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The Effect of Phenothiazine, N. F. (Green) on the Uptake of I^{131} by the Rat Thyroid[1]

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The drug phenothiazine (thiodiphenylamine) is an anthelmintic in common use by veterinarians in the care of hogs, sheep, and cattle and is frequently used in human treatment. As a regular constituent of the diet of livestock it is often used as a preventive. The use of this drug as an anthelmintic has been reviewed by Davey and Innes (*1*). Collier, in a series of papers, has studied various biochemical effects. The latest of these (*2*) dealt with the inhibitory effect of the drug on succinoxidase and cytochrome oxidase in a beef heart preparation.

Our interest in phenothiazine was aroused by the accidental inclusion of the drug in one lot of our stock rat food; thus without our knowledge, our entire rat colony was fed the drug daily for a period of about 2 weeks. Some of these rats were used in a routine class experiment to demonstrate the uptake of iodine by the thyroid. The failure of the gland to take up iodine (I^{131}) and the concurrent discovery of the inclusion of phenothiazine in the diet led to the following preliminary investigations in the influence of the drug on the uptake of I^{131} by the thyroid.

A group of Sprague-Dawley rats weighing between 150 and 200 g were placed on a Remington iodine-deficient diet (*3*). For the times indicated in Tables 1 and 2, commercial phenothiazine, N. F. (Green) was mixed with the diet. The phenothiazine was added to the salt mixture as recommended by the U. S. Department of Agriculture on a basis of 1 g of the drug to 10 g of salt mixture. In the Remington diet the concentration of drug amounted to 0.1% by weight of the total diet. The average daily consumption of the drug by each rat was calculated to be approximately 100 mg/kg of body weight. This figure appears to be between 50 and 75% higher than that recommended for prophylactic feeding in sheep, but it is only a small fraction of the treatment dose used in livestock.

In the first experiment (Table 1), the drug was added to the food after the animals had been maintained on the Remington diet for 3 days; in the second, more complete experiment (Table 2), the animals were fed the Remington diet for 10 days before the addition of phenothiazine. The I^{131} was administered intraperitoneally 24 hr before the animals were killed. For the animals on phenothiazine for only 24 hr, the I^{131} was administered 2 hr after the drugged food was offered, and, since this was done late in the afternoon, it is assumed that very little food had been eaten by the time the radioactive isotope was administered.

It is obvious from these preliminary experiments that phenothiazine, as it is normally administered to stock animals, has a marked effect on the rat thyroid uptake of I^{131}. Although the data recorded in Table 1 are incomplete, they indicate that the drug produces its effect almost immediately; for, when the drug and the iodine isotope were administered within 2 hr of each other, the thyroids, were found to be suppressed to a degree equivalent to those of animals fed phenothiazine for longer periods of time. This immediate effect was further demonstrated by the lack of significant difference in the uptake of I^{131} by the thyroids of animals fed the drug for 2, 3, or 4 days (Table 2). The difference in the uptake between

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TABLE 1

I^{131} UPTAKE BY RAT THYROID—REMINGTON DIET 6 DAYS

Groups	No. of animals	Thyroid wt., mg	% Uptake, total gland[1]	% Uptake,[2] mg	Thyroid/blood ratio[3]
Controls	3	17	9.7	0.56	13,500
Phenothiazine 3 days	2	18	0.9	0.05	137
Phenothiazine 2 days	2	19	1.9	0.10	313
Phenothiazine 1 day	2	15	1.3	0.09	860

[1] Radioactivity in total gland ÷ total radioactivity injected.
[2] Radioactivity/mg of thyroid ÷ total radioactivity injected.
[3] Radioactivity/mg of thyroid ÷ radioactivity/ml of blood.

TABLE 2

I^{131} UPTAKE BY RAT THYROID—REMINGTON DIET 14 DAYS
(All figures represent the average of 4 animals ± S.E.)

Groups	Thyroid wt., mg	% Uptake, total gland[1]	% Uptake,[2] mg	Thyroid/blood ratio[3]
Controls	16.9 ± 1.6	28.5 ± 5.1	1.85 ± 0.33	26,450 ± 1,291
Phenothiazine 4 days	13.6 ± 1.1	1.6 ± 0.03	0.12 ± 0.04	697 ± 120
Phenothiazine 3 days	14.5 ± 1.6	1.2 ± 0.16	0.09 ± 0.16	598 ± 181
Phenothiazine 2 days	12.4 ± 0.6	1.7 ± 0.15	0.14 ± 0.04	574 ± 212

[1] Radioactivity in total gland ÷ total radioactivity injected.
[2] Radioactivity/mg of thyroid ÷ total radioactivity injected.
[3] Radioactivity/mg of thyroid ÷ radioactivity/ml of blood.

the experimental and the control animals was highly significant statistically. This degree of suppression of radioiodine uptake was in the same range as that reported by Rawson *et al.* (*4*) when thiouracil was administered as 0.1% of the diet for 16 days to rats maintained on the same diet used here.

Since this drug is widely used as an anthelmintic for livestock and occasionally as such for man, this effect upon the thyroid should be investigated further. It has not as yet been determined whether its action follows that of a typical antithyroid drug, nor is it known what effect the impurities of the commercial preparation have on this action. It also remains to be seen whether other animals, particularly livestock, react as does the rat.

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Albinism Resulting from Certain Carbonic and Thiocarbonic Acid Derivatives of Hydrazine

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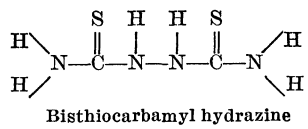
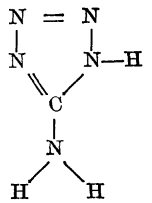
During the course of a research program designed to study the biological activity of various hydrazine derivatives of the carbonic and the thiocarbonic acids and related compounds of high nitrogen content it was discovered that three of these substances, 5-aminotetrazole, bisthiocarbamyl hydrazine, and 1,2-diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole induce albinism in plants. This effect is similar to that described by Hamner and Tukey (*1*), and by Ready *et al.* (*2*) who found that albinism is induced by treatment with 3-(α-imino-ethyl)-5-methyltetronic acid and 3-nitro-4-hydroxybenzoic acid, respectively.

Three series of experiments were conducted to evaluate this unusual phenomenon further. In the first series of tests the three compounds were applied to 400 g of soil in unglazed pots at rates approximately equivalent to 60, 25, and 10 lb/acre. Seeds of corn and soybeans were planted simultaneously. A randomized block design with four replications was used. The second group of experiments was designed to test the persistence of these substances in the soil. When the plants in the first test were 45 days old, they were removed from the pots and the pots were replanted without any further addition of chemical. In a third series of experiments, plant species consisting of both crops and weeds growing in greenhouse flats were sprayed with concentrations of 4000 ppm, 2000 ppm, and 1000 ppm until runoff was obtained.

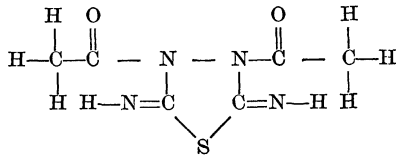
The structural formulas of the three compounds which induced albinism in plants are given as follows:



Bisthiocarbamyl hydrazine



5-Aminotetrazole



1,2-Diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole

The blanching effect produced by bisthiocarbamyl hydrazine and 1,2-diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole started at the base of the lower leaves and advanced toward the tips. The chlorophyll was gradually removed from the leaves resulting in the death of the entire plant. 1,2-Diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole was slower in producing the effect than bisthiocarbamyl hydrazine. Approximately 14 days after treatment with concentrations of 1000 ppm and 4000 ppm of bisthiocarbamyl hydrazine, the corn plants started turning white at the base of the leaves. Plants growing in soil treated with a concentration of 4000 ppm of 1,2-diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole started to show a blanching effect approximately 20 days after treatment; the 1000 ppm concentration had very little effect. However, a concentration of 1000 ppm of bisthiocarbamyl hydrazine turned the plants completely white and finally killed them.

Application of bisthiocarbamyl hydrazine to fresh chlorophyll extractions in light resulted in the disappearance of the green color. The addition of an excess of copper sulfate restored the green color. Since bisthiocarbamyl hydrazine complexes metallic ions very effectively, it is believed that the restoration of green color may be due to the removal of the hydrazine derivative as a stable copper complex or to the formation of a copper chlorophyll complex or to both.

Bisthiocarbamyl hydrazine also affects plastid development. Electron microscopic examinations are being carried on at present to study further the mode of action.

5-Aminotetrazole also caused blanching but to a much lesser degree than either bisthiocarbamyl hydrazine or 1,2-diacetyl-3,5-diamino-1,2,3,5-tetrahydro-1,2,4-thiadiazole. The effect of 5-aminotetrazole was found to be temporary, whereas that of bisthiocarbamyl hydrazine and 1,2-diacetyl-3,5-diamino-1,2,3,5-