Effects of One-Year Administration of Ethylenethiourea upon the Thyroid of the Rat

Stuart L. Graham,* Walter H. Hansen, Kent J. Davis,[1] and Carleene H. Perry

Male and female Charles River rats, approximately 5-weeks-old, were fed ethylenethiourea (ETU) for 2, 6, or 12 months at levels of 0, 5, 25, 125, 250, or 500 ppm in the diet. Body weight, thyroid and other organ weights, thyroidal ^{131}I uptake, hematology, and histology were the criteria studied. Significant decreases in body weight and increases in thyroid weight were seen at the 125-, 250-, and 500-ppm levels. Uptake of ^{131}I was significantly decreased in male rats after 12 months at 500 ppm but was increased in females. After 12 months, microscopic examination of the thyroids revealed the development of nodular hyperplasia at dose levels of 125 ppm and higher; carcinomas were found at dose levels of 250 and 500 ppm. The development of thyroid carcinomas indicates that, under the conditions of this study, ETU is a carcinogen.

Dithiocarbamates have been used as fungicides against a variety of pathogenic plant fungi. Smith *et al.* (1953) reported thyroid changes in rats fed the ethylenebisdithiocarbamate fungicides throughout their life span. These compounds are unstable and one of the degradation products is ethylenethiourea (ETU). A possible degradation scheme for metallic derivatives of the ethylenebisdithiocarbamates has been suggested and is shown in Figure 1. Ludwig and Thorn (1958) speculated that ethylenebisdithiocarbamic acid readily forms ETU under highly alkaline conditions (pH 10.5). It is possible that the ETU obtained under these conditions is formed from ethylenethiuram monosulfide by the loss of a molecule of carbon disulfide.

Until recently, the literature had only one reference to a toxicological study utilizing ETU. Seifter and Ehrich (1948) 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.

Innes *et al.* (1969) revived the interest in ETU when they reported that this compound was tumorigenic for the liver; thyroids were not examined. Graham and Hansen (1972), in a short-term feeding study using male Osborne-Mendel rats, reported increases in thyroid weight, decreases in ^{131}I uptake, and, at high doses (500 and 750 ppm), thyroid hyperplasia. Ulland *et al.* (1972) reported a study in which rats were fed 350 or 175 ppm of ETU in their diets for 18 months, followed by a control diet for 6 months. They concluded that ETU has an action like that of a number of other thio compounds which cause thyroid carcinomas and indirectly affect the liver.

This report describes the effects on the thyroid glands of male and female Charles River rats after 2, 6, and 12 months of ETU administration.

MATERIALS AND METHODS

Five groups of 68 male and 68 female Charles River rats, approximately 5-weeks-old, were started on ETU diets. Control groups were fed the basic diet of Purina Ground Chow, while the test groups were maintained on the commercial diet containing added ETU (Lot No. 4876, K & K Laboratories, Plainview, N. Y.) at levels of 5, 25, 125, 250, or 500 ppm. The rats were individually housed and food and water were provided *ad libitum*. Body weights and food consumption were recorded every 7 days.

At the end of the 2-, 6-, and 12-month feeding periods, ten males and ten females at each ETU level were given, intraperitoneally, 0.2 ml of physiologic saline prepared to contain approximately 5 μCi of ^{131}I. The rats were fasted for 24 hr. Each animal was then given a barbiturate overdose and the thyroids, heart, liver, kidneys, spleen, brain, and testes, in the case of the males, were removed and weighed. The radioactivities of individually weighed thyroid pairs were determined with a γ well counter. Reference standards were made at the time of injection and all counts were compared with this standard as an average of three successive determinations of counts per minute (cpm). The uptake was determined after conventional corrections were made for radioactive decay.

After 3 and 11 months, blood samples were collected from the tail vein of ten male and ten female rats at each dietary level and hemoglobin, hematocrit, leukocyte counts, and leukocyte differential counts were determined.

After the 6- and 12-month feeding periods, tissues were removed from all sacrificed animals and evaluated grossly, and the hematoxylin–eosin-stained (H & E) paraffin sections of the formalin-fixed thyroids were then examined by light microscopy.

All data were compiled and tested for significance by the two-tailed student t test.

RESULTS AND DISCUSSION

Body Weights. Table I shows the effect of ETU on the body weights of rats fed the test diets for 2, 6, and 12 months. The body weights of males fed ETU for 2 months were significantly decreased from control values at the 250- ($p < 0.01$) and 500-ppm ($p < 0.001$) levels; males fed ETU for 6 months had body weights which were decreased significantly at the 25- ($p < 0.001$), 250- ($p < 0.01$), and 500-ppm ($p < 0.001$) levels. After 12 months, only the weights of male rats fed ETU at 500 ppm were significantly lower than the control value ($p < 0.001$).

The body weights of female rats fed ETU for 2 months were significantly decreased from control values at the 25-, 125-, 250-, and 500-ppm levels (Table I), and after 6 and 12 months they were significantly decreased at the 125-, 250-, and 500-ppm levels.

Organ-to-Body Weight Ratios. *2 Months.* The effects of ETU administration for 2 months on organ-to-body weight ratios of rats are shown in Table II. Liver-to-body weight ratios of both males and females, as compared with control ratios, were increased significantly at the 125-, 250-, and 500-ppm levels. In males fed 500 ppm, the kidneys and testes ratios were significantly increased over control values. The greatest effect of ETU was seen in the thyroid gland; thyroid ratios of the male rats fed the 250- and 500-ppm levels and females fed the 125-, 250-, and 500-ppm levels were significantly ($p < 0.001$) elevated over control values.

6 Months. Table III gives the organ-to-body weight ratios of rats fed ETU for 6 months. In males, ratios for liver, kidneys, and testes were significantly elevated above those of the controls at the 125-, 250-, and 500-ppm levels,

Bureau of Foods, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C. 20204.

[1] Present address: Environmental Protection Agency, Washington, D. C. 20460.

Table I. Mean Body Weights (g) ±SE of Rats Fed ETU in the Diet

Dietary level, ppm	No. of rats	2 months of diet	No. of rats	6 months of diet	No. of rats	12 months of diet
			Males			
0	68	419 ± 5	56	617 ± 10	45	703 ± 13
5	68	421 ± 4	58	613 ± 8	48	711 ± 11
25	68	411 ± 4	57	583 ± 8[a]	47	670 ± 12
125	68	421 ± 5	58	601 ± 9	46	692 ± 12
250	67	403 ± 4[b]	58	579 ± 8[b]	44	677 ± 12
500	68	327 ± 4[a]	58	509 ± 7[a]	47	604 ± 15[a]
			Females			
0	68	270 ± 3	58	371 ± 6	47	466 ± 11
5	68	267 ± 3	58	363 ± 7	48	464 ± 11
25	68	259 ± 3[b]	58	357 ± 5	46	442 ± 10
125	68	254 ± 3[a]	58	342 ± 5[a]	48	429 ± 11[c]
250	68	245 ± 3[a]	57	331 ± 5[a]	46	408 ± 9[a]
500	68	231 ± 3[a]	58	326 ± 5[a]	46	406 ± 8[a]

[a] Significantly different from control value, $p < 0.001$. [b] Significantly different from control value, $p < 0.01$. [c] Significantly different from control value, $p < 0.05$.

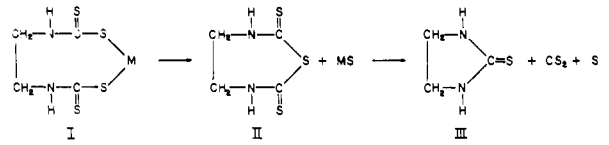

Figure 1. Degradation scheme for metallic derivatives of the ethylenebisdithiocarbamates.

as were thyroid-to-body weight ratios at the 250- and 500-ppm levels. In females, statistically significant increases in liver-to-body weight ratios were seen at the 250- and 500-ppm levels and in thyroid ratios at the 125-, 250-, and 500-ppm levels.

12 Months. The effects of ETU feeding for 12 months on organ-to-body weight ratios of rats are shown in Table IV. Thyroid-to-body weight ratios of both males and females fed the 125-, 250-, and 500-ppm levels were significantly increased as compared with control values. In females, liver and kidney ratios were elevated significantly over control values at both the 250- and 500-ppm levels.

Uptake of Radioactive Iodine. The uptakes of iodine (expressed as cpm/mg of tissue) 24 hr postinjection are

Table II. Mean Organ-to-Body Weight (g/kg) Ratios ±SE of Charles River Rats Fed ETU for 2 Months (Ten Rats per Group)

Dietary level, ppm	Liver	Kidney	Spleen	Heart	Testes	Thyroid[a]
			Males			
0	25.94 ± 0.57	6.48 ± 0.14	1.73 ± 0.09	2.94 ± 0.10	8.22 ± 0.25	44.7 ± 2.3
5	26.50 ± 0.54	6.62 ± 0.13	1.52 ± 0.07	3.13 ± 0.16	7.60 ± 0.15	45.4 ± 3.5
25	26.73 ± 0.55	6.54 ± 0.13	1.67 ± 0.09	2.85 ± 0.09	7.47 ± 0.23[b]	40.1 ± 1.9
125	28.50 ± 0.41[c]	6.72 ± 0.20	1.53 ± 0.11	2.83 ± 0.11	7.47 ± 0.26	55.9 ± 4.9
250	29.58 ± 0.31[b]	6.66 ± 0.16	1.51 ± 0.09	2.92 ± 0.10	7.79 ± 0.22	93.6 ± 5.9[d]
500	29.53 ± 0.76[c]	7.00 ± 0.13[b]	1.62 ± 0.17	2.89 ± 0.13	10.78 ± 0.55[d]	102.9 ± 8.3[d]
			Females			
0	25.40 ± 0.49	6.48 ± 0.14	1.96 ± 0.06	3.10 ± 0.09		60.0 ± 2.9
5	24.93 ± 0.56	6.37 ± 0.13	2.01 ± 0.12	2.86 ± 0.08		66.8 ± 3.5
25	25.50 ± 0.37	6.88 ± 0.15	2.00 ± 0.08	3.02 ± 0.03		66.2 ± 3.3
125	27.60 ± 0.07[b]	6.73 ± 0.22	2.11 ± 0.10	3.14 ± 0.06		78.4 ± 3.3[d]
250	28.72 ± 1.27[b]	6.92 ± 0.32	2.17 ± 0.17	3.12 ± 0.13		105.5 ± 5.3[d]
500	29.34 ± 0.76[d]	6.91 ± 0.43[b]	1.69 ± 0.08[b]	2.86 ± 0.06		171.3 ± 15.5[d]

[a] Thyroid ratios are expressed as mg/kg. [b] Significantly different from control, $p < 0.05$. [c] Significantly different from control, $p < 0.01$. [d] Significantly different from control, $p < 0.001$.

Table III. Mean Organ-to-Body Weight (g/kg) Ratios ±SE of Charles River Rats Fed ETU for 6 Months (Ten Rats per Group)

Dietary level, ppm	Liver	Kidney	Spleen	Heart	Testes	Thyroid[a]
			Males			
0	23.30 ± 0.37	5.66 ± 0.10	1.35 ± 0.08	2.64 ± 0.06	6.28 ± 0.17	51.5 ± 3.3
5	23.61 ± 0.63	5.81 ± 0.23	1.32 ± 0.07	2.65 ± 0.11	6.27 ± 0.33	45.1 ± 2.4
25	24.82 ± 0.13	5.62 ± 0.18	1.31 ± 0.06	2.61 ± 0.09	6.02 ± 0.31	44.3 ± 2.3
125	29.89 ± 0.96[b]	6.52 ± 0.23[c]	1.43 ± 0.08	2.66 ± 0.10	6.83 ± 0.32[d]	61.9 ± 4.1
250	29.35 ± 0.76[b]	6.12 ± 0.18[d]	1.44 ± 0.07	2.75 ± 0.06	6.88 ± 0.16[d]	79.9 ± 6.1[c]
500	30.98 ± 1.15[b]	6.22 ± 0.21[d]	1.31 ± 0.09	2.76 ± 0.08	7.72 ± 0.24[b]	139.5 ± 14.9[b]
			Females			
0	31.00 ± 0.68	6.07 ± 0.20	1.83 ± 0.23	2.81 ± 0.09		59.1 ± 3.1
5	32.14 ± 1.21	6.15 ± 0.19	1.65 ± 0.07	3.04 ± 0.09		65.9 ± 4.4
25	28.68 ± 0.80[d]	5.90 ± 0.18	1.59 ± 0.07	2.90 ± 0.12		59.8 ± 3.8
125	31.74 ± 1.41	6.11 ± 0.11	1.50 ± 0.07	3.00 ± 0.14		72.0 ± 4.6[d]
250	33.75 ± 1.09[d]	6.40 ± 0.18	1.60 ± 0.11	3.11 ± 0.12		93.5 ± 4.1[b]
500	35.58 ± 0.64[b]	6.40 ± 0.27	1.57 ± 0.07	3.01 ± 0.12		174.6 ± 21.5[b]

[a] Thyroid ratios are expressed as mg/kg. [b] Significantly different from control, $p < 0.001$. [c] Significantly different from control, $p < 0.01$. [d] Significantly different from control, $p < 0.05$.

Table IV. Mean Organ Weight-to-Body Weight (g/kg) Ratios ± SE of Charles River Rats Fed ETU for 12 Months (Ten Rats per Group)

Dietary level, ppm	Liver	Kidney	Spleen	Heart	Testes	Thyroid[a]
			Males			
0	26.96 ± 1.22	5.52 ± 0.27	1.55 ± 0.07	2.42 ± 0.09	5.56 ± 0.20	44.3 ± 2.0
5	25.38 ± 0.69	5.24 ± 0.15	1.57 ± 0.07	2.56 ± 0.08	5.99 ± 0.21	43.9 ± 3.3
25	24.89 ± 1.09	5.57 ± 0.21	1.65 ± 0.05	2.44 ± 0.07	5.80 ± 0.28	48.8 ± 2.9
125	31.13 ± 1.24[b]	5.77 ± 0.22	1.50 ± 0.10	2.47 ± 0.10	5.76 ± 0.29	52.9 ± 3.5[b]
250	27.88 ± 1.36	5.73 ± 0.31	1.60 ± 0.14	2.42 ± 0.16	5.76 ± 0.18	87.7 ± 13.2[c]
500	28.32 ± 1.21	5.40 ± 0.20	1.22 ± 0.09[c]	2.32 ± 0.11	6.23 ± 0.26	779.0 ± 231.4[b]
			Females			
0	25.73 ± 1.55	5.29 ± 0.22	1.60 ± 0.09	2.66 ± 0.13		57.3 ± 3.5
5	22.68 ± 0.76[b]	5.32 ± 0.27	1.85 ± 0.19	2.65 ± 0.13		56.4 ± 3.3
25	24.07 ± 0.82	5.52 ± 0.26	1.37 ± 0.06	2.57 ± 0.09		56.8 ± 3.3
125	26.97 ± 1.21	5.53 ± 0.25	1.68 ± 0.11	2.60 ± 0.11		68.6 ± 4.1[b]
250[d]	29.78 ± 1.42[b]	6.21 ± 0.30[b]	1.50 ± 0.08	3.08 ± 0.15[b]		97.7 ± 8.0[e]
500	30.64 ± 1.67[b]	6.24 ± 0.28[b]	1.61 ± 0.10	2.77 ± 0.07		271.5 ± 85.7[b]

[a] Thyroid ratios are expressed as mg/kg. [b] Significantly different from control, $p < 0.05$. [c] Significantly different from control, $p < 0.01$. [d] Nine rats. [e] Significantly different from control, $p < 0.001$.

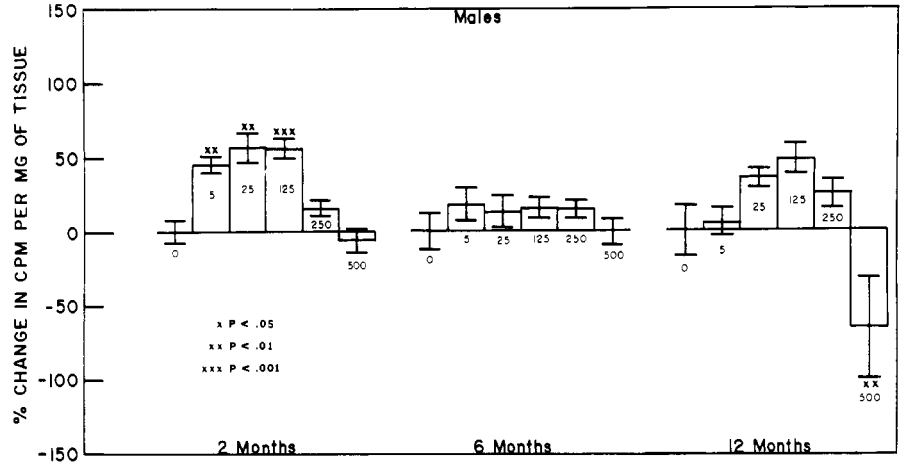

Figure 2. The uptake of ^{131}I by the thyroids (expressed as percent change in cpm/mg of tissue) of male rats. Vertical bars are the SE.

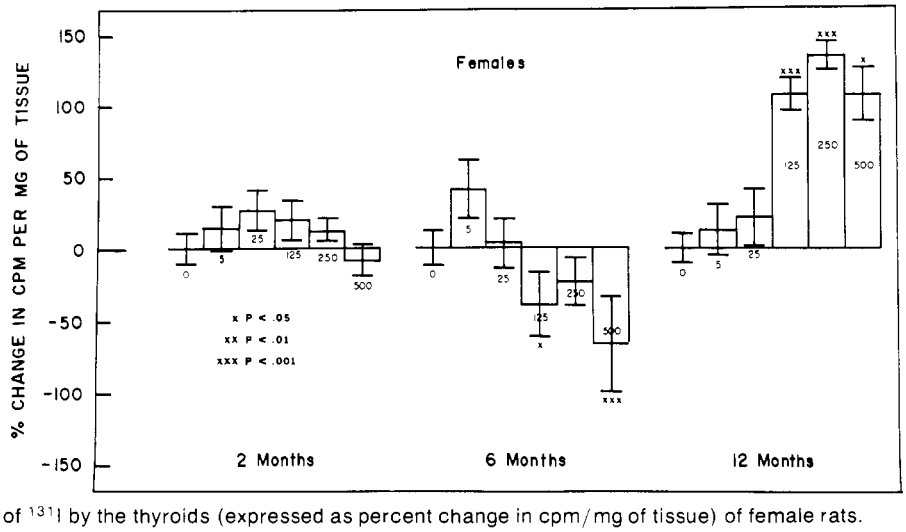

Figure 3. The uptake of ^{131}I by the thyroids (expressed as percent change in cpm/mg of tissue) of female rats.

shown in Figures 2 and 3. The histograms show the percent change from control values.

Male rats fed the ETU diet at the 5-, 25-, and 125-ppm levels for 2 months had statistically significant increases in ^{131}I uptake; the values were 45, 57, and 56% higher, respectively, than control values (Figure 2). There were no significant differences in the uptake at any level in the males that were fed ETU for 6 months; all uptakes except

those at the 500-ppm level were slightly increased over the control value. After 12 months, the uptake was increased in male rats fed the 25-, 125-, or 250-ppm diets. At 500 ppm, however, the uptake was decreased to a value which was 67% lower than that of the controls.

Figure 3 represents the ^{131}I uptake in the thyroids of female rats fed ETU in their diets for 2, 6, or 12 months. There were no statistically significant differences in the uptake at any level in females which were fed for 2 months. However, female rats at 6 months had an initial increase in uptake at the 5-ppm level and gradual decreases at the 125-, 250-, and 500-ppm levels; the decreased values were 40, 23, and 66% lower, respectively, than control values. The uptakes at 12 months were reversed from those seen at 6 months; they were increased at the 125-, 250-, and 500-ppm levels to values which were 107, 134, and 107% of the control value, respectively.

Hematology. No effects were observed on hemoglobins, hematocrits, leukocyte counts, and leukocyte differential counts of male and female rats fed ETU after 3 and 11 months.

Histologic Examination of H & E-Stained Tissues from Rats Fed Ethylenethiourea for 6 and 12 Months. *6 Months.* After 6 months, the thyroids from five male and five female control animals were compared with those from five males and five females fed ETU at 500 ppm. Among the male controls there was slight hyperplasia in the thyroid of one rat and a small keratin cyst or granuloma with squamous metaplasia in another thyroid. The four parathyroids present were essentially normal histologically. In the female controls, which consisted of the thyroids plus sections of five parathyroids, all tissues were normal.

The thyroids of males fed the 500-ppm level were all hyperplastic; eight or more adenomatous nodules were present among the thyroid sections, with no thyroid showing more than three such nodules. In one thyroid, there were three areas of solid thyroid-derived cells which can be considered carcinoma. The four parathyroid sections present were essentially normal.

Female rats fed ETU at 500 ppm also had hyperplastic thyroids; two of these had low-grade carcinomas. Another thyroid showed a large papillary-colloid-cystadenoma and two others had adenomatous foci; the parathyroids were normal.

Sections of brain, Harderian gland, eye with retina and iris, extra-orbital lacrimal gland, salivary gland, salivary lymph node, heart, lung, liver, kidney, urinary bladder, ovary, uterus, spleen, pancreas, adrenal, squamous and glandular stomach, duodenum, jejunum, ileum, colon, bone, bone marrow, and skeletal muscle exhibited no changes which were attributed to ETU ingestion. Thyroid hyperplasia, adenomas, and low-grade carcinomas that were found in rats fed ETU for 6 months were attributed to the treatment.

Figure 4. Illustration of the extent of thyroid enlargement.

12 Months. In the examination of tissues from rats fed ETU for 12 months, the most striking effects were observed in the thyroid. A number of morphologic deviations were evident at all concentrations of ETU; these alterations are summarized in Table V.

The vascularity of the thyroid was increased at all test levels in both male and female rats. Nodular hyperplasia developed in males at 125 ppm and carcinomas were evident at 250 ppm. At the 500-ppm level, carcinomas were found in 77% of the male rats. In females, the parafollicular capillaries and the papillation of acinar epithelium were evident at 5 ppm and appeared especially prominent at 500 ppm. At the 500-ppm level, carcinomas were found in 42% of the female rats.

Figure 4 indicates the extent of thyroid enlargement. The thyroid on the far right is a typical control. A thyroid the size of the one on the far left would be equivalent to a 6-lb thyroid in man. In most sections, the trachea and esophagus could be seen; as the thyroid enlarged, the trachea was constricted.

Figure 5 is a section of a normal thyroid under low power (25×), showing the variation in follicular size and

Table V. Incidence of Histologic Changes Within the Thyroids of Male and Female Rats Fed ETU in the Diet for 12 Months

Dose level, ppm	Animals per group	Mean weight, mg/100 g body weight	Increased vascularity	Papillation of acinar epithelium	Hyperplasia: Diffuse (microfollicular)	Hyperplasia: Nodular (adenoma)	Hyperplasia: Diffuse and nodular (adenoma)	Adenocarcinoma
				Males				
0	13	4.6	4	0	6	0	0	0
5	11	4.6	10	0	4	0	0	0
25	11	5.1	10	1	7	0	0	0
125	11	5.5	10	5	5	0	3	0
250	13	9.0	10	8	3	3	4	3
500	13	80.0	10	12	0	2	0	10
				Females				
0	9	6.0	1	0	1	0	0	0
5	10	6.0	10	2	5	0	0	0
25	10	5.9	9	0	5	0	0	0
125	11	7.2	10	2	8	0	0	0
250	10	10.0	10	7	5	0	0	0
500	12	27.1	10	11	0	6	0	5

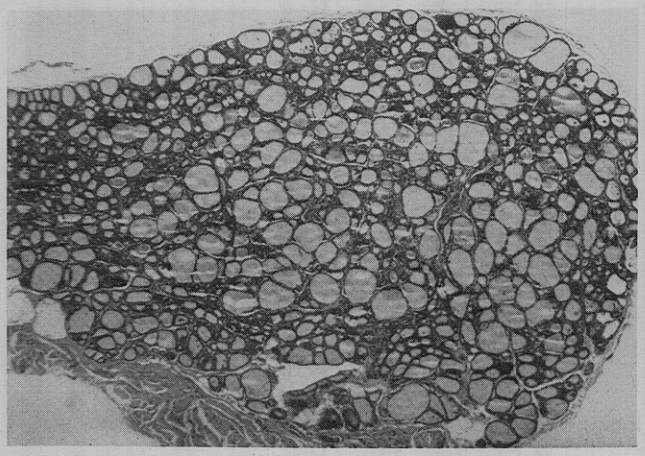

Figure 5. Section of a control thyroid under low power (25×).

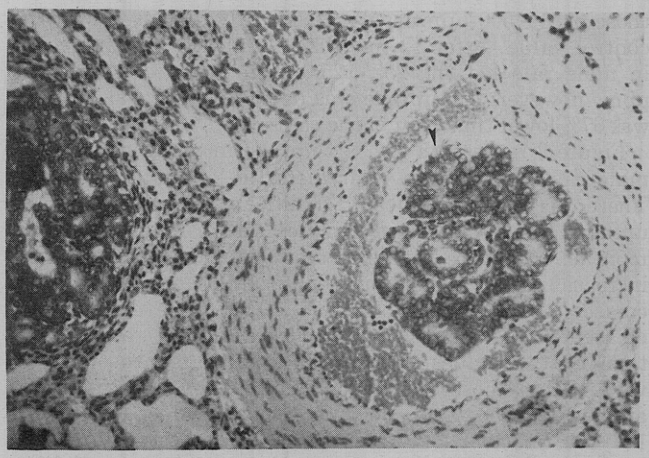

Figure 8. The carcinoma showing more clearly the vascular invasion (125×).

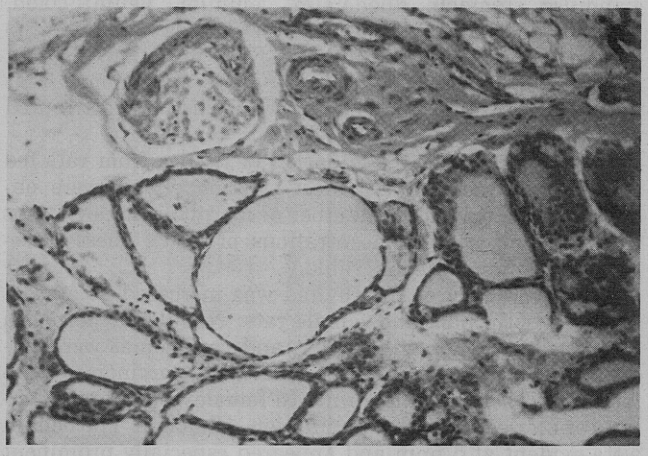

Figure 6. Section of a control thyroid under higher power (125×).

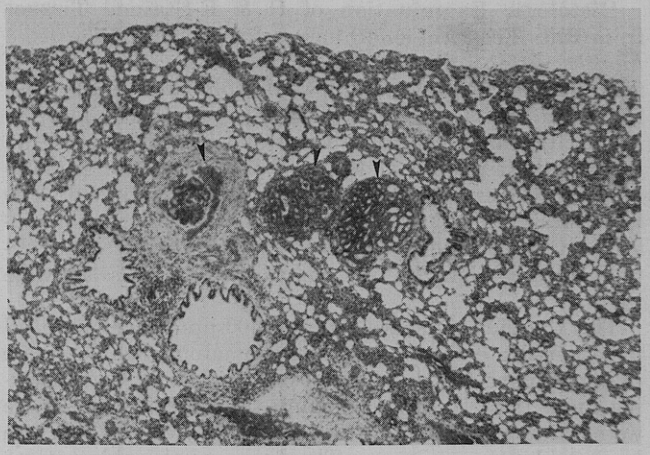

Figure 9. The carcinoma, metastatic in the lung (25×).

shape and the presence of colloid. The variation in size of the follicles is typical of the control rats on this experiment. Figure 6 shows the same normal thyroid at a higher power (125×), and clearly shows the flattened epithelium and the presence of colloid within the follicles.

Figure 7 is a thyroid carcinoma in a rat fed ETU at 500 ppm for 12 months. Figure 8 is a higher power of the same carcinoma, showing clearly the vascular invasion by the tumor. To the right the tall epithelial walls can be seen.

Figure 9 shows the above tumor metastatic in the lung. Tumor cells can be seen within the vessel and out in the parenchyma. Figure 10 is a higher power (125×) of the same metastasis showing the vascular invasion and the tumor adjacent to it. Figure 11 is another exposure of the lung area showing the tumor nearest the bronchus. The acinar formation in the tumor is shown clearly. In instances when metastasis occurred, the lungs only were affected. The overall incidence of metastatic involvement was not determined since lung tissue was not selected for evaluation from all animals. Aberrations in cell growth, such as loss in polarity and anaplasia, violation of the basement membrane, invasion of the surrounding capsule and vessels, and metastasis, were features employed in characterizing malignancy.

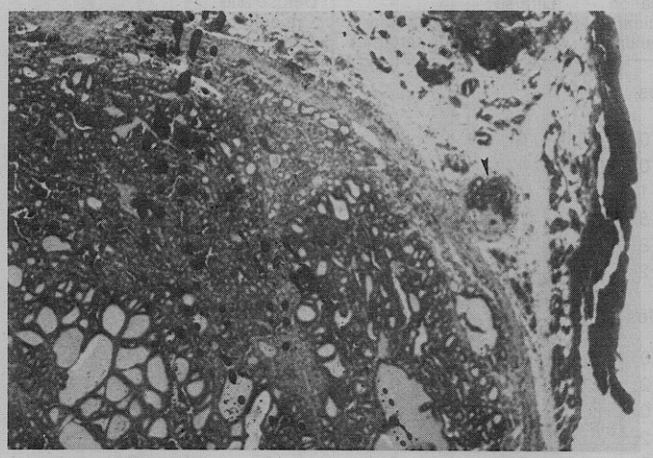

Figure 7. Thyroid carcinoma in a rat fed ETU at 500 ppm for 1 year. Note the vascular invasion (25×).

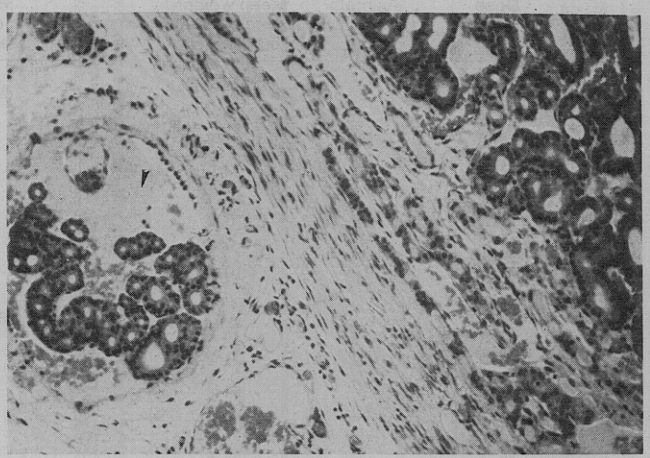

Figure 10. Higher power of the metastasis in Figure 9, showing the vascular invasion and the tumor adjacent to it (125×).

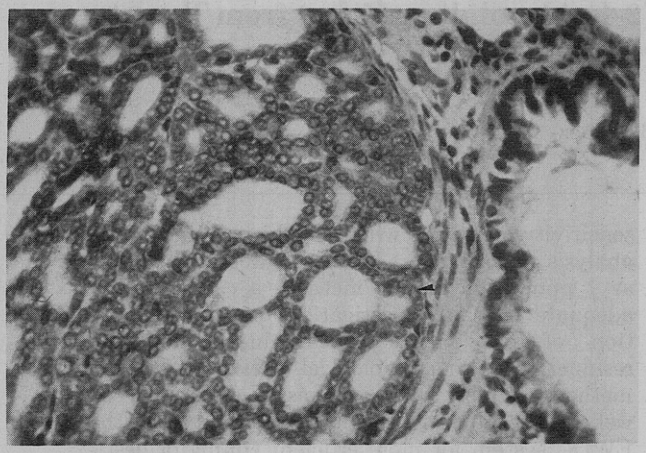

Figure 11. Acinar formation in the tumor nearest the bronchus (125×).

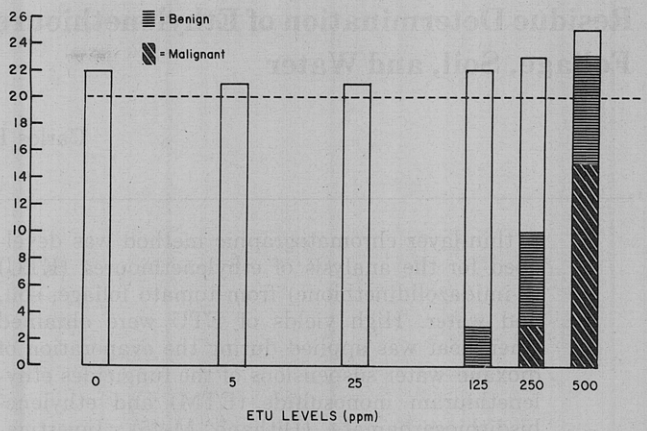

Figure 12. Incidence of thyroid tumors in rats fed ETU for 12 months.

Figure 12 summarizes the incidence of benign and malignant thyroid tumors in rats fed ETU in their diets for 12 months. The dose levels are plotted on a semilogarithmic scale which shows the closer proximity of the three higher levels. The broken line indicates that 20 animals were sacrificed after 1 year on the experiment; the animals indicated above this line are those that died during this period and in which the tissues were examined. At the 125-ppm level, there was a 14% incidence of benign tumors. This incidence was doubled at the 250-ppm level and the incidence of malignant tumors was 13%. At the 500-ppm level, the incidence of benign tumors was 32% and that of malignant tumors increased to 60%. These values reflect the combined data from male and female rats.

The data show that ETU causes statistically significant decreases in body weight and increases in thyroid-to-body weight ratios in male and female Charles River rats exposed to levels of 250 and 500 ppm in their diets. ^{131}I uptake, expressed as cpm/mg of tissue, was significantly decreased in male rats fed 500 ppm of ETU in their diet for 12 months. Females fed the three highest dose levels had hypofunctioning thyroids at 6 months, but at 12 months the uptake was increased.

Increased vascularity and hyperplasia give some indication of an overactive thyroid gland; these effects were seen even at 5 ppm. All thyroids exhibited a series of changes which included diffuse microfollicular hyperplasia, diffuse and nodular hyperplasia, nodular hyperplasia with papillary and cystic deviations, and finally the development of adenocarcinoma. It is possible that ETU initially reduces thyroid activity after which compensation occurs by an increased release of TSH and that this increase in TSH stimulated thyroid weight in an attempt to overcome the blocking effect of ETU. At the 500-ppm dose level, the pituitary was moderately hyperplastic. Sharp elevations in the weight of the thyroid appeared to be more closely associated with the development of malignant tumors which tended to be large rather than with tumors of a benign histological pattern (nodular hyperplasia or adenoma).

The development of thyroid carcinomas indicates that ETU is a carcinogen.

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