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Studies in Detoxication

50. THE ISOLATION OF METHYL AND ETHYL GLUCURONIDES FROM THE URINE OF RABBITS RECEIVING METHANOL AND ETHANOL

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The literature on the metabolism of ethanol has been reviewed recently by Jacobsen (1952*a, b*), and it seems clear that ethanol is mainly oxidized in the body to carbon dioxide via acetaldehyde and acetic acid. Methanol is also oxidized to formaldehyde and formic acid (Lund, 1948*a, b*) and according to Du Vigneaud & Verly (1950) (cf. also Du Vigneaud *et al.* 1951) it can serve indirectly as a precursor of labile methyl groups. It is well known, however, that methanol is more slowly eliminated from the body than ethanol (cf. Bartlett, 1950; Leaf & Zatman, 1952).

Recently we showed that in rabbits most primary aliphatic alcohols were conjugated to a small extent with glucuronic acid (Kamil, Smith & Williams, 1953). The conjugations of methanol and ethanol, however, were so small as to be almost

within the experimental error of the quantitative method used. It was therefore uncertain whether these alcohols were conjugated. In the present paper, we show by the isolation of the glucuronides that these two alcohols do, in fact, conjugate with glucuronic acid in the rabbit.

EXPERIMENTAL

Glucuronic acid was determined by the method of Hanson, Mills & Williams (1944). All animals were kept on a constant diet and the pure alcohols, diluted with water, were administered by stomach tube.

Ethanol was fed at three levels, 2.9, 7.2 and 14.4 ml./3 kg., or 0.05, 0.125 and 0.25 mole/3 kg. rabbit, and methanol at two levels, 3 and 10 ml./3 kg. or 0.075 and 0.25 mole/kg., each dose being studied in three animals. A number of

experiments were also carried out with animals treated with 'Antabuse', tetraethylthiuram disulphide, m.p. 63-65°, (Light and Co. Ltd.), and with mixtures of ethanol and methanol, but the results were erratic and these are not quoted.

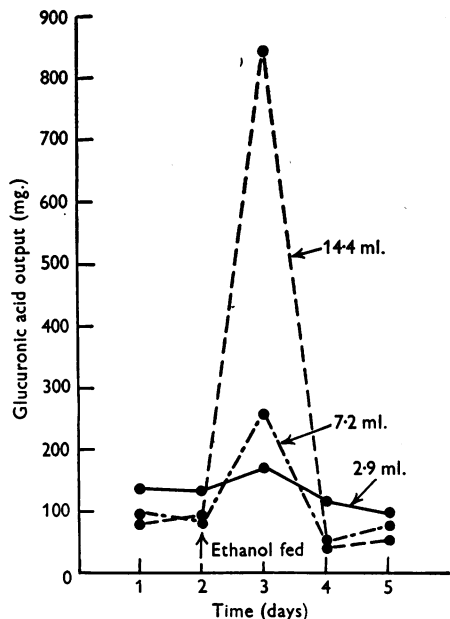


Fig. 1. The effect of ethanol on the glucuronic acid excretion of rabbits. ●—●, Dose 2.9 ml. (rabbit no. 31); ●—●, 7.2 ml. (rabbit no. 25); ●---●, 14.4 ml. (rabbit no. 24).

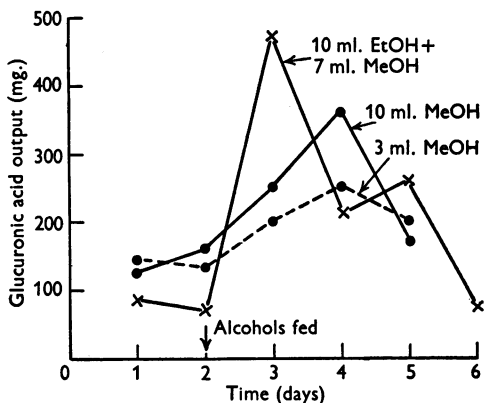


Fig. 2. The effect of methanol and a mixture of ethanol and methanol on the glucuronic acid excretion of rabbits. ●---●, 3 ml. methanol (rabbit no. 31); ●—●, 10 ml. methanol (rabbit no. 21); ×—×, 10 ml. ethanol plus 7 ml. methanol (rabbit no. 32).

In the case of ethanol there was a rise in glucuronic acid excretion for 1 day after dosing and the higher the dose the greater was the excretion of glucuronic acid (Table 1 and Fig. 1). With methanol the glucuronic acid remained slightly above normal for 2-3 days (Fig. 2), and it was con-

sequently difficult to assess the conjugation. When 'Antabuse' was fed to rabbits just before dosing with ethanol, the glucuronic acid output was trebled (Table 1), but 'Antabuse' alone stimulated a small extra glucuronic acid output (Fig. 3).

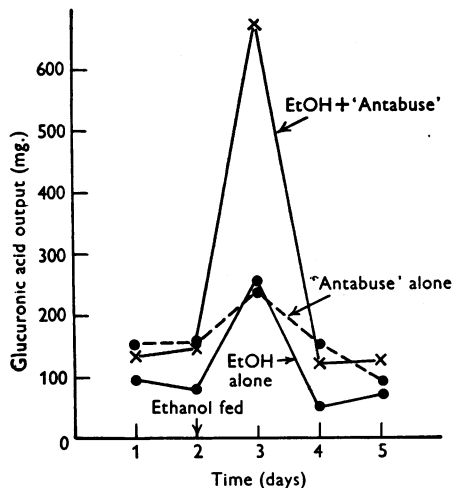


Fig. 3. The effect of 'Antabuse' on the glucuronic acid excretion of rabbits receiving ethanol. ●---●, 1 g. 'Antabuse' alone (rabbit no. 89); ●—●, 7.2 ml. ethanol (rabbit no. 26); ×—×, 7.2 ml. ethanol plus 1 g. 'Antabuse' (rabbit no. 89).

Table 1. The excretion of glucuronic acid by rabbits receiving ethanol orally

Rabbit* no.	Dose of ethanol (ml./kg.)	'Extra' glucuronic acid excreted in 24 hr. after dosing (mg.)	Conjugation (% of dose)
31	0.97	51	0.58
32		32	0.36
33		50	0.56
21	2.4	140	0.64
25		142	0.64
26		183	0.84
4	2.4 + 1 g. 'Antabuse'	372	1.96
11		382	1.83
89		540	2.58
8	4.8	563	1.23
24		775	1.78
27		776	1.78

* All rabbits weighed about 3 kg.

In one experiment, 0.7 g./kg. of vinyl acetate (monomer) was fed, but all the rabbits died within 24 hr. This compound was fed because vinyl alcohol (CH_2CHOH) is a possible dehydrogenation product of ethanol and is isomeric with acetaldehyde.

Isolation of glucuronides

(Melting points are uncorrected.)

Methanol. Six rabbits were each given 10 ml. of methanol in 20 ml. water and the urine collected for the following

48 hr. The glucuronide gum (approx. 1 g.) from the pooled urines was isolated in the usual manner by systematic precipitation with lead acetate (Kamil, Smith & Williams, 1951). The gum was methylated with ethereal diazomethane and the resultant gummy methyl ester acetylated overnight at room temperature with a threefold excess of pyridine and acetic anhydride based on the weight of the ester. After dilution with 200 ml. water, the solution was extracted with 5×10 ml. of CHCl_3 . The extract was washed with 2N-HCl followed by saturated NaHCO_3 and dried. On evaporation a gum was obtained which crystallized (0.35 g.) on rubbing with ethanol. On recrystallization from absolute ethanol the triacetyl β -methyl-D-glucuronide methyl ester formed prisms, m.p. 150–151°, $[\alpha]_D^{20} - 30.3^\circ$ ($c=1$ in CHCl_3). (Found: C, 48.1; H, 5.9. Calc. for $\text{C}_{14}\text{H}_{20}\text{O}_{10}$, C, 48.3; H, 5.7%). This compound was identical (mixed m.p. 151–152°) with an authentic synthetic specimen, m.p. 152–153° and $[\alpha]_D - 28.4^\circ$ ($c=1$ in CHCl_3) kindly given to us by Dr N. E. Artz (Corn Products Refining Co., Argo, Illinois). Goebel & Babers (1935), who first synthesized this compound, gave m.p. 149–150° and $[\alpha]_D - 28.9^\circ$ in CHCl_3 .

Ethanol. Three rabbits were each given 14 ml. ethanol diluted with water. The urine was collected for 24 hr. and contained 1.5 g. of glucuronic acid by the quantitative naphthoresorcinol method. The glucuronide gum (approx. 1 g.) was prepared via the basic lead salt and was then methylated with ethereal diazomethane. The gummy methyl ester was dissolved in pyridine (6 ml.) and acetic anhydride (6 ml.) and after 48 hr. at room temperature was poured into 50 ml. water. The product (0.12 g.) which separated at 0° was recrystallized from absolute ethanol. The triacetyl β -ethyl-D-glucuronide methyl ester formed needles, m.p. 143°, undepressed by a synthetic sample, and $[\alpha]_D^{20} - 32.8^\circ$ ($c=1$ in CHCl_3). (Found: C, 49.75; H, 6.4. $\text{C}_{16}\text{H}_{22}\text{O}_{10}$ requires C, 49.7; H, 6.1%). A synthetic sample of this compound, m.p. 142–143° and $[\alpha]_D - 31.4^\circ$ in CHCl_3 , was kindly provided by Dr N. E. Artz. Dr Artz informs us that this compound has not previously been described in the literature.

DISCUSSION

Both methanol and ethanol are mainly oxidized in the organism to carbon dioxide (cf. Bartlett & Barnet, 1949; Bartlett, 1950). The present work, however, shows that they form small amounts of glucuronides in rabbits. In the case of ethanol, the excretion of glucuronide is complete in 24 hr. (Fig. 1) and, with large doses, the glucuronide excretion may exceed 1% of the dose. Like most

other primary aliphatic alcohols (cf. Kamil *et al.* 1953), ethanol can be metabolized along two routes, namely oxidation and glucuronic acid conjugation. Since the oxidation of ethanol is apparently very rapid, conjugation takes place to only a minor extent. Increasing the dose, however, causes conjugation to increase, but even after 14 ml. the conjugation only accounts for just over 1% of the dose. There is also an increase in conjugation after pre-dosing the animals with 'Antabuse'. This substance is known to inhibit the oxidation of acetaldehyde (see Jacobsen, 1952*b*); this in turn would be expected to slow up the oxidation of ethanol and drive some of it into conjugation with glucuronic acid.

In the case of methanol, since it is slowly oxidized, it could be expected to conjugate with glucuronic acid to a greater extent than ethanol. This appears to be true since the glucuronic acid output after methanol remains above normal for 2–3 days after dosing (Fig. 2). The increase in glucuronic acid each day, however, is small and, owing to the limitations of the quantitative method used, it is difficult to assess by this method exactly how much is conjugated.

SUMMARY

1. Methyl and ethyl β -D-glucuronides have been isolated from the urines of rabbits dosed with methanol and ethanol, respectively, and characterized as the crystalline triacetyl methyl esters. The glucuronides are only very minor metabolites of these alcohols.

2. The excretion of increased amounts of glucuronic acid after feeding single doses of ethanol is complete within 24 hr. of dosing, whereas after methanol the increased excretion continues for 2–3 days.

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