THE PHENOLIC ACIDS OF URINE—A STUDY OF METHYLATION

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Normal human urine contains methanol largely as methoxylated compounds; a small quantity of free methanol is also present. The methoxylated compounds exist partly as ether-soluble compounds, for example methylated phenolic acids, and partly as methoxylated compounds not extracted by ether from acid solution. The latter may be related chemically to quercitrin. Feeding experiments suggest that 3,4-dihydroxyphenolic substances are largely methylated while with other phenolic substances methylation is minimal.

THE present communication is an extension of previous studies.[1,2]. It has been shown that, in man tannic and 3,4-dihydroxybenzoic acids are largely methylated to 4-hydroxy-3-methoxy derivatives. The present paper is concerned with an examination of the nature, in very general terms, of methoxy compounds in human urine and also of the extent to which phenols and phenolic acids, other than those with an *ortho* dihydroxy structure, are methylated.

METHODS

The general principles of the techniques employed have been described[2]. The following fractions have been determined.

(*a*) *Free methanol.* 10 ml. of urine diluted to 15 ml. with water was heated to boiling in a 100 ml. R.B. flask attached to a water cooled condenser and 10 ml. of distillate collected.

(*b*) *Methanol liberated by the action of hot strong sulphuric acid on methoxylated compounds present in untreated urine.* Into a 100 ml. R.B. flask attached to a water cooled condenser (all glass equipment) were introduced 10 ml. of urine and 5 ml. of concentrated sulphuric acid. The mixture was heated to boiling and the distillate collected. Heating was continued until the sulphuric acid reached the fuming stage after which the mixture was allowed to cool. After the addition of 5 ml. of water, the mixture was again heated, the sulphuric acid being allowed to reach the fuming stage. This part of the procedure was repeated, so that three distillates in all were collected. A knife point of sodium bicarbonate was added to the combined distillates. The mixture was heated in apparatus similar to that described above and 10 ml. of distillate collected.

(*c*) *Methanol liberated by the action of hot strong sulphuric acid on the acidic fraction of urine.* 10 ml. of urine and 1 ml. of 10N hydrochloric acid in a test tube were heated in a boiling water bath for 1 hour. After cooling, the urine was extracted three times with 40 ml. quantities of redistilled ether. The combined ether extracts were evaporated to dryness.

10 ml. of water and 5 ml. of concentrated sulphuric acid were added to the residue, the procedure as described in (*b*) being then carried out.

Methanol was determined in the distillate[2].

Results and Discussion

The results obtained from the examination of 10 urines are shown in Table I.

TABLE I

FREE "METHANOL" AND METHOXYLATED COMPOUNDS IN HUMAN URINE AS MG. OF METHANOL/DAY

	Free "methanol"	Methoxy compounds		
		Untreated urine (*b*)	Phenolic and acidic fraction (*c*)	
1 ..	6·4	33·2	20·0	(100)
2 ..	9·1	26·6	15·9	(80)
3 ..	1·3	63·0	25·0	(125)
4 ..	4·6	28·4	18·6	(93)
5 ..	4·8	38·6	28·4	(142)
6 ..	7·2	18·8	11·6	(58)
7 ..	8·2	46·8	31·8	(159)
8 ..	4·6	23·6	18·4	(92)
9 ..	3·8	38·4	31·2	(156)
10 ..	4·2	28·6	18·2	(91)

The figures in brackets refer to the vanillic acid (4-hydroxy-3-methoxybenzoic acid) equivalents

Normal human urine contains a small amount of free methanol. The values are about the same as those obtained by Leaf and Zatman[3]. The origin of urinary methanol is obscure but it may be derived from the *in vivo* hydrolysis of methoxy compounds. Leaf and Zatman found that

TABLE II

THE URINARY EXCRETION OF METHOXY COMPOUNDS (MG. OF METHANOL/8 HOURS) AFTER THE ORAL INGESTION OF 1 G. OF SOME PHENOLIC AND RELATED COMPOUNDS

Compound	Methoxy compounds	
	Phenolic and acidic fraction	
	After compound	Control
Salicylic acid	10·3	5·1
Salicylic acid	9·8	4·8
Salicylic acid	8·7	4·6
m-Hydroxybenzoic acid	11·2	6·6
m-Hydroxybenzoic acid	10·4	6·3
m-Hydroxybenzoic acid	8·6	5·8
p-Hydroxybenzoic acid	12·4	5·5
p-Hydroxybenzoic acid	11·8	5·6
p-Hydroxybenzoic acid	8·6	4·9
3,4-Dihydroxybenzoic acid	24·8	4·9
3,4-Dihydroxybenzoic acid	29·8	5·1
3,4-Dihydroxybenzoic acid	26·8	4·6

the percentage recovery from urine of orally administered methanol was extremely low, which suggests that appreciable quantities of methanol may be metabolised. Normal urine appears to contain methylated phenolic acids and also methoxylated substances which are not readily extracted by ether from acid solution. Although the metabolites of adrenaline

are methoxylated compounds[4,5], the methoxylated compounds encountered in urine are probably mainly of dietary origin. The ether insoluble compounds are probably related chemically to quercitrin.

The urinary excretion of methoxylated compounds after the oral administration of some phenolic substances was studied. The night urine (8 hours) was used to minimise the effect of dietary intake. The results are shown in Table II. Methylation of *o*-, *m*- and *p*-hydroxybenzoic acids and resorcinol does appear to take place but not on the same scale as occurs with the 3,4-dihydroxyphenolic acids.

Substances containing a catecholic structure, for example, tannic acid, are widely consumed by man, and as a result are assumed to be non-toxic. There are, however, reports of liver damage[1], and hepatoma[6] by tannic acid. The position is therefore anomalous.

References

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