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The Effect of Sodium Bromide Ingestion on the Goitrogenic Action of Ethylenethiourea in the Male Rat

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Ethylenethiourea (ETU) is a degradation product of the ethylenebis (dithiocarbamate) fungicides and has been found on crops treated with these compounds(1) as well as in samples of commercial products(2). ETU is goitrogenic(3), teratogenic(4), and when fed athigh levels produces thyroid carcinoma(5). Bromide, which has been found to occur in various foods as a result of fumigation practice(6) or of natural occurrence(7) is also goitrogenic(8) and has been found to augment the antithyroid effect of thiouracil and propylthiouracil(9). The present study was conducted to determine whether dietary bromide would affect the known goitrogenic property of ETU.

MATERIALS AND METHODS

Male Sprague-Dawley rats (125-150 g body weight) were divided into groups of 10 and fed diets for 28 days containing: (a) 0, 200 and 2000 ppm NaBr; (b) 0, 25, 50 and 100 ppm ETU; (c) 200 and 2000 ppm NaBr in combination with 25, 50 and 100 ppm ETU. The ETU and/or NaBr were added to powdered rat diet (Master Lab Cubes, Maple Leaf Mills, Toronto) containing 4% corn oil and fed ad libitum. Food consumption and body weights were monitored weekly. Food was withdrawn 16 hours before sacrifice.

At sacrifice, the thyroid glands were removed, weighed, and frozen pending analysis for ETU. ETU was determined after extraction with ethanol as previously described(10).

Serum thyroxine was determined by radioimmunoassay using the Stat 4 kit obtained from Oxford Laboratories, Foster City, Calif.

All parameters were analysed statistically by Student's t test.

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RESULTS AND DISCUSSION

The effect of ETU and of NaBr on the body weight gain and food consumption is shown in Table I. Rats fed up to 100 ppm ETU in the diet for 28 days showed a significant depression of body weight gain. There was a slight, but not significant, decrease in food consumption in the groups fed 50 and 100 ppm ETU. This growth retardation has been previously observed in rats fed ETU where there were significant decreases in food intake at levels of 100, 500 and 750 ppm for 30 and 90 days but only at the 500 and 750 ppm levels after 60 and 120 days(11). Rats fed 2000 ppm NaBr (Table I) showed growth retardation although this was not significant. The food intake remained constant at the two levels of NaBr. Similar results were obtained by Van Logten et al(8) on adult female rats, but a second study on weanling rats indicated that higher dose levels of NaBr resulted in growth retardation despite constant, or in some cases, elevated food consumption(9). When fed in combination, the NaBr seemed to diminish this effect of growth retardation since only animals fed 100 ppm ETU in combination with 200 ppm NaBr and animals receiving 50 or 100 ppm ETU in combination with 2000 ppm NaBr showed similar effects. The greatest reduction in body weight gain was seen at 2000 ppm and this was accompanied by a significant reduction in food consumption. At these dosage levels it would appear that body weight and food intake changes are not reliable indicators of thyroid function.

The effects of ETU and NaBr on liver, kidney and brain weights are shown in Table II. Liver wet weights were unaffected by any treatment. However when calculated as a percentage of body weight, ETU at all levels caused increased liver/body weight ratios. Sodium bromide treatment alone at 2000 ppm resulted in decreased liver/body weight ratio. When ETU and NaBr were fed together, larger liver/body weight ratios than control animals were observed. Since these results paralleled the changes in body weight gain of the various groups, and the fact that the weights of the liver were not affected, it is doubtful that either NaBr or ETU had a real effect on the liver. Kidney weights expressed either as wet weights or as a percentage of body weight were not affected by either NaBr or ETU treatment alone. Kidney weights were decreased at 100 ppm ETU fed in combination with 200 and 2000 ppm NaBr but these effects were probably related to decreased body weight gain. Since the effects seen on kidney weights expressed as percent body weight were not consistent they were considered as incidental findings.

TABLE I

Food Consumption and Growth of Rats Fed Various Levels of Sodium Bromide and Ethylene Thiourea for 28 Days[a].

NaBr in Diet (ppm)	ETU in Diet (ppm)	Initial Body Weight (g)	Body Weight Gain (g)	Food Consumption[a] (g)
0	0	194 ± 9	187 ± 26	25.0 ± 1.8
	25	197 ± 13	163*± 18	25.4 ± 1.8
	50	196 ± 11	164*± 19	24.0 ± 0.9
	100	195 ± 9	160*± 28	23.4 ± 2.0
200	0	195 ± 10	183 ± 42	25.7 ± 1.6
	25	195 ± 9	180 ± 49	25.0 ± 3.1
	50	195 ± 9	170 ± 22	23.3 ± 3.4
	100	195 ± 8	157*± 20	23.6 ± 1.7
2000	0	195 ± 9	169 ± 36	24.5 ± 2.7
	25	195 ± 9	169 ± 41	24.3 ± 7.0
	50	195 ± 11	154*± 22	24.3 ± 1.8
	100	193 ± 8	132*± 50	22.4*± 1.4

[a] Values represent mean ± S.D. of 10 animals.

* Significantly different from controls at $P \leq 0.05$.

TABLE II

Liver, kidney and brain weights of rats fed various levels of sodium bromide and ethylene thiourea for 28 days[a].

NaBr in Diet (ppm)	ETU in Diet (ppm)	Liver Weights(g)	Liver (% Body Weight)	Kidney Weight(g)	Kidney (% Body Weight)
0	0	10.3	2.9	1.33	0.37
	25	10.2	3.1*	1.29	0.38
	50	10.4	3.1*	1.27	0.35
	100	10.1	3.1*	1.24	0.38
200	0	9.9	2.9	1.32	0.38
	25	10.1	3.0	1.36	0.44
	50	10.2	3.0	1.31	0.38
	100	10.3	3.2*	1.21*	0.36
2000	0	9.3	2.8*	1.23	0.36
	25	9.8	3.0	1.27	0.40
	50	10.1	3.1*	1.29	0.39
	100	9.4	3.1*	1.20	0.38

[a] Values represent the mean of 10 animals.

* Denotes significant difference from controls at $P \leq 0.05$.

TABLE III

Effect of dietary NaBr and ETU on thyroid weights, serum thyroxine and ETU content of the thyroid.

NaBr in Diet (ppm)	ETU in Diet (ppm)	Thyroid[a] Weight (mg)	Thyroid/Body Weight[a] (mg/100 g)	Serum T_4[b] (ug/100 ml)	ETU in[c] Thyroid (ppm)
0	0	20.1	5.43	4.9	<0.5
	25	17.8	5.42	5.7	14.4
	50	18.3	5.59	6.1	41.6
	100	20.4	6.29	5.4	39.6
200	0	17.3	5.11	6.4	<0.5
	25	16.8	5.05	6.8	15.0
	50	17.3	4.94	8.7*	59.4
	100	22.7	6.82*	4.5	57.9
2000	0	16.6	5.12	7.7*	<0.5
	25	15.3*	4.61	7.4*	19.0
	50	19.6	5.87	9.3*	13.8
	100	24.8*	8.40	6.9	46.1

[a] Values represent the mean of 8 animals.

[b] Values represent the mean of 6 animals.

[c] Values represent the mean of 7 animals.

* Denotes significant difference from controls at $P \leq 0.05$.

In this experiment (Table III), ETU did not cause any change in the thyroid weight either absolute or relative to body weight. In previous experiments a significant increase in relative thyroid weight was observed in rats fed ETU at 100 ppm for 30 and 60 days but not after 90 and 120 days (11), nor after 125 ppm ETU for 18 or 24 months (12). It would thus appear that at 100 ppm the thyroid gland may or may not become goitrous. Graham et al (11) observed slight hyperplasia and increased vasculature indicative of increased TSH stimulation in the glands of rats treated at the 100 ppm level. An increase in relative thyroid weight and hyperplasia of the gland were observed in weanling female rats fed 0.1% ETU for 8 days (3). The increased 24 hr. ^{131}I uptake in rats fed 50 ppm ETU (11) and 25 or 125 ppm ETU (12) and decreased uptakes at higher doses (11, 12) substantiate the theory that goitrogens administered over a long period trigger a more complex response than simple inhibition of hormone synthesis. The initial glandular response may well be decreased hormone synthesis, but this in turn may result in increased TSH release which could have a twofold effect on the gland, increasing the turnover of a diminishing hormone pool and stimulating growth of the gland itself. The distinction between goitrogens which act primarily by inhibiting trapping (thiocyanates and perchlorates), and those which act by inhibiting synthesis (thionamides and sulphonamides) may be apparent only with short term administration. With longer term feeding, the thiocyanates and perchlorates may also affect synthesis, although to a lesser extent (13).

Thioureas are thought to be similar to propylthiouracil in blocking thyroid hormone synthesis in the gland, and the demonstration that ETU inhibits an extractable thyroid peroxidase (11) would substantiate this. Early reports indicated that PTU acts extrathyroidally, blocking the conversion of thyroxine to triidothyronine in rat kidney slices (14), in vivo in rats (15) and in man (16, 17). It has been suggested that this inhibition may somehow be associated with decreased effectiveness of exogenously administered T_4 (17). The extrathyroidal inhibition of deiodination might be a result of the inhibition of one of two possible deiodinase enzymes or in the initial phases through interference with protein binding which could alter availability of the hormone to the sites of deiodination. PTU does not alter protein binding of thyroid hormones in humans (17,18,19) or in rats (20), thus supporting an enzyme inhibitory mechanism. The results of this would be unchanged or increased T_4 levels and decreased T_3 levels leading to increased TSH levels (17). PTU does not appear to have a

direct stimulatory effect on the pituitary(17). High doses of thiourea administered for six days did not alter the excretion pattern of ^{131}I administered as $^{131}I.T_4$ to thyroidectomized rats(21), whereas PTU treated rats showed a decreased urinary ^{131}I excretion indicating decreased peripheral deiodination(21). The slight increase in serum T_4 levels observed (Table III) might indicate that ETU does have an extrathyroidal effect when administered at low levels for a prolonged period.

The concentration of ETU in the gland (Table III) paralleled the dietary level at 25 and 50 ppm, but not at 100 ppm. NaBR did not significantly alter the accumulation of ETU in the gland. There is a possibility that the other components of the fungicide may have some effect on the ETU uptake by the gland and is a phenomenon that should be closely examined.

With the adminstration of NaBr the thyroid weight decreased slightly but not significantly with dosage (Table III). However the serum thyroxine content was significantly increased at the 2000 ppm level. Van Logten observed histologic changes indicative of increased glandular activity in rats fed 19,200 NaBr(8). It is interesting that although the gland was decreased in size, it was producing significantly increased amounts of T_4. This could indicate alterations in turnover rate or peripheral metabolism of T_4. Williams et al(9) have suggested that the non-iodide halides might act by competing with iodide for uptake in the gland or by competition for kidney tubular reabsorption thereby increasing the rate of iodide excretion.

200 ppm NaBr along with 50 ppm ETU resulted in significant increase in serum T_4 with slight decrease in thyroid weight (Table III). The same level of NaBr along with 100 ppm ETU resulted in a significant increase in gland weight and no change in the serum T_4 level. A similar biphasic response was observed with 2000 ppm NaBr and 25 ppm ETU and 2000 ppm NaBr and 100 ppm ETU. In the first response of decreased gland weight and increased serum T_4, NaBr and ETU would appear to have increased the release of T_4 rather than altering deiodination. If the extrathyroidal conversion of T_4 to T_3 had been blocked an increased TSH release might be expected. The reverse response of increased gland weight and decreased serum T_4 at the higher level of ETU could be due to blocked synthesis of T_4 or depressed conversion of T_4 to T_3 with the resultant TSH mediated response.

The different responses observed by various authors at the lower doses of ETU emphasize the need to clearly describe the possible effects and then carefully design the experiment to test them. There are undoubtedly many mechanisms involved, and these appear to be dose related with some operating at low doses and others at high doses. The iodide content of the diet may also vary from laboratory to laboratory, and it has been shown that low doses of PTU may or may not be goitrogenic depending on the iodide content (13) thus leading to different results. There are also time related changes in development of goitre, for example, PTU has been shown to act first on peripheral metabolism and later on synthesis with both actions causing increased TSH release (22).

There appear to be some species differences in response, and man has been shown to react in a similar fashion to rats when given PTU(23). These differences emphasize that extrapolations should not be made from one goitrogenic compound to another, or one species to another without testing being done to justify conclusions that might be drawn.

In summary it would appear that over the dose range examined, the goitrogenic effect of ETU is augmented only slightly by the inclusion of NaBr in the diet. This finding is in contrast to that of Williams et al (9) who observed a somewhat greater augmentation of the goitrogenic effect of the uracils when NaBr was given.

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