The Excretion of Ethylenethiourea by Rat and Guinea Pig

by

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Ethylenethiourea (ETU) has been found as a contaminant in commercial ethylenebisdithiocarbamate fungicides (JOHNSON and TYLER, 1962; BONTOYAN et al., (1972). It has been reported to possess goitrogenic (SEIFTER and EHRICH, 1948; GRAHAM and HANSEN, 1972), carcinogenic (ULLAND et al., 1972) and teratogenic (KHERA, 1973) properties. Since the metabolic fate of ETU in animals is unknown, the present study was initiated to determine the rate of excretion after oral dosing of ETU and thus obtain preliminary data on the rate of metabolism.

MATERIALS AND METHODS

Male Wistar rats (body weight 280-320 g) and male Hartley strain quinea pigs (body weight 239-313 g) were fasted for 24 h prior to dosing. A single dose of 20 mg/kg of ETU in aqueous solution (2.0 mg/ml) was administered by gastric intubation. The animals were given food and water ad libitum 5 h after dosing. Urine and fecal samples were collected and stored at 0°C prior to analysis. The animals were killed by carbon dioxide asphyxiation 96 h after dosing, and tissue samples excised and frozen.

For the analysis of ETU, urine samples were diluted with distilled water to 100 ml and aliquots (1.0-2.0 ml) added to 50% aqueous ethanol (20 ml).

Feces were mixed with an equal weight of water and 200 ml of ethanol. The mixture was homogenized on a Sorvall Omni-Mixer and filtered through Whatman No. 1 paper. The filtrate was diluted to 250 ml with ethanol. Aliquots of the ethanol dilution (10.0 ml) were added to distilled water (10.0 ml) and analysed.

Tissues were homogenized with ethanol (1:10 w/v) and the homogenate filtered. Aliquots of the filtrate representing 1 g of tissue were diluted with an equal volume of distilled water and analysed. Both thyroid glands were homogenized in ethanol (10 ml) and the suspension was filtered. The entire filtrate was analysed after the addition of distilled water (10 ml).

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ETU analysis was carried out by gas-liquid chromatography after benzylation, extraction, and trifluoroacetylation as previously described (NEWSOME, 1972). Recoveries on spiked samples were 90% or greater. The method was capable of detecting 2 ug/sample or 0.04% of the administered dose in urine and fecal specimens. With tissues, 0.005 ppm of ETU was the minimum detectable limit.

RESULTS AND DISCUSSION

In both rats and guinea pigs, a rapid elimination of ETU in the urine was observed. Approximately 50% of the administered dose was excreted unchanged within 24 h (TABLE 1).

TABLE 1

Percentage of Administered Dose of ETU Excreted in Urine[1]

Time Interval After Dosing (h)	Rat	Guinea Pig
0-6	18.60±2.38	17.75±1.72
6-24	42.68±2.36	27.18±0.66
24-48	3.65±0.50	2.48±0.43
48-72	0.42±0.12	trace
72-96	trace	

[1] Values are mean ± S.E. of 6 animals.

The excretion of ETU in feces was negligeable as compared to that in urine. Rats eliminated 1.06% in the feces within 48 h, while guinea pigs excreted 0.78%. After 48 h only trace amounts were detected in feces. These results are similar to those reported for the excretion of thiourea, another goitrogenic agent (SHULMAN Jr. and KEATING, 1950). The lower total recovery of ETU in the guinea pig (48.2%) as compared to that in the rat (66.4%) may be due to interspecies metabolic differences.

The data in TABLE 2 shows a relatively uniform distribution of ETU in selected tissues. Mean residue levels in liver, kidney, heart, and muscle ranged from 0.010 ppm to 0.086 ppm in the rat and guinea pig. However, the concentration in the thyroid of both species was markedly elevated. The higher concentration of ETU in the thyroid may be associated with the goitrogenic effects reported by SEIFTER and EHRICH (1948), and GRAHAM and HANSEN (1972). In thiourea treated rats it was found that sulfur metabolites, but not thiourea per se were concentrated in the thyroid (MALOOF and SOODAK, 1957).

TABLE 2

Residues in Tissues 96 h After Oral Dosing of ETU

Tissue	Rats (ppm ETU)	Guinea Pig (ppm ETU)
Liver	0.010±0.005	0.024±0.004
Kidney	0.046±0.004	0.086±0.008
Heart	0.038±0.006	0.038±0.008
Thyroid	0.824±0.042	0.751±0.106
Muscle	0.012±0.000	0.011±0.000

[1]Values are the mean ± S.E. of 6 animals.

Since the present analytical method measures only the parent compound, it is possible that the tissues contain undetected metabolic residues. Further studies are required to establish whether enzymes exist which are capable of desulfurating ETU in a manner similar to that described for thiourea (MALOOF and SPECTOR, 1959).

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