Metabolism of 2-Thiophenecarboxylic Acid in Rats (36012)

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Since Fang (1) observed the hypoglycemic action of 2-thiophenecarboxylic acid (2-TCA) in diabetic rats, multiple pharmacological effects of this simple compound were discovered. These included the antilipolytic action in normal and diabetic rats (2, 3), hypocalcemic and hypophosphatemic actions in intact and parathyroidectomized rats (4, 5). It was further demonstrated that several compounds structurally related to 2-TCA were hypocalcemic (4). The mechanism of the hypocalcemic action of 2-TCA had been elucidated in tissue culture to be its specific inhibition on calcium transfer out of osseous tissue (6).

Blicke (7) reviewed the old literature and stated that 2-TCA was excreted in urine by rabbits, dogs, and human subjects, partly unchanged and partly as 2-thenoylglycine (2-TG). Since 2-TCA is not toxic to rats (4), it is of interest to study the metabolism of 2-TCA in rats. Results presented in this report have confirmed that in rats 2-TCA is almost completely excreted in urine and 2-TG is the major metabolite. Also noted in this study is that 2-TCA significantly increases the volume of urine excreted.

Materials and Methods. Male Holtzman rats, weighing 145 to 170 g, were maintained on Purina Rat Chow and tap water *ad libitum*. 2-Thiophenecarboxylic acid was purchased from Aldrich Chemical Co., Inc. Milwaukee, WI and was recrystallized twice from ethanol solution. 2-Thenoylglycine was synthesized from 2-thiophenecarbonyl chloride and sodium glycinate in a slightly alkaline medium by means of a reaction similar to the synthesis of hippuric acid (8) and was recrystallized twice from warm acidic solution (1 *N* HCl). A neutral aqueous solution of their sodium salts was used for all injections. The ultraviolet absorption of 2-TCA and 2-TG was carried out using Zeiss Q-II spectrophotometer.

Urine samples of rats. At the start of the experiment (8:00 to 9:00 a.m.), rats were lightly anesthetized with ether to facilitate injection. Ether was also used to cause voiding of urine present in the bladder. After administration of the compound (dose: 2 or 4 mmoles/kg) or normal saline (5 ml/kg), the animals were individually caged without food and water. Urine was collected on wax paper and transferred to graduated centrifuge tubes for volume measurement.

2-TCA and its metabolite in rat urine were characterized by thin-layer chromatography (TLC). TLC plates were prepared by spreading on glass plates with a slurry of 35 g of silica gel (GF-254 from E. Merck AG, Darmstadt) in 70 ml of water. Five microliters of urine were spotted on the TLC plates for development in 95% ethanol:acetic acid (98:2, v/v). The separate spots observed under ultraviolet light were removed with a spatula and extracted with exactly 5 ml of 0.001 *N* NaOH. The metabolite concentration was determined by spectrophotometry.

Results. Rat urinary metabolites of 2-TCA. Rats receiving 2-TCA by oral or parenteral route eliminated the compound solely in the urine. Figure 1 illustrates the typical pattern of urine samples from a rat injected sc with 2-TCA (2 mmoles/kg). After TLC development, 2 spots of metabolites could be detected in urine as soon as 10 min after the administration of 2-TCA. The fast moving spot gradually disappeared from urine sam-

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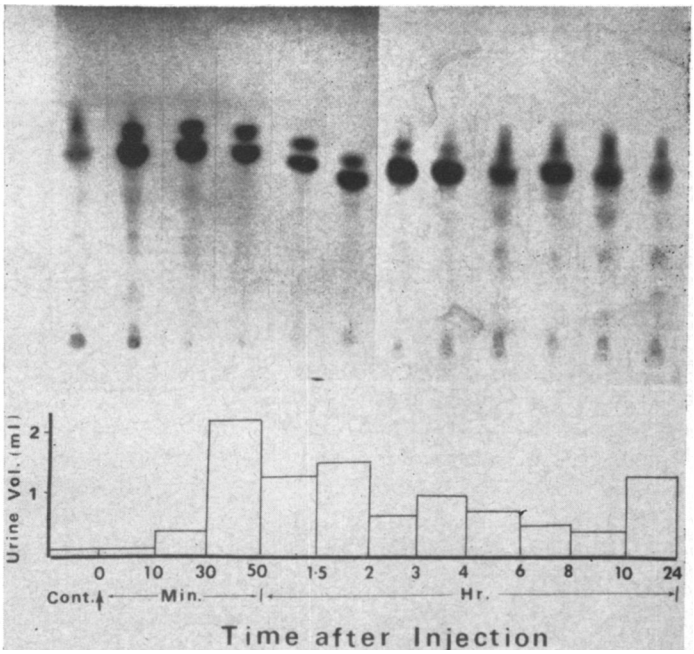

FIG. 1. Urinary excretion pattern by a rat injected sc with 2-thiophenecarboxylic acid (2 mmoles/kg): The thin-layer chromatogram was photographed by reflected ultraviolet light.

ples collected at 3 hr, and later. Urine samples collected later than 10 hr after a single injection of 2-TCA returned to the picture of a pretreatment (control) urine, indicating a complete excretion of the compounds. Urine samples collected from rats receiving a larger dose of 2-TCA (4 mmoles/kg) still retained the slow moving spot even after 10 hr. Intravenous or intraperitoneal administration of 2-TCA resulted in a similar pattern of urinary excretion, but oral administration of 2-TCA tended to prolong the excretion period at lower concentrations of metabolites. A marked diuretic effect of 2-TCA could be seen 30 min after a single injection. When both urine volume and urinary concentration of metabolites were taken into calculation, the amount and rate of metabolites excreted were the highest in urine sample collected between 30 and 50 min after a single injection.

The urinary metabolites of 2-TCA could be identified by thin-layer chromatography as shown in Fig. 2. Rat urine showed two spots (R_f = 0.64 and 0.54, respectively) after injection of 2-TCA. Addition of 2-TCA in control urine *in vitro* gave only one spot, R_f = 0.64. The other spot was identified to be 2-thenoylglycine (2-TG). Urine sample from rat injected with 2-TG gave only one spot, R_f = 0.54, which was identical to the spot of 2-TG added in control urine *in vitro*.

2-TCA and 2-TG had different ultraviolet absorption spectra (Fig. 3). The absorption maximum of 2-TCA was at 246 nm (molar absorptivity, ϵ_{246} = 8210) while the absorption maximum of 2-TG was at 252 nm (ϵ_{252} = 8990). Extraction of the two separated spots from rat urine after injection of 2-TCA gave spectra similar to 2-TCA and 2-TG (Fig. 3). Control urine would not interfere with the measurement of ultraviolet absorbance of either compound and was used as blank. The identities of the two metabolites were further verified by determining the melting points of crystals obtained from TLC extraction; 2-TCA melted at 128–130° and 2-TG at 175–177°.

Urine samples were collected for 0–4 and 4–24 hr periods in groups of rats receiving sc injection of normal saline or 2-TCA, 2 or 4 mmoles/kg. The metabolites in urine (5 μl) were separated by TLC, extracted in NaOH and measured by spectrophotometry. Table I

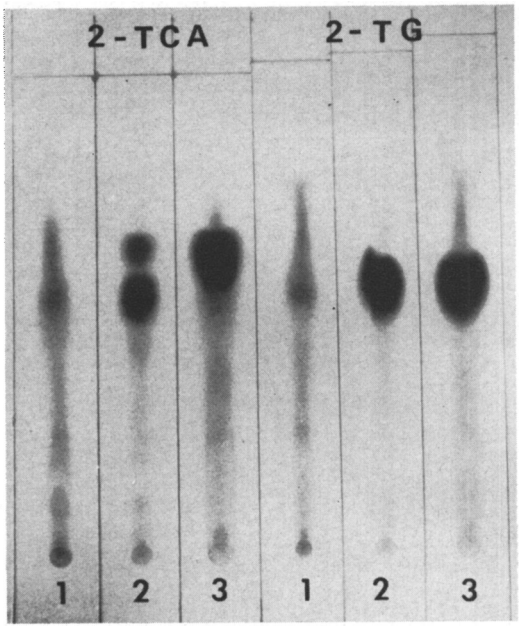

FIG. 2. Thin-layer chromatograms of urinary metabolites of 2-thiophenecarboxylic acid (2-TCA) and 2-thenoylglycine (2-TG) photographed by reflected ultraviolet light: (1) pretreatment urine, (2) urine from rat after sc injection of 2-TCA or 2-TG, and (3) addition of 2-TCA or 2-TG in control urine *in vitro*.

shows that 2-TCA significantly increased excretion of urine during the 0–4 hr period compared to saline control animals. About 80% of the injected 2-TCA (2 or 4 mmoles/kg) was converted into 2-TG and the remaining 20% was excreted without conjugation. The ratio between 2-TG and 2-TCA recovered in each urine sample, however, varied with time after injection and also the injected dose. Urine samples collected in 4–24 hr periods contained mainly 2-TG. In rats receiving 2 mmoles/kg, 96% of the injected 2-TCA was recovered in urine collected in the 0–4 hr period. In rats injected with a larger dose of 2-TCA, 4 mmoles/kg, the recovery was nearly complete (98%) in their 24 hr urine collection.

When 2-TG was given to rats by a single oral dose of 12 mmoles/kg or daily injections of 2 mmoles/kg for consecutively 7 days, the animals showed no overt sign of toxic reaction as judged by the general appearance of animals and their growing weight. 2-TG showed the same degree of diuretic effect of 2-TCA, but it was ineffective in lowering blood calcium, phosphorus, glucose, and free fatty acids.

Discussion and Summary. As with the rabbit, dog, and man, 2-thiophenecarboxylic acid in the rats was eliminated by route of urinary excretion. About 80% of the injected 2-TCA was recovered as metabolite by means of glycine conjugation and 20% remained unchanged. The conjugation process seemed to take place immediately after ingestion of the compound in the rats and the excretion of metabolites in urine was rapid. The peak rate of excretion occurred between 30 and 50 min after injection and was in company with a diuresis. After a

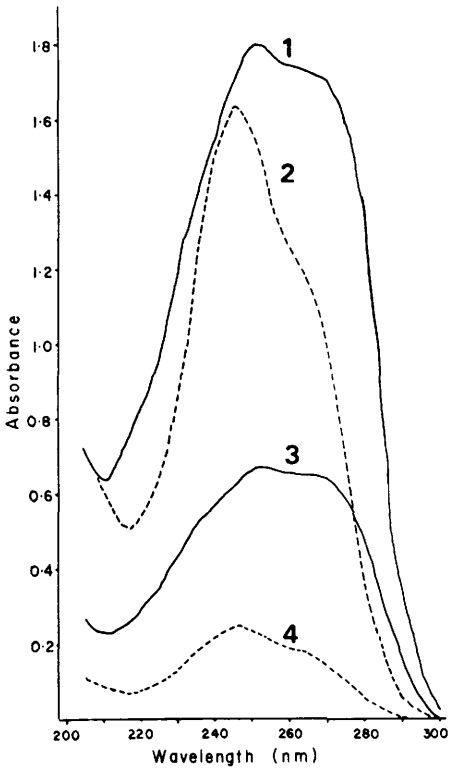

FIG. 3. Ultraviolet absorption spectra of 2-thiophenecarboxylic acid (2-TCA) and 2-thenoylglycine (2-TG): (1) 2-TG, (2×10^{-4} *M*); (2) 2-TCA (2×10^{-4} *M*; (3) 2-TG extracted from TLC spot ($R_f = 0.54$) of urine from rat injected sc with 2-TCA; and (4) 2-TCA extracted from TLC spot ($R_f = 0.64$) of the same urine sample.

TABLE I. Recovery of 2-Thiophenecarboxylic Acid Metabolites in Rat Urine.

Group[a]	After injection (hr)	Urine vol	Metabolite recovery (%)[b] 2-TCA	2-TG	Total
Saline	0–4	1.3 ± 0.2			
	4–24	4.7 ± 1.1			
2-TCA, mmoles/kg, sc					
2	0–4	5.8 ± 0.9[c]	18.5 ± 2.8	78.4 ± 8.5	96.3 ± 6.4
	4–24	4.8 ± 0.9	—	1.1 ± 0.4	1.1 ± 0.4
4	0–4	6.6 ± 1.4[c]	19.8 ± 2.9	60.3 ± 5.7	80.2 ± 5.9
	4–24	5.1 ± 1.2	<1	16.4 ± 2.0	17.5 ± 2.3

[a] Each group consists of 5 rats.
[b] Mean ± SD.
[c] $p < .001$, compared to saline control group.

single injection of 2-TCA (dose: 2 or 4 mmoles/kg), nearly all of the injected compound could be recovered in urine collected in a 10- or 24-hr period.

Since 2-thenoylglycine is virtually a nontoxic compound in rats, this mechanism of detoxification assured the nontoxicity of 2-thiophenecarboxylic acid in these animals. As 2-thenoylglycine is a pharmacologically inactive compound, the metabolism of 2-TCA in body and the rapid excretion in urine explains the relatively short duration of the hypocalcemic effect of 2-TCA in rats (4).

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