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GLYCURONIC ACID AND GLYCURONAMIDE DETOXICATING ACTIVITY

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Abstract—The action of glycuronic acid and glycuronamide on phenol and *p*-amino-phenol glycuronide excretion in rats have been studied.

Glycuronamide and, to a lesser extent, glycuronic acid increase the urinary excretion of phenol and *p*-aminophenol glycuronides in rats receiving these compounds. The mechanism of detoxication and the difference of activity glycuronic acid and glycuronamide are discussed.

AS DEMONSTRATED by Dutton and Storey,[1, 2] uridindiphosphoglycuronic acid (UDPGA) acts as a glycuronic acid residue donor in the synthesis of glycuronides of many physiological or non physiological substances. Since free glycuronic acid cannot be utilized for the synthesis of UDPGA in animal tissues, it should have no action on the synthesis of glycuronides. But several biochemical and pharmacological researches by Otobe,[3] Takatsu,[4] Shioya,[5] Della Pietra[6, 7] and so on, have stated that exogenous glycuronic acid or some of its derivatives (glycuronolactone, glycuronamide) can detoxicate many toxic substances.

The purpose of the present study is to demonstrate the detoxicating action of glycuronic acid and glycuronamide against phenol or *p*-amino-phenol in rats and to compare the relative detoxicating activities.

MATERIALS AND METHODS

Animals. Male albino rats (Morini strain, Italy) 200 g wt., have been used; the animals were maintained on Morini diet.

Drugs. Phenol (300 mg/kg), *p*-aminophenol (300 mg/kg), glycuronic acid (200 mg/kg) and glycuronamide (200 mg/kg) were administered in aqueous solution (pH 6) by stomach tube. Either phenol and *p*-aminophenol were administered one hour after glycuronic acid or glycuronamide.

Urine collection. For each animal, 24-hr urines were collected using individual metabolic cages; urine collection started from the phenol or *p*-aminophenol administration.

Assay procedure. Each 24-hr urine was diluted to 30 ml; glycuronic acid was determined according to Fishman,[8] with some modifications.[6, 7] Phenol was determined by the method of Porteous and Williams.[9] For *p*-aminophenol the method was that of Brodie and Axelrod.[10] For the determination of total conjugated phenol or *p*-aminophenol, urine aliquots were hydrolysed for 1 hr in a boiling bath with 5 parts H_2SO_4 IO N. Glycuronized phenol or *p*-aminophenol was determined after enzymatic

digestion or urine aliquots with β-glycuronidase (Sigma): 15 mg enzyme powder for each ml of urine.

Other experimental conditions are described in[6, 7].

RESULTS AND DISCUSSION

The obtained results are reported in Table 1 and in Table 2.

TABLE 1. TOTAL GLYCURONIC ACID, FREE, TOTAL CONJUGATED AND GLYCURONIZED PHENOL EXCRETED IN 24-HR URINES*

Treatment	Free Phenol μmole	Total Conjugated Phenol μmole†	Glycuronized Phenol μmole‡	Total Glycuronic acid μmole
Control	2 ± 0·3	3 ± 0·2	2 ± 0·3	61 ± 10
Phenol	17 ± 3	38 ± 6	28 ± 5	123 ± 15
Glycuronic acid + Phenol	23 ± 6	93 ± 10	69 ± 8	139 ± 18
Glycuronamide + Phenol	26 ± 6	159 ± 20	119 ± 15	360 ± 25

* Each value is the mean of duplicate determinations in single urines of forty rats ± S.E.

† Phenol after acid hydrolysis of urine minus free phenol.

‡ Phenol after enzymatic digestion of urine with β-glycuronidase minus free phenol.

As shown in Table 1 glycuronamide, and to a lesser extent, glycuronicacid increase the excretion of total phenol, and especially of glycuronized phenol in rats receiving phenol. In these animals the excretion of glycuronic acid is instead stimulated only by glycuronamide and insignificantly by glycuronic acid.

From the data of Table 1 is also evident that in rats receiving glycuronamide + phenol, glycuronic acid excreted is much higher than glycuronized phenol. It is possible that glycuronic acid might have been utilized also for the synthesis of gluronnides of phenol metabolites (cate phol, hydroquinone and so on). The same considerations can be extended to the results obtained in rats receiving *p*-aminophenol, as shown in Table 2.

TABLE 2. TOTAL GLYCURONIC ACID, FREE, TOTAL CONJUGATED AND GLYCURONIZED *p*-AMINOPHENOL EXCRETED IN 24-HR URINES*

Treatment	Free *p*-Aminophenol μmole	Total Conjugated *p*-Aminophenol μmole†	Glycuronized *p*-Aminophenol μmole‡	Total Glycuronic acid μmole
Control	1·8 ± 0·2	0·9 ± 0·2	0·9 ± 0·2	61 ± 10
p-Aminophenol	8 ± 1	62 ± 5	47 ± 2	139 ± 15
Glycuronic acid + *p*-Aminophenol	16 ± 1·5	82 ± 10	64 ± 8	149 ± 15
Glycuronamide + *p*-Aminophenol	17 ± 1·5	111 ± 10	94 ± 10	221 ± 20

* Each value is the mean of duplicate determinations in single urines of forty rats ± S.E.

† *p*-Aminophenol after acid hydrolysis of urine minus free *p*-aminophenol.

‡ *p*-Aminophenol after enzymatic digestion of urine with β-glycuronidase minus free *p*-aminophenol.

About the detoxicating mechanism of exogenous glycuronic acid or glycuronamide the suggested hypotheses are:

(1) Glycuronic acid (or its derivatives) could be metabolized by pentose pathway to glucose which, in turn, could be utilized for the synthesis of UDPGA.[11]

(2) Glycuronic acid could be phosphorylated to glycuronic I-phosphate and this compound might react with UTP to form UDGPA. These reactions have been demonstrated only in *Phaseolus Aureus* by Feingold and *et al*;[12, 13] according to Strominger[11] the same reactions might also be present in animal tissues after stimulation by exogenous glycuronic acid.

(3) The hydrolysing enzyme β-glycuronidase, according to Fishman,[14] could also catalyze the glycuronide synthesis from free glycuronic acid.

(4) The most probable mechanism of detoxicating action of free glycuronic acid is suggested by Marsh's research;[15, 16, 17] this demonstrates that glycuronic acid is oxidized to D-glucaric acid, which is a strong inhibitor, also *in vitro*[18, 19] of β-glycuronidase. According to these results, exogenous glycuronic acid can reduce the hydrolysis of endogenous glycuronides with the effect of an increase in their excretion. The higher detoxicating efficacy of glycuronamide can depend on its pharmacological properites. In fact Shioya[20] has demonstrated that glycuronic acid is absorbed by the intestinal mucous membrane less quickly than glycuronamide; moreover glycuronic acid is destroyed by the intestinal flora, whereas glycuronamide is not.[21]

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