Effects of Short-Term Administration of Ethylenethiourea Upon Thyroid Function of the Rat

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Dithiocarbamates have been used as fungicides against a variety of plant pathogenic fungi. Smith et al. (1) reported that thyroid changes occurred in rats fed the ethylenebisdithiocarbamate fungicides throughout their life span. Petrosini et al. (2) investigated the degradation of the zinc ethylenebisdithiocarbamate (zineb) and zinc dimethyldithiocarbamate (ziram) under different storage conditions and found the degradation products ethylenethiourea (ETU), carbon disulfide, and zinc sulfide. Seifter and Ehrich (3) fed ETU to weanling rats at 0.1% of the diet (1000 ppm) for 8 days and noted decreased growth, increased thyroid weight, and marked thyroid hyperplasia. The present report describes a short-term study of the effect of ETU on thyroid function to determine whether the thyroid effects ascribed by Smith et al. (1) to ethylenebisdithiocarbamate fungicides, as measured by ^{131}I uptake per unit mass of tissue, may be due to the breakdown product ETU.

Materials and Methods

Five groups of 20 male Osborne-Mendel rats were fed ETU mixed in ground Purina Laboratory Chow at levels of 0, 50, 100, 500, and 750 ppm for 30, 60, 90, and 120 days. The acute oral LD50 for ETU was found to be 1832 mg/kg (confidence interval from 1379 to 2562). The rats were individually housed and food and water were provided ad libitum. Body weight and food consumption were recorded every 7 days.

At each sacrifice period, 20 rats from each group were injected ip with 0.2 ml of normal saline containing approximately 5 μCi of ^{131}I. Half of these rats were killed with a barbiturate overdose at 4 hr post-injection and the other half at 24 hr post-injection. Thyroid pairs were removed and weighed, and radioactivity was determined with a gamma well-type scintillation counter. Three successive 2-min. counts of ^{131}I activity were taken for each thyroid pair.

Thyroid glands of rats fed ETU for 90 days were cut, placed in formalin, stained with hematoxylin-eosin, and graded for degree of epithelial hyperplasia, follicular enlargement, and loss of colloid according to the method of Seifter and Ehrich (3).

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Results and Discussion

The effect of ETU on the body weights of rats is shown in Table 1. At dietary concentrations of 500 and 750 ppm, the weights of the treated rats were significantly lower than their respective controls at all feeding periods. The weights of those fed for 60 days at 100 ppm were also significantly decreased. Rats fed 750 ppm weighed an average of 45% less than control rats. The effects were relatively greater early (at 30 days) than later (120 days).

Food consumption was significantly lower in animals fed ETU at levels of 100, 500, and 750 ppm for 30 and 90 days. After 60 and 120 days, food consumption was decreased only in the animals fed 500 and 750 ppm.

The ratios of thyroid weight to body weight (mg thyroid/100 g of body weight) of rats fed ETU at 500 and 750 ppm were significantly higher than those of control rats (Table 2) at all feeding periods. The ratios of the 100-ppm feeding level were only slightly higher than controls. At the 2 higher levels, the ratios ranged from 2 to 5 times the control values; maximum effect was reached at 500 ppm. The mean weights of the thyroid pairs are recorded in Table 3.

The effect of ETU feeding upon the uptake in thyroid of radioactive iodine, measured 4 and 24 hr after the injection of the isotope, is shown in Tables 4 and 5. The 4 hr post-injection count was made to determine the magnitude of the effect during this period as compared to the 24-hr period. Literature reports have shown that the counts are approaching a peak at 24 hr (4).

Four hours after the injection of ^{131}I, the uptake had decreased significantly in rats fed ETU at 500 and 750 ppm at all feeding periods. Non-significant increases in uptake were observed at the 50-ppm level for both the 30- and 90-day feeding periods.

The uptake of iodine 24 hr after injection was decreased in those animals fed ETU at all levels for 30, 60, and 120 days; however, this decrease is significant only at the levels of 100, 500, and 750 ppm. After the 90-day feeding period, the uptake increased slightly in rats fed the 50-ppm level, while at the 500- and 750-ppm levels, there were significant decreases which ranged from 6 to 13 times lower than control values.

On histologic examination, the sections of thyroid glands of rats fed 50 ppm of ETU were not different from those of controls and the sections from rats fed ETU at 100 ppm showed very slight to slight hyperplasia. At levels of 500 and 750 ppm, the thyroid glands showed moderate to marked hyperplasia, and the lack of colloid material and the heightened epithelial walls were quite noticeable.

TABLE 1

Effect of ETU Administration on Body Weight of Rats[a]

Dietary Level (ppm)	Mean Body Weight (g) After Days of ETU Diet 30	60	90	120
0	341 ± 5	358 ± 6	401 ± 8	421 ± 9
50	330 ± 6	355 ± 8	386 ± 10	423 ± 12
100	330 ± 7	337 ± 7[b]	389 ± 9	432 ± 13
500	265 ± 4[b]	288 ± 7[b]	325 ± 10[b]	381 ± 7[b]
750	168 ± 8[b]	204 ± 7[b]	206 ± 10[b]	270 ± 15[b]

[a] Values are given as the mean ± 1 SE of 10 rats.

[b] Significantly different from control group at 5% level or less when tested by the Student t test.

TABLE 2

Effect of ETU Administration on Thyroid Weights of Rats[a]

Dietary Level (ppm)	Thyroid to Body Weight Ratio (mg/100 g) After Days of ETU Diet 30	60	90	120
0	6.1 ± 0.3	5.6 ± 0.3	6.3 ± 0.3	5.2 ± 0.2
50	6.3 ± 0.3	6.9 ± 0.3	6.6 ± 0.4	6.1 ± 0.2[b]
100	8.0 ± 0.2[b]	7.2 ± 0.4[b]	6.9 ± 0.3	6.9 ± 0.3[b]
500	13.3 ± 0.5[b]	24.3 ± 1.5[b]	22.4 ± 1.2[b]	25.9 ± 1.8[b]
750	16.3 ± 1.1[b]	24.1 ± 1.5[b]	24.4 ± 1.7[b]	27.1 ± 2.0[b]

[a] Values are given as the mean ± 1 SE of 10 rats.

[b] Significantly different from control group at 5% level or less when tested by the Student t time.

These changes can be seen in Fig. 1, which shows a section of thyroid from a rat fed the 500-ppm diet and a section from the thyroid of a control rat. Adenomas were also observed in sections from rats fed at the 500- and 750-ppm levels. Microscopically the hyperplasia shows some indications of increased circulation. The number of blood vessels present per low power field were not counted, but the incidence of vessels was greater in the enlarged thyroid glands, indicating a response to an increased blood level of thyroid-stimulating hormone (TSH).

We have shown that short-term ETU feeding increases thyroid weight, decreases ^{131}I uptake, and at high doses, produces thyroid hyperplasia. Our findings agree with those of Christensen et al. (5) who administered disulfiram to rats both by feeding at 500 ppm in the diet for 30 days and by oral intubation at 500 mg/kg/day for 3 days. They found that the thyroid ^{131}I trapping activity (as measured by ^{131}I uptake per unit mass of tissue) was reduced to 44.8% of that of the control. Increased thyroid mass was also noted.

The observation of Rosenberg (6) that compounds which inhibited peroxidase activity also altered thyroid function lent support to the concept that a thyroid peroxidase might be involved in the organic binding of iodine. Alexander (7) demonstrated the presence in thyroid extracts of an iodide peroxidase which oxidized iodide to iodine. The activity of the enzyme was inhibited in vitro by thiouracil, cyanide, azide, aminotriazole, and p-aminobenzoate. In a preliminary study we have found that ETU inhibits iodide peroxidase in vitro and this may be one mechanism by which ETU acts in vivo.

An increase in thyroid size with hyperplasia associated with a reduction in colloid content of the follicles may reflect either a hyperfunctioning or a hypofunctioning gland (8). We found a decrease in body weight together with a significant increase in thyroid mass, indicating an overactive thyroid gland. It is possible that ETU reduces thyroid activity initially with compensation occurring later by an increased release of TSH. This increase of TSH produced a hyperplasia of the thyroid in an attempt to overcome the blocking effect of ETU (9).

Previous long-term feeding studies (10), involving the ethylenebisdithiocarbamates indicated that thyroid changes occurred in rats, and the question has now arisen as to how much of the effect was due to the presence of ETU in the feed. For this reason we plan to reinvestigate the parent compounds, using materials of known purity.

TABLE 3

Effect of ETU Administration on Thyroid Weights of Rats[a]

Dietary Level (ppm)	Mean Thyroid Weight (mg) After Days of ETU Diet			
	30	60	90	120
0	20.7 ± 0.9	20.5 ± 0.9	23.2 ± 1.4	21.9 ± 0.9
50	20.5 ± 1.1	24.6 ± 1.5[b]	25.1 ± 1.3	25.8 ± 1.2[b]
100	26.2 ± 0.8[b]	24.2 ± 1.4[b]	26.6 ± 1.0	27.9 ± 1.9[b]
500	35.1 ± 1.3[b]	70.6 ± 5.5[b]	72.4 ± 4.1[b]	93.9 ± 8.8[b]
750	27.7 ± 3.3[b]	48.7 ± 1.6[b]	50.9 ± 5.1[b]	73.4 ± 6.9[b]

[a] Values are given as the mean ± 1 SE of 10 rats.

[b] Significantly different from control group at 5% level or less when tested by the Student t test.

TABLE 4

^{131}I Uptake (cpm/mg Tissue) 4 Hours Post-Injection[a]

Dietary Level (ppm)	Days of ETU Diet 30	60	90	120
0	494 ± 74	301 ± 44	1395 ± 172	1375 ± 89
50	612 ± 52	238 ± 34	1615 ± 219	1161 ± 138
100	468 ± 32	260 ± 36	1487 ± 224	1114 ± 110
500	204 ± 12[b]	84 ± 15[b]	839 ± 124[b]	430 ± 47[b]
750	213 ± 75[b]	100 ± 26[b]	791 ± 55[b]	422 ± 77[b]

[a] Values are given as the mean ± 1 SE of 5 thyroid pairs.

[b] Significantly different from control group at 5% level or less when tested by the Student t test.

TABLE 5

^{131}I Uptake (cpm/mg Tissue) 24 Hours Post-Injection[a]

Dietary Level (ppm)	Days of ETU Diet 30	60	90	120
0	910 ± 89	1852 ± 155	2056 ± 197	1802 ± 154
50	722 ± 52	1606 ± 200	2214 ± 215	1482 ± 142
100	493 ± 58[b]	1365 ± 129[b]	1858 ± 193	1288 ± 87[b]
500	81 ± 13[b]	144 ± 20[b]	412 ± 49[b]	364 ± 87[b]
750	129 ± 34[b]	138 ± 29[b]	274 ± 14[b]	319 ± 52[b]

[a] Values are given as the mean ± 1 SE of 5 thyroid pairs.

[b] Significantly different from control group at 5% level or less when tested by the Student t test.

Summary

In summary, five groups of male Osborne-Mendel rats were fed ETU for 30, 60, 90, and 120 days at levels of 0, 50, 100, 500, and 750 ppm in the diet. At the end of each feeding period two groups on each level were injected with approximately 5 μCi of ^{131}I to measure iodine uptake by the thyroid 4 and 24 hours after injection.

Significant decreases in body weight occurred in the animals fed the diet at 500 and 750 ppm of the diet, and significant increases in thyroid to body weight ratios were seen at the 100, 500, and 750 ppm levels at 30 days, after 90 days at the two high levels, and at all levels after 60 and 120 days.

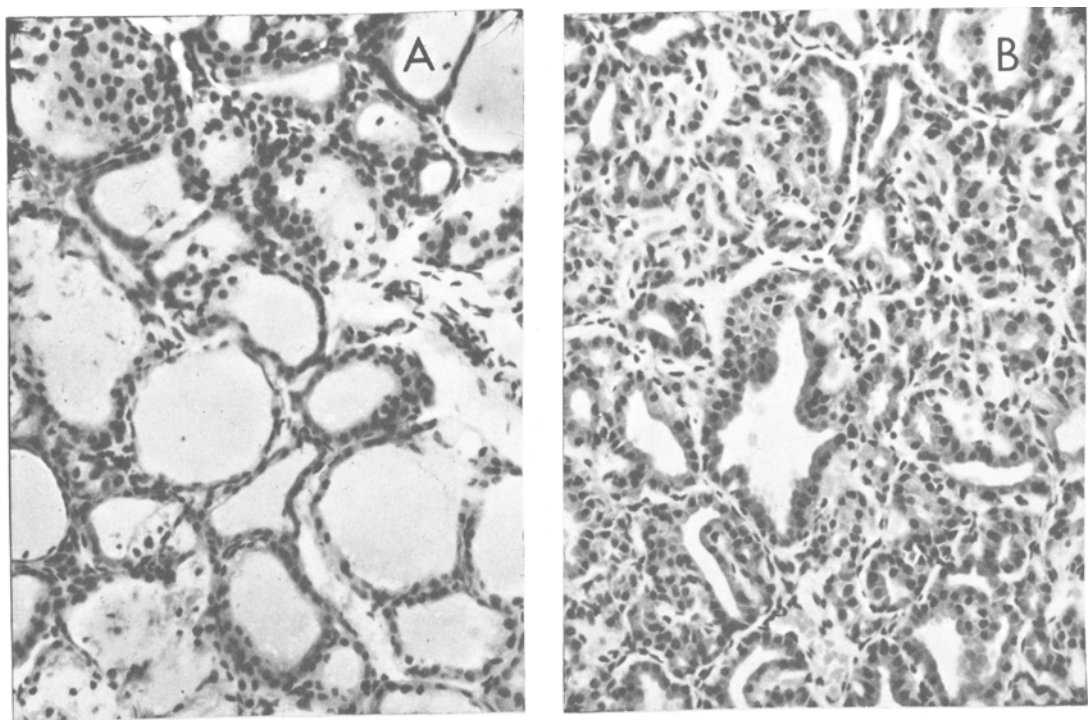

FIG. 1. Hyperplasia, loss of colloid, and heightened acinar epithelial cells of the thyroid of a rat fed 500 ppm ETU for 90 days (A) compared to the thyroid of a control rat (B). Hematoxylin-eosin, X250.

Twenty-four hours after the injection of ^{131}I the thyroidal uptake (counts per minute per milligram tissue) was significantly decreased at the 100, 500, and 750 ppm levels at all feeding periods.

No difference from controls could be detected in the histologic appearance of sections of the thyroid glands of rats fed ETU at 50 ppm of the diet. Thyroid glands from rats fed at 500 and 750 ppm showed moderate to marked hyperplasia.

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