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Pathways for Formation of Catechol and 1,2,4-Benzenetriol in Rabbits

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Benzene, an established human leukemogen (International Agency for Research on Cancer 1982), was once widely used as an industrial solvent (Browning 1965) and is currently an important material for organic synthesis (International Agency for Research on Cancer 1982). Its metabolism in man and animals has also been studied extensively, and phenolic compounds (e.g., phenol, catechol, etc.) were identified as major metabolites in urine after benzene exposure. One point yet to be elucidated is the pathway for formation of catechol (or 1,2-benzenediol). Early in 1959, Williams summarized extensive studies of his group to suggest that catechol will be formed via phenol whereas a later study from his group failed to identify catechol in the urine of men and rabbits after oral administration of ^{14}C-phenol (Capel et al. 1972). Sensitive HPLC methods have been recently developed in our laboratory to measure urinary phenolic metabolites and t,t,-muconic acid (Inoue et al. 1988a, b and c). The methods were applied to show that phenol is not a precursor of catechol in rabbits. Evidence is also presented that 1,2,4-benzenetriol is formed only from quinol (1,4-benzenediol) and not from catechol.

MATERIALS AND METHODS

Male Japanese white rabbits, weighing about 2 kg, were purchased from Funabashi Farm (Funabashi, Japan). The animal room was lighted from 0800 till 2000, and kept dark thereafter till 0800 next morning. The rabbits were allowed free access to laboratory chow and water through out the experiment. In the experiment, the rabbits (5 animals per group) were individually housed

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in metabolic cages and given 50 mg/kg of either phenol, quinol or catechol in saline intraperitoneally [i.e., one third to one fourth LD_{50} (Sweet 1987)] at 1800. Urine excreted thereafter was collected separately from faeces for two 24-hr periods (i.e., for 48 hrs). At each time of sampling, the urine was combined with the wash of the funnel in the bottom of the cage to a volume of 200 mL.

HPLC analyses of urine for catechol (Inoue et al. 1988a), quinol (Inoue et al. 1988a), t,t-muconic acid (Inoue et al. 1988b) and 1,2,4-benzenetriol (Inoue et al. 1988c) were conducted as previously described. In brief, the urine sample for catechol and quinol determination was acid-hydrolyzed by heating and extracted with carbon disulfide-diethyl ether (1:1 by volume), and the extract after removal of the solvent by evaporation was taken up in acetonitrile-water (3:7 by volume) and subjected to HPLC analysis on a Hitachi No. 3056 column with a mobile phase of acetonitrile-acetic acid-water (15.0:1.5:83.5 by volume). For 1,2,4-benzenetriol determination, the sample was heated for acid-hydrolysis in the presence of pyrogallol. The hydrolyzate was kept overnight in dark and the supernate was analyzed on a Spherisorb ODS 5um column with a mobile phase of methanol-water-acetic acid (20:971:9 by volume). In the case of t,t-muconic acid measurement, the urine was mixed with an equal volume of methanol and the supernate was injected into a HPLC equipped with a Spherisorb ODS 5um column. The mobile phase was a mixture of methanol-acetic acid (1:9 by volume). All metabolites were measured spectrophotometrically, i.e., catechol, quinol, 1,2,4-benzenetriol and t,t-muconic acid at 280, 280, 290 and 265 nm with a detection limit of 0.5, 1.0, 0.5 and 0.1 mg/L, respectively. The recovery rate was between 96-101%.

RESULTS AND DISCUSSION

When phenol (50 mg/kg) was given i.p., there was a significant ($p<0.01$) increase in the urinary level of quinol, but not that of catechol nor t,t-muconic acid. The increase in the level of 1,2,4-benzenetriol was insignificant ($p>0.05$) in the 1st 24-hr period but up to double the control level ($p<0.05$) in the 2nd 24-hrs (Table 1). The administration of catechol did not result in any increase of the metabolite levels except for the level of the compound itself (i.e., catechol). In contrast, when quinol was given, a significant increase ($p<0.01$) was detected in the urinary 1,2,4-benzenetriol level in addition to that of quinol. t,t-Muconic acid level did not show any change. The increase in urinary catechol in the 2nd 24-hr period

Table 1. Urinary excretion of 1,2,4-benzenetriol, and t,t-muconic acid after i.p. administration of phenol, catechol and quinol in rabbits

Compound given[a] (No.[b])	Metabolite measured	Amount (mg/kg) excreted in 1st 24 hrs	2nd 24hrs
Phenol (5)	1,2,4-Benzenetriol	0.26±0.06	0.63±0.49*
	t,t-Muconic acid	ND[c]	ND
	Catechol	1.09±0.37	1.18±0.60
	Quinol	3.26±0.61**	1.63±0.72
Catechol (5)	1,2,4-Benzenetriol	0.55±0.65	0.40±0.32
	t,t-Muconic acid	ND	ND
	Catechol	9.73±2.32**	1.47±0.72
	Quinol	0.79±0.11	0.95±0.38
Quinol (5)	1,2,4-Benzenetriol	3.13±0.45**	0.27±0.08
	t,t-Muconic acid	ND	ND
	Catechol	1.19±0.23	1.57±0.38*
	Quinol	18.31±3.90**	1.20±0.21
None[d] (15)	1,2,4-Benzenetriol	0.31±0.22	
	t,t-Muconic acid	ND	
	Catechol	0.96±0.60	
	Quinol	1.19±0.69	

Numbers in the table are mean±SD. Asterisks indicate a significant (** for P<0.01 and * for P<0.05) increase over the corresponding control value. .SR3

a/ The dose given i.p. was 50 mg/kg of either phenol, catechol or quinol.

b/ Number of animals in parentheses.

c/ Below the detection limit. The detection limit for 1,2,4-benzenetriol, t,t-muconic acid, catechol and quinol is 0.1, 0.02, 0.1 and 0.2 mg/24 hrs, respectively.

d/ Controls.

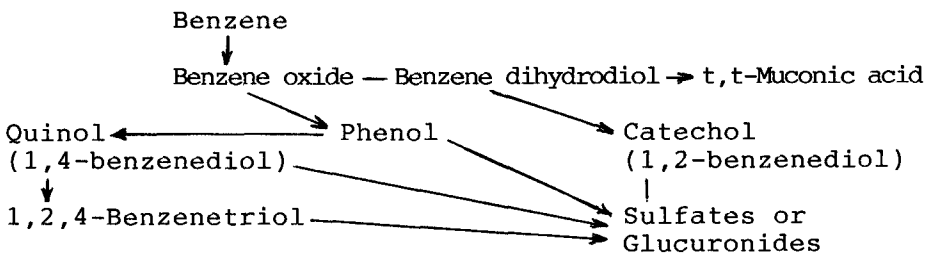

Figure 1. Oxidative metabolism of benzene in rabbits

after quinol administration was statistically significant ($p<0.05$) but the increment was less than two thirds of the control level.

The present in vivo study with rabbits clearly demonstrated that the formation of catechol (1,2-benzenediol) from phenol is negligible if any, that 1,2,4-benzenetriol is formed via phenol and quinol (1,4-benzenediol) and not via catechol (1,2-benzenediol), and that none of the three phenolic compounds tested is a precursor of t,t-muconic acid. Thus, the results in connection with previous observation (Cooper and Snyder 1988) can be summarized as shown in Fig. 1.

The present observation on the lack of conversion of phenol to catechol in vivo is in accordance with the finding by Capel et al. (1972) that no radioactive catechol was detected in the urine of rabbits given 25 mg ^{14}C-phenol/kg p.o. and disagrees with that of Parke and Williams (1953) in which 0.5-1.0% of the radioactivity was recovered in catechol fraction of the urine of rabbits dosed with 50 mg ^{14}C-phenol/kg p.o. Sawahara and Neal (1981) incubated ^{14}C-phenol with rat liver liver microsomes and found catechol as a minor metabolite in addition to quinol as a major metabolite, whereas no excretion of radioactive catechol was detected in the urine of rats after p.o. administration of ^{14}C-phenol (Capel et al. 1972; Kao et al. 1979). It may be possible to speculate that the phenol level in liver cells after phenol administration to experimental animals should be much lower than the phenol levels used in in vitro experiment so that phenol will be almost exclusively converted to quinol in vivo.

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