Effects of Some Substituted Thioureas on Alpha-naphthylthiourea (ANTU) Toxicity to Rats. (18394)

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It has been reported from this laboratory that relatively nontoxic thioureas related in structure to ANTU are effective in reducing mortality in rats when given before, simultaneously with or after toxic doses of the rodenticide(1). Two alkyl singly N-substituted thiourea derivatives, allyl thiourea and isopropyl thiourea, were most effective in reducing mortality in rats when given simultaneously with or one hour after the injection of two LD_{50}'s (5 mg/kg) of ANTU. It was suggested that these compounds confer their protection against ANTU poisoning by virtue of their similar structure and act as competitive antagonists against the rodenticide.

We have recently obtained three additional substituted thioureas, ethyl thiourea, n-butyl thiourea and 1-ethyl-1-phenyl thiourea, and have investigated the effects of these compounds on ANTU toxicity to rats.

Materials and methods. The compounds† used in this study included 2 singly substituted thioureas (ethyl thiourea and n-butyl thiourea) and one 1,1 disubstituted thiourea (1-ethyl-1-phenyl thiourea). Wistar, male, albino rats, weighing from 200 to 300 g, were used throughout these experiments. The animals were born and raised in our animal colony and maintained for no less than one month on a diet previously described(2). Injections of ANTU and the compounds tested were given by the intraperitoneal route with constant nontoxic quantities of propylene glycol (1.0 ml/kg) as the vehicle. Simultaneous injections of ANTU and test-compounds were made separately in different areas of the abdomen. Tests were conducted in groups of 8 animals. Control animals were injected on each test-day with the same dose of ANTU (2 LD_{50}'s‡ = 5.0 mg/kg) as given to rats subjected to treatment. One set of controls served for each compound tested. Both control and experimental (treated) groups were observed for a period of 5 days following the injection of ANTU.

Results and discussion. Of the 3 compounds tested, 1-ethyl-1-phenyl thiourea (EPTU) was most effective as an antidote when given as much as 2 hours after ANTU (Table I). No animal treated with this compound died. Both ethyl thiourea and n-butyl thiourea showed significant reductions in mortality when administered in doses of 25 mg/kg and 50 mg/kg, but when compared with EPTU at the higher dose, little protection was given when administered 2 hours after ANTU, as shown in Table II. This may have been due to increased toxicity at the higher dose level.

Rats that had been given ANTU followed by EPTU had an amazing recovery. A rat poisoned with ANTU usually shows signs of uneasiness in an hour and, at the end of 2 hours, is quite dyspneic. Death usually occurs within 12 hours. This course of events was followed partly by the rats given EPTU 2 hours after ANTU. This is to say that prior to the EPTU injection the rats were dyspneic and almost completely inactive. Within one hour after EPTU injection normal respiration was restored and by the end

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1. Meyer, B. J., and Saunders, J. P., *J. Pharm. and Exp. Therap.*, 1940, v97, 452.

† Ethyl thiourea, n-butyl thiourea and 1-ethyl-1-phenyl thiourea were supplied through the generosity of Sharples Chemicals, Inc., Philadelphia, Pa. E. I. du Pont de Nemours and Co., Wilmington, Del., kindly furnished the ANTU.

2. Meyer, B. J., and Karel, L., *J. Pharm. and Exp. Therap.*, 1948, v92, 15.

‡ LD_{50} = 2.47 mg/kg, calculated by the method of Bliss(3).

3. Bliss, C. I., *Quart. J. Pharm. Pharmacol.*, 1938, v11, 192.

TABLE I. Effect of Substituted Thioureas When Given to Rats Simultaneously With, One or Two Hours After ANTU.*

Compound	Dose† mg/kg*	When given	Mortality Control	Mortality Experimental	P‡
Ethyl thiourea	25	Simultaneously	7/8	1/8	<.02
,,	25	1 hr after ANTU		3/8	>.10
,,	25	2 hr after ANTU		1/8	<.02
,,	50	Simultaneously	7/8	1/8	<.02
,,	50	1 hr after ANTU		2/8	<.05
,,	50	2 hr after ANTU		6/8	>.10
n-butyl-thiourea	25	Simultaneously	7/8	0/8	<.01
,,	25	1 hr after ANTU		1/8	<.02
,,	25	2 hr after ANTU		3/8	>.10
,,	50	Simultaneously	7/8	2/8	<.05
,,	50	1 hr after ANTU		0/8	<.01
,,	50	2 hr after ANTU		4/8	>.10
l-ethyl-l-phenyl thiourea	50	Simultaneously	7/8	0/4	<.01
,,	50	1 hr after ANTU	14/16	0/16	<.01
,,	50	2 hr after ANTU		0/16	<.01

* Inj. intraper. 5.0 mg/kg equivalent to an expected mortality of ca 90%.
† Inj. intraper. in a constant non-toxic quantity of propylene glycol (1.0 ml/kg).
‡ P <.05 indicates significant difference between experimental and control groups.

of 2 hours the animals were moving around the cage. The next morning they could not be distinguished from normal animals. Rats given EPTU simultaneously with the rodenticide developed no symptoms of ANTU poisoning.

Of all the compounds studied in this laboratory(2,4), EPTU appears to be the most effective antidote against ANTU poisoning in rats. Although EPTU has an LD_{50} approximately 1/100 that of ANTU,§ the structures of the two compounds are quite similar. We have reported previously that lethal quantities of certain thiourea derivatives having structures similar to that of ANTU produce pulmonary edema and pleural effusion, which are the main symptoms of ANTU poisoning. The compounds studied here are not exceptions, and it seems probable that thiourea derivatives confer antidotal protection by acting as competitive antagonists against ANTU. Because of the effectiveness of EPTU as an antidote against ANTU poisoning in rats, it was hoped that EPTU could be used in cases of ANTU poisonings of domestic animals. However, preliminary experiments in this laboratory with a limited number of dogs have shown EPTU to be ineffective.

TABLE II. Comparison of Protective Action of 50 mg Doses of Ethyl Thiourea (I), n-butyl Thiourea(II), and l-ethyl-l-phenyl Thiourea (III). (P = Probability by chi-square test(5). P <.05 indicates significant difference between 2 drugs).

Drug	Treatment	Chi square (X^2)	P
I vs II	Simultaneous	.33	>.50
I vs III	,,	.08	>.70
II vs III	,,	.50	>.30
I vs II	1 hr after ANTU	1.641	>.20
I vs III	,, ,, ,,	3.857	<.05
II vs III	,, ,, ,,	.13	>.70
I vs II	2 hr after ANTU	.27	>.50
I vs III	,, ,, ,,	7.87	<.01
II vs III	,, ,, ,,	9.13	<.01

Summary. 1. l-ethyl-l-phenyl thiourea (EPTU) administered in an intraperitoneal dose of 50 mg/kg was effective in significantly reducing mortality and toxic symptoms in rats when given simultaneously with, one hour or 2 hours after two LD_{50}'s (5 mg/kg) of ANTU. All animals receiving EPTU survived. 2. Ethyl thiourea and n-butyl thiourea also conferred significant but less uniform protection when given in doses of 25 and 50 mg/kg.

§ LD_{50} = 297 ± 63 mg/kg, calculated by the method of Bliss.

4. Karel, L., and Meyer, B. J., *J. Pharm. and Exp. Therap.*, 1948, v93, 414.

5. Fisher, R. A., and Yates, F., Table VIII, *Statistical Tables for Biological, Agricultural and Medical Research*, Hafner, New York, 1949.

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