alcohols. With octan-2-ol the conjugation was low. The oxidation products of some of the secondary alcohols (*iso*propanol, butan-2-ol and heptan-2-ol) were proved to be ketones.

4. The conjugation of three tertiary alcohols, butyl, amyl and hexyl, was higher than that of the corresponding primary and secondary alcohols.

5. The results suggest that conjugation is an alternative metabolic process to oxidation. The order of conjugation is tertiary > secondary > primary, which is the reverse of the order for ease of oxidation.

6. In most cases the glucuronides were isolated and characterized as triacetyl methyl esters.

7. Aliphatic alcohols do not appear to form ethereal sulphates.

The expenses of this work were in part defrayed by a grant from the Medical Research Council. One of us (I.A.K.) is grateful to the Egyptian Government (Ministry of Public Health) for a grant which enabled him to participate in the work.

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