consecutive hours was noted in 5 instances; in one case there was no free acid in 11 of the 12 specimens. However, hydrochloric acid usually reappeared towards the conclusion of the nocturnal period. Free acid had been present continuously during the control periods in the 9 patients with duodenal ulcer. The inhibition of secretion consisted usually of a pronounced decrease in the concentration of acid. However, the volume of gastric content also diminished markedly in 3 patients.

Comment. The present data indicate that the fasting gastric secretion may decrease greatly, albeit temporarily, following the intramuscular administration of 5000 mg of a dialyzed preparation of enterogastrone. This finding is in contrast to a single previous experiment in which gastric secretion was unaffected by the injection of 5000 mg of an apparently less concentrated product. Although the injections produced severe pain immediately, the decrease in acid usually did not become apparent until two or three hours had elapsed. As in preceding studies, the effect consisted chiefly of a lowered concentration of acid; the volume of secretion also diminished significantly in three of the present series. The mechanism of this reduction and its significance remain obscure. It may be noted that the intramuscular administration of a non-specific protein, sterile lactalbumin, did not reduce the nocturnal gastric secretion in 10 patients with peptic ulcer; indeed, an increased output of acid was noted in 6.[9] The inhibition is not attributable to an elevation in body temperature, since the temperature increased in only three patients and then very slightly; the reduction in acid in one of this group was slight. The temporary duration of the inhibition, the tremendous quantities required, the accompanying severe pain, and the uncertainty as to the nature of the material and the mechanisms involved do not warrant further clinical trial of the present dialyzed preparation of enterogastrone. Nevertheless, the data would appear to justify the continued search for a much more effective and safely administered product, whose chemical nature and physiological behavior may be studied more precisely.

Conclusions. 1. The output of hydrochloric acid in the 12-hour nocturnal gastric secretion of 4 patients with peptic ulcer was unchanged following the intramuscular administration of 200 to 2000 mg of a dialyzed preparation of enterogastrone. 2. Gastric secretion was reduced slightly in one and markedly in five of six patients with peptic ulcer, given 5000 mg. The transitory inhibition consisted chiefly of a decrease in the concentration of acid and, to a lesser extent, of the volume of secretion.

[9] Kirsner, J. B., Levin, E., and Palmer, W. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 90.

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Thiosemicarbazide: A New Toxic Derivative of Thiourea.*

SALLY H. DIEKE. (Introduced by C. P. Richter.)
From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

Previous papers from this laboratory[1-4] have described the acute toxicity of thiourea and a number of its singly substituted derivatives, notably alpha-naphthyl thiourea (ANTU), which has proved to be an effective rodenticide for the field control of Norway rats.[1] The subject of the present paper is thiosemicarbazide ($NH_2NHCSNH_2$), in

* Carried out under a contract between the Medical Division, Chemical Corps, U. S. Army, and The Johns Hopkins University. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the author.

TABLE I.
Acute Toxicity of Thiosemicarbazide.
(Adult animals, except as noted).

Species	No. used	Administered*	$LD_{50} \pm$ S.E.† mg/kg B.W.
Wild Norway rat	Adult 56	s.t.	13 ± 2.1
(Rattus norvegicus)	Young 21	"	19 ± 1.3
Laboratory rat	Strain I 49	"	11 ± 2.0
(Rattus norvegicus)	Strain II 44	"	18 ± 2.0
Alexandrine rat	26	"	23 ± 1.4
(Rattus rattus)			
Cotton rat	31	"	16 ± 2.2
(Sigmidon hispidus hispidus)			
Guinea pig	37	i.p.	24 ± 2.0
(Cavia cobaya)			
Dog	6	s.t.	10 (5-15)
(Canis familiaris)			
Cat	4	"	20 (15-?)
(Felix libyca domestica)			

* s.t. = by stomach tube; i.p. = by intraperitoneal injection. The vehicle was, in every instance, a 10% solution of gum acacia in water.

† LD_{50}'s and their standard errors were obtained by the method of Litchfield and Fertig.[7] Whenever data were not sufficiently extensive to justify statistical treatment, an estimate of the LD_{50} is followed by the range between the highest dose observed to kill none and the lowest dose killing all.

which an amino group replaces the naphthyl radical of ANTU. This compound differs in several respects from the other toxic thioureas, owing presumably to the fact that it is a derivative of hydrazine (NH_2NH_2) as well as of thiourea (NH_2CSNH_2).

The rapid death of white rats following administration of thiosemicarbazide was first observed by Dr. Emanuel Waletzky of the American Cyanamid Company; Dr. R. O. Roblin of that company then sent a sample to this laboratory for testing on wild Norway rats as a possible new rodenticide. Observations that this substance was toxic have also been made by Astwood[5] and by Jensen and Kjerulf-Jensen[6] in the course of their studies on the chronic feeding of various chemicals to determine their goiterogenic activity.

Acute Toxicity. Table I summarizes the data obtained with the 6 different species of animals available for assay. The thiosemicarbazide was administered either by stomach tube or by intraperitoneal injection, in water containing 10% gum acacia, according to the technic previously used for assaying ANTU.[3] Two strains of laboratory Norway rats were used to compare with the wild Norways, because previous work had shown that very marked differences in susceptibility to thiourea existed between domestic rats from different colonies.[2] The rats designated as strain I came from our own colony, which shows uniformly high susceptibility to thiourea poisoning; while those of strain II came from a colony of Wistar rats maintained at the Army Chemical Center, Edgewood, Maryland. The wild Norway rats were freshly trapped speci-

1 Richter, C. P., *J. A. M. A.*, 1945, **129**, 927.

2 Dieke, S. H., and Richter, C. P., *J. Pharm. and Exp. Therap.*, 1945, **83**, 195.

3 Dieke, S. H., and Richter, C. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 22.

4 Dieke, S. H., Allen, G. S., and Richter, C. P., *J. Pharm. and Exp. Therap.*, 1947, **90**, 260.

5 Astwood, E. B., *J. Pharm. and Exp. Therap.*, 1943, **78**, 79.

6 Jensen, K. A., and Kjerulf-Jensen, K., *Acta Pharmacol.*, 1945, **1**, 280.

7 Litchfield, J. T., Jr., and Fertig, J. W., *Bull. Johns Hopk. Hosp.*, 1941, **69**, 276.

mens from the alleys of Baltimore. The Alexandrine and cotton rats came from our own colonies. All the rats were fed purina fox chow while in this laboratory.

Table I shows that all the acute median lethal doses obtained fell between 10 and 25 mg/kg. For comparison, the equivalent values for ANTU ranged from 2.5 mg/kg for domestic Norway rats up to about 500 mg/kg for cats (and over 4 grams/kg for monkeys and chickens).[3] This indicates that thiosemicarbazide does not share with ANTU a specific toxicity to Norway rats, but is more widely toxic to warm blooded animals in general.

One monkey (*Macaca mulatta*) was also available for testing the toxicity of thiosemicarbazide: it received 200 mg/kg by stomach tube and died within 24 hours.

The acute toxicity of thiosemicarbazide to young Norway rats was tested, since previous work showed that young rats were 6 or 7 times less susceptible to ANTU than were adults.[3] Thiosemicarbazide was given to 21 young wild Norway rats weighing between 36 g and 110 g and all sexually immature. The LD50 obtained with this limited number of rats (See Table I) was less than twice that found for adults, which indicates that compared to ANTU, the response to thiosemicarbazide is substantially less variable with age. This is borne out by the results of acceptance tests, described below.

Both male and female animals of every species were used at most assay levels. A separation of the data gave no indication of any difference in response that could be attributed to sex.

In all the animals included in Table I, with the significant exception of some laboratory Norway rats, the effects noted after poisoning were very similar. Within an hour after dosing the animals appeared to be in a highly excited and apprehensive condition. The rats and guinea pigs frequently squealed, the cats miaowed, and the dogs barked or growled. Violent convulsions then started, often terminating within a minute or two with the teeth bared and the limbs stiffly extended, only to begin again within a matter of minutes. Intense salivation was observed in all animals receiving fatal doses, and vomiting in the dogs, cats, and monkey. Death or what appeared to be complete recovery occurred within 6 hours, except for the monkey which survived for almost a day.

On the other hand, laboratory Norway rats of both strains often reacted differently to thiosemicarbazide, especially after low but nevertheless fatal doses. Those from our colony (Strain I) frequently had only mild convulsions or none at all, and deaths usually did not occur until after 18 to 24 hours. At autopsy these rats showed pulmonary edema accompanied by pleural effusion (as found in typical ANTU or thiourea poisoning). The other laboratory rats (Strain II) usually died in convulsions, but some of these also had none and died overnight with pulmonary edema.† In none of the other animals receiving thiosemicarbazide (including the monkey) was any pleural effusion found, nor was any significant amount of pulmonary edema evident either grossly or in histological sections of the lungs. This interesting finding is reminiscent of results previously reported,[2] in which poisoning of rats from our colony with the parent compound thiourea produced pulmonary edema, while equivalent doses had no effect on the lungs of wild Norway rats.

Acceptability. The voluntary consumption of thiosemicarbazide appears to be good, on the basis of tests made with individually caged rats. A concentration of 0.5% in either water solution or in a bait of yellow cornmeal killed 18 of 19 wild Norways, all of 8 Alexandrines, and 7 of 8 cotton rats. At 1% in bait all of 12 wild Norways died, and at 2% 7 out of 8. With 20 laboratory Norways (Strain I), divided into 3 groups, all of 8 died after drinking a concentration of 0.25% in water, and 4 of 8 at a 0.1% concentration, while none of 4 died from drinking a 0.05% solution. No hesitation to drink these solutions was observed, despite the fact that thiosemicarbazide has a bitter taste to humans and most probably also to rats.[8]

† Dr. Waletzky states (private communication) that rats from his colony, originating from Carworth Farms for the most part, exhibited violent convulsions and died in 1 to 3 hours. These rats would thus seem to resemble wild Norways more than the rats from our colony.

Of the 39 wild Norway rats included in this test, 29 were immature, weighing between 54 and 130 g. All but one of these young rats died after ingesting doses as low as 15 mg/kg, which indicates that this poison should be effective against young as well as adult wild Norway rats.

Tolerance. Thirty-six adult wild Norway rats and 6 adult laboratory Norway rats (Strain I) were used to investigate the possible development of tolerance to thiosemicarbazide. The wild Norways all received an initial dose of 7.5 mg/kg by stomach tube (approximately ½ an LD50 dose). Seven received 4 subsequent doses of 7.5, 7.5, 20, and 50 mg/kg, also by stomach tube, on alternate days thereafter. Convulsions were observed in 2 of these 7 rats following the 20 mg/kg dose, but all survived. All died within 3 hours after receiving 50 mg/kg, in violent convulsions.

Fourteen other wild Norways received 30 mg/kg on their second dose: 3 out of 3 died when the interval between doses was 14 days, 4 out of 4 when 3 days had elapsed, but only 3 out of 7 at 2 days after the initial poisoning.

When the second dosing was accomplished by offering poisoned water (0.25%) for voluntary consumption, 4 out of 4 died when the interval was one day, and at 2 days 7 out of 10. No wild Norway survived a dose higher than 60 mg/kg at any time. It would seem, then, that a tolerance to 4 or 5 median lethal doses can develop in wild Norway rats, and that this tolerance is most marked about 2 days after a sublethal dose. This is not serious from a practical standpoint, as the consumption of 5 cc of poisoned water (at 0.25%) or a few grams of poisoned food would provide ample thiosemicarbazide to offset such a tolerance, even in large sized rats.

In contrast to the above results, somewhat larger tolerances were established in the laboratory rats (Strain I). These rats received by stomach tube an initial dose of 5 mg/kg (or about ½ an LD50) and subsequent doses on alternate days of 5, 5, 20, 50, 100, 150, 200, and 300 mg/kg. One rat died (with pulmonary edema) from the initial dose, but no other fatalities occurred until 2 of the remaining 5 succumbed to 100 mg/kg. Two others died following 200 mg/kg and the last after 300 mg/kg. Thus 1 rat developed a tolerance to about 20 times the LD50 dose, on a regimen which with ANTU produces tolerances of 80 times with little difficulty, even in wild Norway rats.[1]

Antidote. The copious salivation found in animals poisoned with thiosemicarbazide suggested that atropine might be an antagonist. Preliminary experiments with 8 rats, one dog and one cat were unsuccessful; in fact, although the salivation was suppressed, the animals appeared to die rather sooner when treated with amounts of atropine which were not lethal to controls than when they received the thiosemicarbazide alone.

On the other hand the administration of 20 mg/kg of sodium pentobarbital at the onset of the first violent convulsion (about one hour after poisoning) followed by another similar amount when necessary, was effective in preventing the death of 2 dogs and one cat, each of which had received 5 times an LD50 dose. The convulsions were not entirely prevented by this amount of pentobarbital, but their intensity was lessened. Six hours after poisoning all 3 animals looked well although a little groggy; the next day recovery appeared to be complete.

The treatment of 16 laboratory rats (Strain II) with 40 mg/kg of sodium pentobarbital (and subsequent booster doses when necessary), at the onset of convulsions following either 3 or 6 LD_{50}'s of thiosemicarbazide produced some interesting results. Whereas the controls all died within 3 hours, none of the rats kept unconscious by the barbiturate died within the first 5 hours. Only 3 out of 8 survived the lower dose, however, and only one of the 8 receiving the higher dose: the rest died overnight with pulmonary edema and pleural effusion. It would therefore appear that pentobarbital is an antidote for those species in which thiosemicarbazide has only a convulsant action, but not when it also has an ANTU-like action on the lungs. Since 2 dogs survived relatively large doses when treated with this barbiturate, it may be that, unlike ANTU, thiosemicarbazide would not

[1] Richter, C. P., in press.

cause pulmonary edema in this species as it does in the laboratory Norway rat.

Comparison with other poisons. Some of the effects produced by thiosemicarbazide indicate the relationship of this compound to hydrazine. According to Fränkel,[9] hydrazine causes excitement and occasionally convulsions in higher animals, but does not damage erythrocytes as does phenyl hydrazine. O. Loew[10] reports convulsions and rapid death in guinea pigs after fatal doses of hydrazine. Underhill and Karelitz,[11] who studied the anemia caused in dogs by hydrazine sulfate, observed more or less constant salivation, vomiting and diarrhea; they mention no convulsions, however, nor does Bodansky[12] whose dog also vomited and salivated for several days.

To check the relative effects of thiosemicarbazide and the two compounds from which it derives, 14 laboratory rats (Strain II) were given thiourea and 14 hydrazine (as the sulfate), at the same time and in the same way that the LD50 of thiosemicarbazide was determined. The acute LD50 for thiourea fell at about 20 mg/kg, and that for hydrazine sulfate at about 450 mg/kg.‡ None of the rats receiving either thiourea or hydrazine sulfate had convulsions; the main autopsy findings in those succumbing to thiourea were pulmonary edema and pleural effusion, while hydrazine sulfate caused increased salivation and a grossly evident liver damage. A chocolate brown blood, as found after poisoning with phenyl hydrazine, was not observed.

The differences between thiosemicarbazide and the other toxic thioureas previously studied are several. Thiosemicarbazide has a strong convulsant action, which ANTU and other similar thioureas do not share, and appears to be quite generally toxic to a number of species of animals, rather than almost specifically toxic to Norway rats. It is not markedly less toxic to young than to adult Norway rats, and sublethal doses do not produce the high tolerances characteristic of ANTU. Furthermore, pentobarbital gives promise of being an adequate antidote, at least for those species not affected by the thiourea moiety of the molecule.

From a practical standpoint, thiosemicarbazide might have a wider applicability than ANTU because it dissolves in water and so can be used as a water poison as well as in bait. It can also presumably be used against other rodents than wild Norway rats: an evaluation of the success of such use must await field trials. On the other hand it is very probably toxic to man as well as to other animals and will therefore require far more caution in use. It acts very quickly and apart from a bitter taste, similar to that of phenyl thiourea and probably also subject to genetically controlled "taste blindness",[13] carries with it no warning. The existence of an antidote under these circumstances is comforting but may not be of much practical value.

Compared to other universal poisons such as sodium fluoroacetate (1080), the value of thiosemicarbazide would seem to lie in its lower toxicity, which under some circumstances might be an advantage, since it would lessen the hazard to larger animals, including man. The consumption of a few grams of bait is sufficient to kill rats, and since the animals feel unwell within a short time after poisoning they rarely take more than that. No individual rat in our tests has voluntarily consumed sufficient poison to kill a (much larger) dog or cat by secondary poisoning, assuming no loss of toxicity of the substance while in the rat's body.

Thiosemicarbazide is a relatively stable compound under ordinary conditions of light-

[9] Fränkel, S., *Die Arzneimittel-Synthese*, 6th ed., Berlin, 1927, pp. 84-85.

[10] Loew, O., *Ber. d. Deut. Chem. Ges.*, 1890, **23**, 3203.

[11] Underhill, F. P., and Karelitz, S., Jr., *J. Biol. Chem.*, 1923, **58**, 147.

[12] Bodansky, M., *J. Pharm. and Exp. Therap.*, 1924, **23**, 127.

‡ This last finding confirms a previous report[4] as to the relatively low toxicity of hydrazine sulfate to laboratory rats, and also agrees well with results obtained with a few wild Norway rats, for which strain the LD_{50} probably falls between 250 and 500 mg/kg.

[13] Blakeslee, A. F., and Fox, A. L., *J. Hered.*, 1932, **23**, 97.

ing, humidity and temperature. It melts at 180°C with decomposition, but below that temperature it appears not to decompose spontaneously. It is soluble in cold water to about 1 or 2% and in hot water to about 10%. It is also soluble in alcohol.§

Summary. Thiosemicarbazide, a derivative of both thiourea and hydrazine, has proved to differ from other toxic thioureas previously studied. Instead of producing fatal pulmonary edema in a limited number of animal species and not harming others at equivalent dose levels, thiosemicarbazide caused convulsions and death within 1 to 3 hours in the 6 species tested, when given in amounts ranging from 10 to 30 mg/kg. In some individual laboratory rats, however, including those in which the convulsions had been suppressed by administration of a barbiturate, death was delayed and accompanied by the development of pulmonary edema. Thiosemicarbazide may have promise as a practical rodenticide, because of its general toxicity to rats of several species and because it appears to be readily accepted in lethal amounts when offered to rats in either water solution or bait.

It is a pleasure to acknowledge the kindness of Dr. Wayland G. Hayes of the Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia, who provided the nucleus of our Alexandrine rat colony, and of Dr. R. O. Roblin, American Cyanamid Company, who has helped in many ways throughout the course of this study.

§ The analytical data on thiosemicarbazide given in this paragraph were very kindly provided by Dr. J. R. Vaughan of the American Cyanamid Company.

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Estimation of Dicumarol, 3, 3¹-Methylenebis (4-Hydroxycoumarin) in Biological Fluids.*

JULIUS AXELROD, JACK R. COOPER, AND BERNARD B. BRODIE.

From the Third New York University Research Service, Goldwater Memorial Hospital, Department of Biochemistry, New York University College of Medicine, and the Laboratory of Industrial Hygiene, New York City.

Although the physiological activity of dicumarol has been assayed by its effect on the prothrombin time, no chemical method for its estimation has been available. A rapid chemical method would make possible a study of certain problems concerning dicumarol about which little is known. These are its physiological disposition in man, its relationship to vitamin K in the clotting mechanism, and its formation in spoiled sweet clover.

A chemical method is described below which involves isolation of the drug from the body by extraction into heptane. The drug is returned to alkali and measured spectrophotometrically at 315 mμ where the drug exhibits a pronounced peak (Fig. 1).

Reagents. 1. Standard solution of dicumarol 100 mg per liter. 100 mg of dicumarol are dissolved in 1 liter of 0.1 N NaOH. This solution is stable for at least one month when stored in the refrigerator.

2. 3 N HCl.

3. Heptane. (Paragon Testing Company). A technical grade of heptane is purified by successive washings with 1 N NaOH and 1 N HCl followed by 2 washings with water.

4. 2.5 N NaOH.

Procedure. Add 1 to 3 ml of plasma or urine (sample containing up to 50 γ of dicumarol) and 0.5 ml of 3 N HCl to 20 ml of heptane in a 60 ml glass-stoppered bottle. Adjust the aqueous volume to about 3.5 ml if necessary by the addition of water. Shake for 30 minutes on a shaking apparatus and

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