TABLE I. Mean Paralyzing and Toxic Doses of Mephenesin and MC 2303 in mM/kg.

	Intraperitoneal		Oral	
	ED_{50}	LD_{50}	ED_{50}	LD_{50}
Mephenesin	1.08 ± 8.8%	2.83 ± 3.8%	4.17 ± 5.1%	10.53 ± 5.1%
MC 2303	1.16 ± 7.9%	2.77 ± 5.9%	3.4 ± 5 %	7.67 ± 5.4%

TABLE II. Number of Animals Protected Against Extensor Phase of Maximal Electroshock Pattern. 10 animals per group.

Drug	Dose, mM	Time in min 10	40	70	120	180
Mephenesin	3.40	10	4	1	0	—
	2.30	8	1	1	0	—
	1.54	4	1	1	0	—
	.99	6	0	—	—	—
	.66	3	0	—	—	—
MC 2303	4.20	10	10	10	5	4
	2.76	10	10	6	0	—
	1.88	10	10	6	1	2
	1.25	10	7	1	0	—
	.81	8	2	0	—	—
	.54	3	0	—	—	—

lar to mephenesin in potency and activity, and appears to have a longer duration of action.

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Thiosemicarbazide Toxicity in Mice.* (19354)

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Domagk(1) has reported that the 3-thiosemicarbazones of substituted benzaldehydes are active *in vitro* in inhibiting the growth of *Mycobacterium tuberculosis.* Promising results have been reported for a number of these compounds when tested *in vivo* in mice(2-4). However, thiosemicarbazide (TSC) itself, although active *in vitro* in low concentrations against *M. tuberculosis,* var. *bovis,* strain BCG(5) has not proven effective when tested *in vivo* because of its extreme toxicity. Indeed, Dieke(6) has suggested using TSC as a rodenticide. She found that dose levels of 10-30 mg/kg caused convulsions and death within one to 3 hours in rats, dogs, cats, guinea pigs, and monkeys.

It has been shown(7) that the ciliated protozoan, *Tetrahymena geleii,* is inhibited by TSC and that the inhibition may be reversed at the lower dose levels by small amounts of pyridoxamine. Tests to determine whether or not the toxicity of TSC to mice can also be reversed by pyridoxamine were therefore carried out.

Methods. The animals employed in these experiments were in most cases 18-20 g C57 black mice. A few experiments were performed on Swiss and A/C3H hybrid mice, the results of which were in complete accord with those using the C57 black strain. The compounds tested were administered in water solution with the concentration adjusted so that a total dose was contained in one ml. Accurate oral doses were administered by a syringe with an attached blunted, curved 15-gauge hypodermic needle, which was introduced into the esophagus. Following administration of the desired compounds the animals were observed closely for 4 hours and again at 12- and 24-hour intervals. The time of death of each animal was recorded. The great majority of deaths occurred within the first 4 hours. It was observed that striking uni-

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TABLE I. Reversal of Thiosemicarbazide Toxicity. No. deaths/No. mice treated.

mg thiosemicarbazide/kg body wt	mg pyridoxamine/kg body wt								
	0	6.5	16	32	65	190	320	800	1600
12	2/5	1/5	0/5	—	—	—	—	—	—
65	5/5	—	—	5/5	2/5	0/5	0/5	—	—
160	5/5	—	—	—	—	—	—	1/5	—
320	5/5	—	—	—	—	—	—	—	2/5

formity occurred in the time of onset of convulsions in animals receiving the same dose of TSC alone. Often the first seizure was fatal. If not, successive seizures occurred at intervals of about 10 minutes until death ensued. All experiments were repeated many times. In all 810 mice were used.

Results. Doses of 20 mg of TSC/kg resulted in fatal convulsions in all animals tested. This dosage is in good agreement with the "maximal acceptable drug intake daily" for mice (16 mg/kg) as reported by Hamre *et al.*(4) and with the toxic range (10 to 30 mg/kg) found by Dieke(6) for 6 species of mammals other than mice. The importance of the presence of sulfur in the molecule in determining its specificity is demonstrated by the results with semicarbazide and aminoguanidine. If the sulfur is replaced by oxygen as in semicarbazide, the toxicity is reduced to about one-twentieth of that of TSC, whereas if an imino group is substituted as in aminoguanidine, no acute toxicity is encountered even with a dose of 650 mg/kg.

Table I gives data showing that complete reversal of the acute toxicity of TSC may be achieved by intraperitoneal injections of pyridoxamine (B_6NH_2). These data are representative of a large number of experiments. Uniform reversal of high doses of TSC (8 and 16 fold greater than the minimal toxic dose) was not obtained with the amounts of B_6NH_2 tested. However, when deaths did occur, they were delayed significantly beyond the time of death observed with TSC alone. Toxic levels of semicarbazide were reversed only sporadically with B_6NH_2. Neither pyridoxine nor pyridoxal was as effective as pyridoxamine in reversing the toxicity of TSC. In mice given daily intraperitoneal injections of 65 mg of TSC/kg, preceded by 320 mg of B_6NH_2/kg; for 9 days, no deaths, weight loss or other signs of toxicity occurred.

The possibility that the reversal of TSC toxicity by B_6NH_2 was due to a chemical interaction in the peritoneal cavity was ruled out by the fact that intraperitoneally injected TSC could be reversed by subcutaneous injections of B_6NH_2. The toxicity of TSC administered either orally or intraperitoneally was reversed by B_6NH_2 administered by either route. These data suggest that the site of reversal is in the tissues or the blood stream. It was found that B_6NH_2 was effective in overcoming TSC toxicity if injected within 3 hours before, or 30 minutes after TSC administration. If the B_6NH_2 was given 5 hours before the TSC, the initial convulsions and deaths were delayed significantly. Some protection was afforded when B_6NH_2 was given at the time at which the first death occurred in the control group (45 minutes after the TSC injection).

It was thought possible that the toxic effect of TSC might be enhanced if the B_6 content of the tissues was lowered by dietary restriction. Therefore groups of 40 mice were fed a diet deficient in B_6 (vit. B complex test diet, Nutritional Biochemical Corp.). Control groups of 10 mice were fed the same diet plus adequate amounts of B_6NH_2. After 27 days on these diets, the deficient animals had lost about one g/mouse, while the control animals had gained about 2 g/mouse. The toxic dose of TSC was then determined in these two groups of animals and compared to the toxic levels in animals fed the routine diet of Purina Chow. No significant difference in the TSC toxicity was observed.

The possibility existed that TSC toxicity might be due to the active removal of essential metabolites containing a carbonyl group, by thiosemicarbazone formation, and that these metabolites might be protected by administration of competing carbonyl compounds. Accordingly the following materials

were tested by intraperitoneal injection for their ability to reverse TSC toxicity or to potentiate the effectiveness of suboptimal doses of B_6NH_2: Na pyruvate (200 mg/kg), α-ketoglutaric acid (400 mg/kg), and glucose. These compounds had no effect. The administration of glutamic acid (400 mg/kg) and γ-amino butyric acid (up to 1625 mg/kg)(8) were also without effect. The convulsant effect of TSC does not appear to be due either to hypoglycemia or to hypocalcemia since glucose (650 mg/kg) and $CaCl_2$ (65 mg/kg) were inactive.

Discussion. It is not possible with the evidence at hand to come to a definite conclusion as to the mode of action of pyridoxamine in reversing TSC toxicity, nor is it clear why TSC is toxic. However, it would appear unlikely that pyridoxamine acts through its normal role of coenzyme for reactions involving transaminations, deaminations or amino acid decarboxylations. If this had been the case, one would have expected that TSC toxicity would have been greatly enhanced by B_6 deficiency. This was not found to occur. Also, the very large amount of B_6NH_2 required for reversal as compared to the amount needed for normal metabolism suggests that some sort of direct chemical inactivation takes place. In accord with this suggestion is the observation that an approximately equimolar amount of B_6NH_2 is required to neutralize a given dose of TSC. It also appears that the reversal of TSC toxicity by B_6NH_2 in mice takes place by a somewhat different mechanism than is the case with *Tetrahymena*(7). In *Tetrahymena,* protection is afforded by catalytic rather than stoichiometric amounts of B_6NH_2. Since keto acids are capable of detoxification of TSC in *Tetrahymena* cultures this may be correlated with the known function of B_6NH_2. In the mice, however, administration of large amounts of keto acids (pyruvic and α-ketoglutaric acids) produced no detectable alteration in the toxicity of TSC.

In *Tetrahymena,* TSC and semicarbazide are approximately equal in their inhibitory powers, and at the lower concentrations both are reversed by B_6NH_2. This is in contrast to the findings in the mice, where semicarbazide was only 1/20 as active as TSC, and was poorly reversed by B_6NH_2.

It would be of interest to extend the observations presented in this report to *in vivo* and *in vitro* studies of *M. tuberculosis*. Valuable information may be gained concerning the mechanism of action of TSC and of various thiosemicarbazones now being considered as agents in the chemotherapy of tuberculosis. It is also possible that B_6NH_2 may be found useful as an antidote for untoward reactions which may occur during clinical use of these compounds, if it is found that the inhibition of *M. tuberculosis* by TSC is not reversed by B_6NH_2.

Summary. 1. Fatal convulsions follow the administration of doses of 20 mg of thiosemicarbazide (TSC)/kg or more to mice. 2. Semicarbazide is approximately 1/20 as toxic as thiosemicarbazide, while aminoguanidine produces no toxic symptoms at a dose of 650 mg/kg. 3. The toxicity of thiosemicarbazide is completely reversed by stoichiometric doses of pyridoxamine administered either orally, intraperitoneally or subcutaneously. The administration of pyridoxamine is effective either preceding or following the dosage with TSC at appropriate intervals. 4. Dietary B_6NH_2 deficiency does not influence the toxicity of TSC. 5. Pyruvic acid, α-ketoglutaric acid, γ-aminobutyric acid, glutamic acid, glucose and calcium chloride did not reverse the toxicity of TSC nor enhance the activity of suboptimal doses of pyridoxamine.

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