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# TOXICITY STUDY OF A RUBBER ANTIOXIDANT, 2-MERCAPTOBENZIMIDAZOLE, BY REPEATED ORAL ADMINISTRATION TO RATS

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**ABSTRACT** — The chemical structure of 2-mercaptobenzimidazole (2-MBI), which is widely used as a rubber antioxidant, is partially similar to those of thiourea (TU) and ethylenethiourea (ETU), both potent thyrotoxic compounds. In order to determine the oral toxicity of 2-MBI, a 28-day repeated dose toxicity study in Wistar rats followed by observation over a 14-day recovery period was conducted at dose levels of 2, 10 and 50 mg/kg 2-MBI administered by gavage. No toxic deaths occurred due to 2-MBI treatment. Decreases of body weight gain and food consumption in the 50 mg/kg dose group were observed during the second half of the treatment period. In addition, hematological examination and serum biochemical tests revealed decreased white blood cells and hemoglobin and increased serum urea nitrogen, cholesterol, phospholipid, γ-glutamyl transpeptidase and the $Na^+$ /$K^+$ ratio in the 50 mg/kg dose group. Marked thyroid enlargement (to10 fold the control weight), histopathologically associated with diffuse hyperplasia of follicles with decreased colloid and thickening of the fibrous capsule, was found. Reduction in thymus weight was also observed in a dose-dependent manner without significant histopathological alteration.

The non-observed effect level (NOEL) of 2-MBI in this gavage study was found to be less than 2 mg/kg/day based on the significant decrease in thymus weight in the 2 mg/kg 2-MBI treatment group.

In an ancillary study, measurement of serum levels of $T_3$, $T_4$ and TSH, and thyroid weight after gavage treatment with 0.15 and 0.3 mmol/kg of three antithyroid compounds for 14 days revealed a more potent antithyroid effect for 2-MBI than for TU or ETU.

**KEY WORDS** : 2-mercaptobenzimidazole, Rats, Gavage administration, Thyroid toxicity, Thymus involution, Rubber antioxidant

## INTRODUCTION

2-Mercaptobenzimidazole (2-MBI), widely used as a rubber accelerator and/or antioxidant, is structurally similar to ethylenethiourea, a carcinogen/teratogen, and methimazole, a hyperthyroid drug (IARC,1974; Paynter *et al.*,1988). Antithyroid effects of 2-MBI have been demonstrated with oral administration of 8.3 mg/ kg to rats causing thyroid enlargement and a decrease in circulating thyroxin ($T_4$) (Janssen *et al.*,1981) . A dose of 7.5 mg/kg inhibits iodine uptake into the thyroid by 95% (Searle *et al.*, 1950). 2-MBI has been also used to assess the effects of decreased thyroid function in carcinogenesis in rats (Kellen,1972) It is thought to block the biosynthesis of thyroxin by inhibiting thyroid peroxidase (Deorge,1986; Taurog,1976). This thioureylene compound has also been shown to be a potent inhibitor of deiodinase which catalyses the conversion of 3,3',5-triiodothyronine ($T_3$) to 3,3'-diiodothyronine (Visser *et al.*,1979). Administration of 2-MBI to rats has been

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shown to result in reproductive toxicity (Barilyak,1974) and chromosomal aberrations in rat fetal cells (Barilyak and Melnik,1979). Recently, Yamano *et al.* (1995) investigated the adverse effects of 2-MBI on pregnant rats and their fetuses and observed major fetal malformations but only at a dose lethal to most treated dams. They concluded that maternal toxicity preceded fetal toxicity.

In a 13-week inhalation toxicity study of 2-MBI in F344 rats, Gaworski *et al.* (1991) encountered thymic atrophy and adrenal cortex necrosis in addition to a potent antithyroid effect. Exposure to 2-MBI *via* other routes has also been demonstrated due to use of rubber products processed with this antioxidant and vulcanization accelerator (Airaudo *et al.*, 1990). Thus Airaudo *et al.*(1990) demonstrated some anesthetic drugs to be contaminated with 2-MBI in the range of 2.8 - 11.8 ppm, the contamination sources being rubber plunger-seals of syringes and /or drug packing containers. Recently, we have also detected 11.5- 67.7 ppm of 2-MBI in commercial farming rubber boots, a possible source of human exposure to 2-MBI (Isama *et al.*, submitted). 2-MBI is known to be a metabolite of the immunomodulator, 3-(p-chlorophenyl)-2,3-dihydro-3-hydroxythiazolo[3,2a]benzimidazole-2-acetic acid (Janssen *et al.*, 1981).

The present 28-day repeated dose oral toxicity study of 2-MBI followed by 2-week recovery examination in Wistar rats was conducted to evaluate adverse effects and their reversibility, and the non-observed-effect level (NOEL). Considering its potential as an environmental endocrine disrupter, toxicological investigations of 2-MBI with various exposure routes appear to be important. In an ancillary 14-day repeated dose oral toxicity study, the antithyroid potency of 2-MBI was compared with those of thiourea (TU) and ethylenethiourea (ETU), both thyroid toxic and tumorigenic compounds (Paynter *et al*, 1988), employing circulating thyroid hormone and TSH levels as parameters.

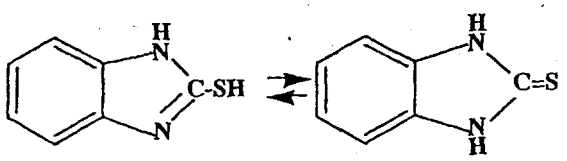


**Fig. 1.** Chemical structure of 2-mercaptobenzimidazole (2-MBI).

## MATERIALS AND METHODS

### *Chemicals*

2-MBI (MW:150.20, CAS No. 583-39-1, RTECS No. DE1050000) was obtained from Ouchi Shinko Chemical Ind., Ltd.(Osaka, Japan) as a slight yellow powder, soluble in methanol, ethanol and acetone and practically insoluble in water and chloroform, and was used without further purification. Reagents employed for hematological and biochemical analyses were purchased from Wako Pure Chemicals Industries (Osaka, Japan), Boeringer Mannheim-Yamanouchi (Tokyo, Japan), Shinotest Laboratory (Tokyo, Japan) and Midorijuji Co. Ltd. (Kobe, Japan). Corn oil was purchased from Sigma ( MO, USA). Thiourea and ethylenethiourea were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

### *Experimental animals and diets*

Specific pathogen-free Wistar male and female rats (4 weeks old) were purchased from SLC Co. (Shizuoka, Japan) and acclimated for one week prior to the initiation of the study.The basal pellet diet (F-2) was purchased from Funabashi Farm ( Funabashi, Japan). Food and tap water were available *ad libitum* throughout except in the acute toxicity study.

### *Housing Conditions*

The animal room was maintained at 24±1℃ and 55±5% humidity with a 12 hr light/dark cycle. Rats were housed in aluminum hanging cages (3 rats/cage) for the acute toxicity study and in plastic cages (5 rats /cage) using chip bedding for the subacute toxicity study.

### *Experimental design*

2-MBI was dissolved or suspended in corn oil and administered to rats by the i.g. route using disposable gavage tube (Fuchigami Kiki, Tokyo, Japan).

Acute oral toxicity study: The acute oral toxicity study of 2-MBI in male and female rats was conducted according to the method reported by Lorke (1983). 2-MBI was administered by gavage in 1 ml per 100 g body weight under fasted conditions. In the first stage test, 3 rats per group (mean body weights: males, 92 g, females, 80 g, fasted for 16 hr before administration) were treated with 10, 100 and 1000 mg/kg of 2-MBI . Clinical signs and mortality were monitored for 10 hr on the day of administration and then twice a day up to day 14 when the test was terminated. Based on the results of the mortality in the 1st stage test, the acute toxicity of 140, 225, 370 and 600 mg/kg of 2-MBI was

examined using 2 rats of each sex / dose group as a 2nd stage test (Lorke,1983) in which clinical signs and mortality were again monitored for 14 days.

Twenty-eight-day repeated dose oral toxicity study: For the dose-determining study, male and female rats (5 rats/group) were orally administered 80 mg (approximately 1/4 dose of the oral $LD_{50}$), 40 ,20, 10 and 5 mg/kg of 2-MBI for a consecutive two weeks. Taking into account the observed reduction in body weight gain in groups receiving more than 40 mg/kg and the 2 weeks longer treatment period for the 28-day repeated oral toxicity study, doses of 0 (control, corn oil alone), 2, 10 and 50 mg/kg of 2-MBI were administered by gavage to groups of 10 male and 10 female rats for 28 consecutive days. Half the rats in each group (5 males and 5 females) were used for blood clotting time tests. Additional sub-groups of 10 rats each of both sexes receiving 0 and 50 mg/kg were maintained without treatment for 14 days subsequent to termination of 2-MBI administration in order to assess recovery and /or appearance of delayed adverse effects.

In the ancillary 14-day repeated dose oral toxicity study, 5 male rats were treated with either 25 mg (0.15 mmol) or 50 mg (0.3 mmol)/kg of 2-MBI by gavage for 2 weeks to examine the effect of 2-MBI on circulating thyroid hormone and TSH levels. For comparison, TU and ETU, both well characterized antithyroid agents, were also administered to rats following the same protocol.

Clinical signs were monitored throughout the study. The male and female animals were weighed and randomly allocated to 12 groups (n=5) three days prior to the initiation of the treatment. On the first day of treatment, and then twice weekly throughout the study, body weights were measured and the most recently obtained body weight values were used for the calculation of administration doses per kg body weight. Food consumption was measured once a week.

***Stability test of 2-MBI in corn oil***

The stability of 2-MBI in corn oil(2%) was examined by HPLC after extracting the test compound with methanol. The HPLC conditions applied were as follows: a Shimadzu LC-6A liquid chromatograph (Shimadzu Co. Ltd., Kyoto, Japan) attached to a Shimadzu SPD-M6A photodiode array UV-VIS detector (Kyoto, Japan) with a Wako Wakosil-II 5C18 HG Prep (4.6 mm i.d. ×250 mm) column were used with a mobile phase of 0.1% phosphoric acid/methanol (50/50, v/v). The flow rate and the detection wavelength were 1ml/min and 304 nm, respectively.

2-MBI in corn oil (2%) was confirmed to be stable for at least one week at room temperature. Thus, test samples for gavage administration were prepared once a week. The purity of 2-MBI used was >95%.

***Clinical parameters***

In the 28-day repeated oral dose toxicity study, blood was collected from the orbital plexus under ethylether anesthesia, and the hematological parameters, red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean hemoglobin concentration (MHC), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and white blood cells (WBC) were assessed with a Sysmex M-2000 System (Toa Medical Electronics Co., Kobe, Japan). Differential white blood cell counts were performed using a Microx (Tateishi Electric Co., Japan).

Serum biochemical analyses were conducted for 24 items; total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRN), glucose (GLC), non-esterified fatty acid (NEFA), phospholipid (PL), triglyceride (TG), total cholesterol (T-CHO), free cholesterol (F-CHO), alkaline phosphatase (ALP), amylase (AMY), cholinesterase (CHE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), $\gamma$ -glutamyl transpeptidase ($\gamma$ -GTP), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), inorganic posphorus (Pi), sodium ($Na^{+}$), potassium ($K^{+}$), and chlorine ($Cl^{-}$) using an Auto Clinical Analyzer, Hitachi Model 7150 (Hitachi Ltd., Tokyo, Japan) . $T_3$/$T_4$ and TSH levels in serum were measured by RIA using analytical kits.

At autopsy, the weights of the brain, heart, lungs, liver, kidneys, spleen, adrenals, testes, ovaries, pituitary, thymus, submaxillary glands and thyroid glands of each animal were measured. These organs and the esophagus, stomach, small and large intestine, pancreas, ischiatic nerve, urinary bladder, seminal vesicles, uterus, prostate and, mesenteric lymph nodes as well as samples of spinal cord, skeletal muscle, and bone marrow (femur and sternum) were fixed in 10% buffered formalin solution for routine histological processing. Paraffin sections were stained with hematoxylin and eosin for histopathological examination.

***Statistical analysis***

All quantitative data, except for the histopathological findings, were statistically analyzed by one-way analysis of variance (ANOVA) techniques with Dunnett's or Scheff's multiple comparison procedures. Significance was established at the $p<0.05$ level.

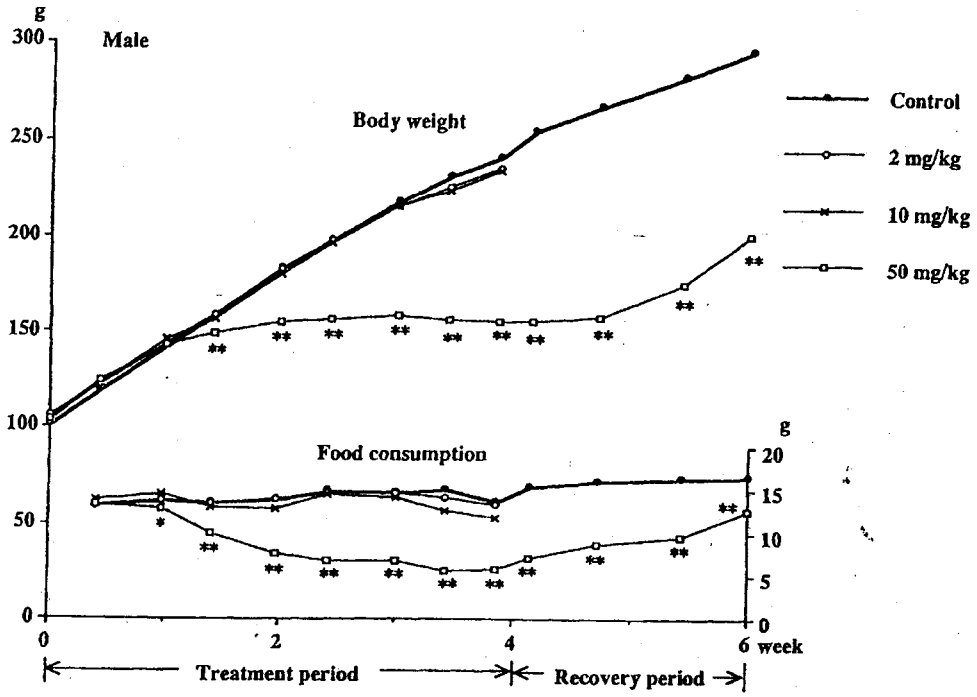


**Fig. 2.** Body weight and food consumption curves for male rats treated with 2-mercaptobenzimidazole.

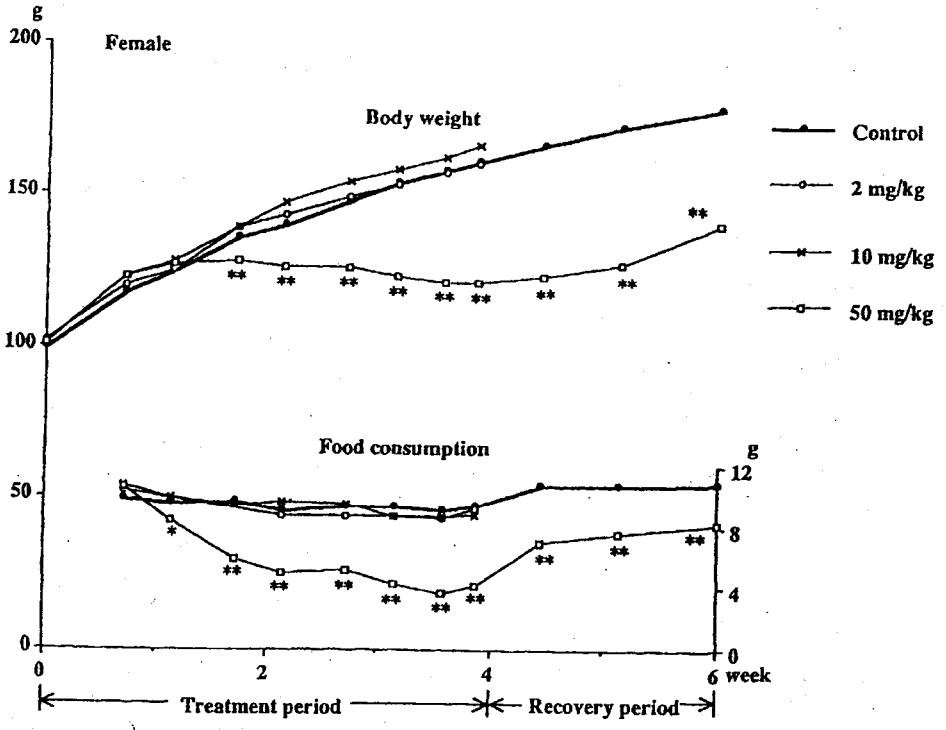


**Fig. 3.** Body weight and food consumption curves for female rats treated with 2-Mercaptobenzimidazole.

**Table 1.** Hematological findings for rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| Groups(Dose) No. of animals | | Treatment | | | | Recovery | |
|---|---|---|---|---|---|---|---|
| | | Control 5 | 2 mg/kg 5 | 10 mg/kg 5 | 50 mg/kg 5 | Control 5 | 50 mg/kg 5 |
| Male | | | | | | | |
| RBC | $x10^{12}/l$ | 9.00 ± 0.10 | 9.07 ± 0.37 | 8.61 ± 0.19 | 8.66 ± 0.25 | 9.86 ± 0.19 | 7.49 ± 0.13** |
| Hb | g/dl | 15.8 ± 0.4 | 15.9 ± 0.4 | 15.4 ± 0.4 | 15.7 ± 0.4 | 16.3 ± 0.3 | 13.7 ± 0.2** |
| HCT | % | 47.1 ± 0.4 | 46.7 ± 1.6 | 45.8 ± 1.6 | 44.0 ± 1.5** | 48.0 ± 0.6 | 39.8 ± 0.9** |
| PLT | $x10^{12}/l$ | 0.81 ± 0.08 | 0.84 ± 0.09 | 0.79 ± 0.18 | 0.63 ± 0.05** | 0.84 ± 0.13 | 1.06 ± 0.08* |
| WBC | $x10^{9}/l$ | 7.36 ± 0.83 | 6.66 ± 0.36 | 6.58 ± 0.35 | 6.46 ± 0.83 | 8.22 ± 0.68 | 6.30 ± 0.40** |
| PT | Sec. | 14.6 ± 0.6 | 14.3 ± 0.8 | 14.8 ± 0.8 | 14.9 ± 0.9 | 16.3 ± 0.5 | 15.2 ± 0.3** |
| APTT | Sec. | 24.7 ± 2.1 | 24.2 ± 4.1 | 25.6 ± 2.7 | 35.6 ± 0.8* | 30.0 ± 6.7 | 25.2 ± 2.6 |
| Female | | | | | | | |
| RBC | $x10^{12}/l$ | 9.01 ± 0.14 | 9.02 ±0.12 | 8.77 ± 0.58 | 9.00 ±0.51 | 8.58 ± 0.20 | 6.64 ± 0.08** |
| Hb | g/dl | 16.2 ± 0.2 | 16.2 ± 0.3 | 15.9 ± 1.0 | 16.3 ± 0.7 | 15.6 ± 0.3 | 12.7 ± 0.5** |
| HCT | % | 46.2 ± 1.0 | 45.8 ± 1.0 | 45.1 ± 3.1 | 45.1 ± 2.6 | 44.6 ± 0.9 | 34.8 ± 0.6** |
| PLT | $x10^{12}/l$ | 0.72 ±0.08 | 0.71 ± 0.21 | 0.78 ± 0.16 | 0.67 ± 0.15 | 1.04 ± 0.06 | 1.22 ± 0.08** |
| WBC | $x10^{9}/l$ | 8.78 ± 1.05 | 7.62 ± 1.33 | 5.98 ± 1.06** | 5.24 ±0.31** | 5.84 ± 0.65 | 6.24 ± 1.2 |
| PT | Sec. | 13.9 ± 0.6 | 14.0 ± 0.8 | 14.1 ± 0.4 | 14.9 ± 0.9 | 14.7 ± 0.5 | 14.4 ± 0.4 |
| APTT | Sec. | 26.5 ± 0.9 | 26.3 ± 4.9 | 27.5 ± 7.3 | 44.3 ± 9.0 ** | 24.1 ± 3.1 | 19.8 ± 1.3* |

Data are mean ± S.D. values

*,**: Significantly different from the relevant control at $p<0.05$, $p<0.01$, respectively

**Table 2.** Biochemical findings for male rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| Groups(Dose) No. of animals | | Treatment | | | | Recovery | |
|---|---|---|---|---|---|---|---|
| | | Control 5 | 2 mg/kg 5 | 10 mg/kg 5 | 50 mg/kg 5 | Control 5 | 50 mg/kg 5 |
| TP | g/dl | 5.98 ± 0.16 | 6.01 ± 0.15 | 6.20 ± 0.22 | 6.99 ±0.08** | 6.32 ± 0.16 | 5.79 ±0.10** |
| ALB | g/dl | 4.25 ± 0.07 | 4.24 ± 0.12 | 4.33 ± 0.10 | 4.86 ± 0.06** | 4.38 ± 0.05 | 3.94 ± 0.09** |
| A/G | | 2.47 ± 0.14 | 2.39 ± 0.09 | 2.32 ± 0.19 | 2.29 ± 0.18 | 2.26 ± 0.15 | 2.14 ± 0.12 |
| BUN | mg/dl | 8.89 ± 1.26 | 8.09 ± 0.89 | 6.65 ± 0.31** | 12.77 ± 0.49** | 12.1 ± 1.40 | 11.1 ± 0.4 |
| CRN | mg/dl | 0.28 ± 0.03 | 0.27 ± 0.04 | 0.24 ± 0.02 | 0.28 ± 0.02 | 0.34 ± 0.07 | 0.24 ± 0.02* |
| GLC | mg/dl | 118 ± 4 | 120 ± 13 | 124 ± 6 | 124 ± 6 | 130 ± 7 | 108 ± 8** |
| NEFA | mEq/l | 0.78 ± 0.13 | 0.84 ± 0.15 | 0.83 ± 0.12 | 0.82 ± 0.07 | 0.82 ± 0.05 | 0.95 ± 0.12 |
| PL | mg/dl | 103 ± 3 | 108 ± 9 | 107 ± 9 | 226 ± 16** | 121 ± 5 | 160 ± 12** |
| TG | mg/dl | 76 ± 15 | 82 ± 14 | 74 ± 23 | 65 ± 10 | 157 ± 23 | 85 ± 12** |
| T-CHO | mg/dl | 52 ± 4 | 54 ± 6 | 65 ± 7* | 180 ± 11** | 59 ± 2 | 105 ± 11** |
| F-CHO | mg/dl | 7.6 ± 1.6 | 7.1 ± 2.3 | 10.0 ± 1.7 | 46.4 ± 3.5** | 11.4 ± 1.6 | 23.1 ± 3.7** |
| ALP | mU/ml | 323 ± 29 | 312 ± 9 | 206 ± 24** | 187 ± 26** | 198 ± 11 | 262 ± 28** |
| ALT | mU/ml | 38 ± 9 | 40 ± 5 | 32 ± 7 | 29 ± 5 | 33 ± 7 | 33 ± 7 |
| AST | mU/ml | 82 ± 8 | 81 ± 4 | 69 ± 8* | 54 ± 3** | 62 ± 16 | 64 ± 6 |
| CHE | mU/ml | 174 ± 15 | 186 ± 21 | 536 ± 53 | 2013 ± 289** | 173 ± 32 | 480 ± 42** |
| γ-GTP | mU/ml | 1.29 ±0.40 | 1.33 ± 0.22 | 1.42 ± 0.37 | 1.98 ± 0.09** | 0.01 ± 0 | 0.01 ± 0 |
| LAP | mU/ml | 47 ± 2 | 45 ± 1 | 47 ± 2 | 65 ± 4 | 42 ± 2 | 48 ± 2** |
| LDH | mU/ml | 539 ± 104 | 503 ± 118 | 483 ± 128 | 374 ± 47 | 340 ± 47 | 334 ± 129 |
| Ca | mg/dl | 9.9 ± 0.1 | 9.9 ± 0.2 | 9.9 ± 0.2 | 9.8 ± 0.3 | 10.5 ± 0.1 | 10.2 ± 0.2* |
| Mg | mg/dl | 2.1 ± 0.1 | 2.2 ± 0.1 | 2.0 ± 0.2 | 2.0 ± 0.1 | 2.0 ± 0.1 | 2.0 ± 0.1 |
| Pi | mg/dl | 7.9 ± 0.3 | 7.7 ± 0.3 | 7.1 ± 0.3** | 5.4 ± 0.2** | 7.3 ± 0.3 | 7.6 ± 0.3 |
| Na | mEq/l | 137 ± 1 | 137 ± 1 | 138 ± 1 | 142 ± 1** | 136 ± 1 | 136 ± 1 |
| K | mEq/l | 5.2 ± 0.2 | 4.9 ± 0.3 | 4.7 ± 0.2** | 3.5 ± 0.1** | 4.3 ± 0.3 | 5.0 ± 0.2** |
| Cl | mEq/l | 100 ± 0 | 99 ± 1* | 98 ± 2 | 98 ± 1* | 99 ± 1 | 103 ± 1** |

Data are mean ± S.D. values

*,**: Significantly different from the relevant control at $p<0.05$, $p<0.01$, respectively

**Table 3.** Biochemical findings for female rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| Groups(Dose) | | Treatment | | | | Recovery | |
|---|---|---|---|---|---|---|---|
| No. of animals | | Control 5 | 2 mg/kg 5 | 10 mg/kg 5 | 50 mg/kg 5 | Control 5 | 50 mg/kg 5 |
| TP | g/dl | 5.92 ± 0.16 | 5.81 ± 0.15 | 5.88 ± 0.06 | 6.70 ± 0.23** | 6.29 ± 0.25 | 6.31 ± 1.05 |
| ALB | g/dl | 4.30 ± 0.12 | 4.29 ± 0.10 | 4.29 ± 0.05 | 4.60 ± 0.14** | 4.44 ± 0.19 | 3.78 ± 0.08** |
| A/G | | 2.67 ± 0.18 | 2.83 ± 0.11 | 2.71 ± 0.10 | 2.19 ± 0.13** | 2.41 ± 0.15 | 1.66 ± 0.5* |
| BUN | mg/dl | 10.17 ± 1.09 | 9.95 ± 0.91 | 7.35 ± 0.92 | 18.08 ± 5.23 | 11.4 ± 1.4 | 10.7 ± 1.0 |
| CRN | mg/dl | 0.29 ± 0.04 | 0.29 ± 0.04 | 0.27 ± 0.03 | 0.41 ± 0.07** | 0.32 ± 0.01 | 0.30 ± 0.02 |
| GLC | mg/dl | 116 ± 5 | 115 ± 6 | 116 ± 7 | 120 ± 5 | 117 ± 10 | 110 ± 10 |
| NEFA | mEq/l | 0.69 ± 0.07 | 0.64 ± 0.08 | 0.60 ± 0.05 | 0.94 ± 0.20 | 0.82 ± 0.14 | 0.86 ± 0.16 |
| PL | mg/dl | 154 ± 8 | 139 ± 9 | 123 ± 7** | 265 ± 22** | 173 ± 14 | 187 ± 14 |
| TG | mg/dl | 52 ± 2 | 49 ± 12 | 41 ± 4 | 65 ± 12** | 55 ± 11 | 64 ± 15 |
| T-CHO | mg/dl | 85 ± 7 | 74 ± 5 | 73 ± 6 | 208 ± 22** | 95 ± 4 | 121 ± 13** |
| F-CHO | mg/dl | 19.0 ± 2.0 | 15.8 ± 1.4 | 15.2 ± 1.7 | 59.1 ± 4.0** | 23.3 ± 1.5 | 30.0 ± 3.1** |
| ALP | mU/ml | 200 ± 29 | 200 ± 28 | 138 ± 7* | 183 ± 51 | 131 ± 12 | 153 ± 18 |
| ALT | mU/ml | 33 ± 5 | 32 ± 5 | 28 ± 4 | 29 ± 5 | 29 ± 7 | 28 ± 7 |
| AST | mU/ml | 74 ± 7 | 75 ± 6 | 68 ± 4 | 62 ± 4** | 63 ± 4 | 61 ± 5 |
| CHE | mU/ml | 1230 ± 235 | 1350 ± 146 | 1380 ± 152 | 2096 ± 41** | 1566 ± 293 | 1421 ± 147 |
| γ-GTP | mU/ml | 0.70 ± 0.18 | 0.57 ± 0.21 | 0.69 ± 0.24 | 1.25 ± 0.31** | 0.03 ± 0.02 | 0.10 ± 0.21 |
| LAP | mU/ml | 46 ± 3 | 44 ± 1 | 45 ± 1 | 68 ± 3** | 43 ± 3 | 48 ± 3* |
| LDH | mU/ml | 338 ± 25 | 362 ± 74 | 334 ± 33 | 328 ± 76 | 323 ± 82 | 268 ± 83 |
| Ca | mg/dl | 9.8 ± 0.1 | 9.6 ± 0.3 | 9.5 ± 0.1 | 9.6 ± 0.5 | 9.9 ± 0.4 | 10.0 ± 0.2 |
| Mg | mg/dl | 2.0 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 2.05 ± 0.06 | 2.07 ± 0.09 |
| Pi | mg/dl | 6.4 ± 0.3 | 6.1 ± 0.2 | 6.1 ± 0.3 | 5.5 ± 0.2** | 5.1 ± 0.3 | 6.8 ± 0.3** |
| Na | mEq/l | 138 ± 0 | 138 ± 1 | 140 ± 1 | 143 ± 2** | 137 ± 0 | 135 ± 1** |
| K | mEq/l | 4.5 ± 0.2 | 4.4 ± 0.2 | 3.9 ± 0.1** | 3.1 ± 0.1** | 4.4 ± 0.2 | 4.6 ± 0.1 |
| Cl | mEq/l | 101 ± 1 | 102 ± 1 | 96 ± 0 | 97 ± 3 | 104 ± 1 | 104 ± 1 |

Data are mean ± S.D. values
*,**: Significantly different from the relevant control at $p<0.05$, $p<0.01$, respectively

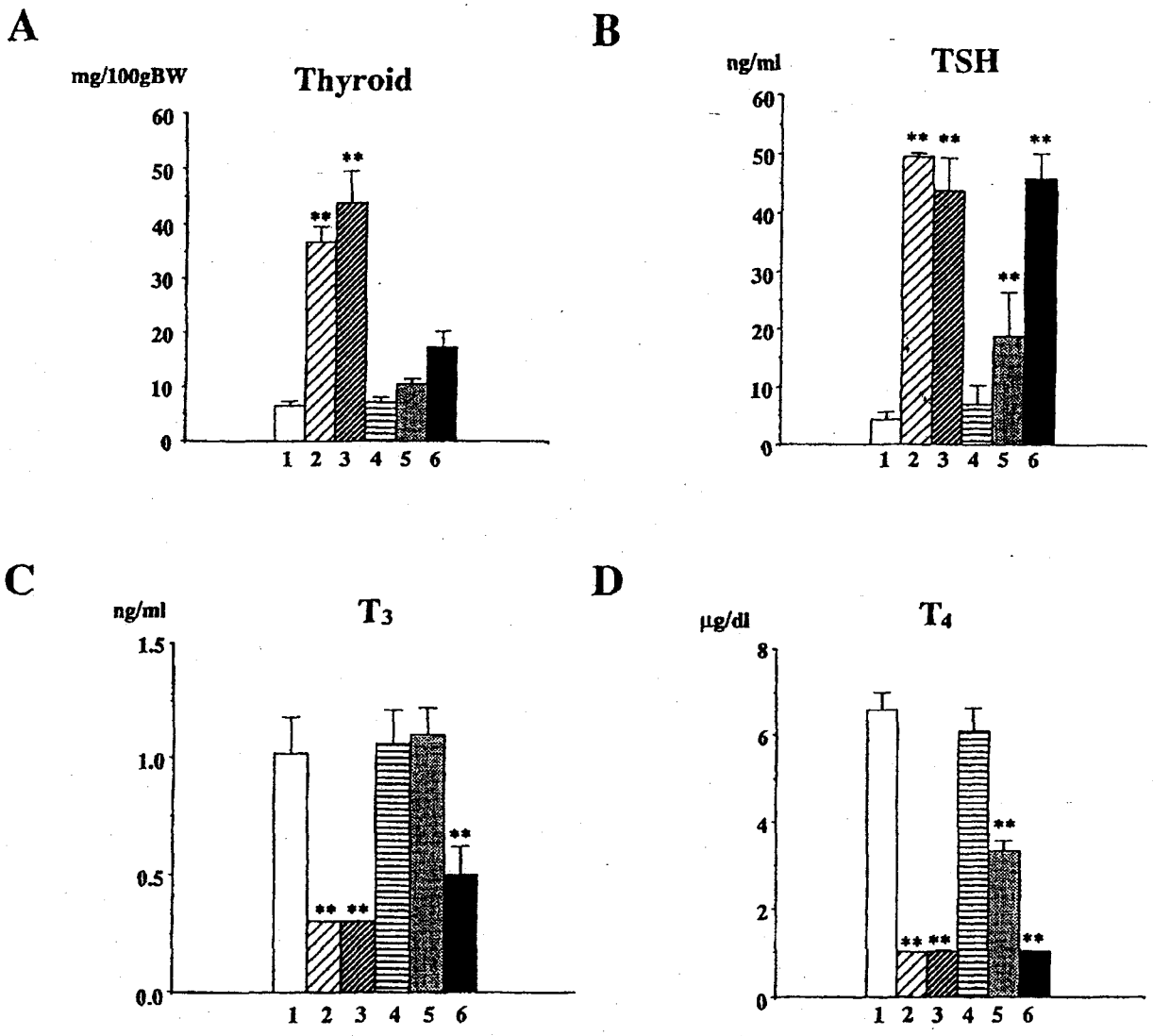


**Fig. 4.** Thyroid weights and serum thyroid-related hormone levels in male rats after gavage treatment with antithyroid thioureylene compounds 2-MBI, TU and ETU for 14 days.

A: Relative thyroid weights (mg/100g body weight)
B: Serum levels of TSH C: Serum levels of T3
D: Serum levels of T4
1 : Control (Corn oil alone) 2 : 0.15mmole 2-MBI/kg
3 : 0.3mmole 2-MBI/kg 4 : 0.3mmole TU/kg
5 : 0.15mmole ETU/kg 6 : 0.3mmole ETU/kg

**Table 4.** Organ weights for male rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| Groups(Dose) | | Treatment | | | | Recovery | |
|---|---|---|---|---|---|---|---|
| No of animals | | Control 5 | 2 mg/kg 5 | 10 mg/kg 5 | 50 mg/kg 5 | Control 5 | 50 mg/kg 5 |
| Body weight | g | 224 ± 8 | 223 ± 7 | 218 ± 14 | 153 ± 3** | 285 ± 11 | 186 ± 8** |
| Brain | g | 1.73 ± 0.04 | 1.79 ± 0.04 | 1.73 ± 0.07 | 1.65 ±0.02* | 1.83 ± 0.04 | 1.67 ± 0.04** |
| Heart | g | 0.72 ± 0.02 | 0.70 ± 0.02 | 0.60 ± 0.03** | 0.39 ±0.02** | 0.80 ± 0.06 | 0.56 ± 0.02** |
| Lung | g | 0.83 ± 0.04 | 0.87 ± 0.03 | 0.86 ± 0.05 | 0.63 ±0.02** | 0.92 ± 0.04 | 0.77 ± 0.07** |
| Liver | g | 6.39 ± 0.30 | 6.72 ± 0.32 | 8.09 ± 0.79** | 6.50 ± 0.24 | 8.36 ± 0.52 | 5.29 ± 0.30** |
| Kidney | g | 1.47 ± 0.09 | 1.56 ± 0.05 | 1.57 ± 0.11 | 1.04 ±0.07** | 1.82 ± 0.11 | 1.15 ± 0.03** |
| Spleen | g | 0.44 ± 0.04 | 0.46 ± 0.02 | 0.43 ± 0.02 | 0.22 ± 0.01** | 0.57 ± 0.03 | 0.42 ± 0.02** |
| Testis | g | 2.55 ± 0.10 | 2.59 ± 0.07 | 2.48 ± 0.10 | 2.40 ± 0.09* | 2.80 ± 0.05 | 2.49 ± 0.11** |
| Pituitary | mg | 7.3 ± 1.5 | 6.9 ± 1.2 | 8.3 ± 0.9 | 7.6 ± 1.0 | 8.3 ± 1.0 | 6.7 ± 2.0 |
| Thyroid | mg | 9.4 ± 1.3 | 11.6 ± 1.5 | 39.0 ± 5.2* | 81.2 ± 12.4** | 11.8 ± 1.5 | 47.4 ± 8.2** |
| Adrenal | mg | 32.0 ± 3.0 | 32.7 ± 3.1 | 31.3 ± 3.0 | 31.6 ± 3.1 | 32.8 ± 1.9 | 28.1 ± 3.4* |
| Submaxillary G. | g | 0.36 ± 0.04 | 0.38 ± 0.04 | 0.33 ± 0.05* | 0.20 ± 0.04** | 0.47 ± 0.05 | 0.27 ± 0.02** |
| Thymus | g | 0.34 ± 0.05 | 0.26 ±0.02** | 0.23 ± 0.04** | 0.13 ± 0.02** | 0.33 ± 0.02 | 0.18 ± 0.01** |
| Brain | g% | 0.78 ± 0.04 | 0.80 ± 0.03 | 0.80 ± 0.03 | 1.08 ± 0.03** | 0.64 ± 0.02 | 0.90 ± 0.04** |
| Heart | g% | 0.31 ± 0.01 | 0.31 ± 0.01 | 0.27 ± 0.02** | 0.26 ± 0.01** | 0.28 ± 0.01 | 0.30 ± 0.02* |
| Lung | g% | 0.37 ± 0.01 | 0.39 ± 0.01 | 0.40 ± 0.02** | 0.41 ± 0.01** | 0.33 ± 0.01 | 0.41 ± 0.03** |
| Liver | g% | 2.85 ± 0.08 | 3.01 ± 0.10 | 3.71 ± 0.17** | 4.25 ± 0.14** | 2.93 ± 0.09 | 2.85 ± 0.07 |
| Kidney | g% | 0.65 ± 0.02 | 0.70 ± 0.02* | 0.72 ± 0.02** | 0.68 ± 0.05 | 0.64 ± 0.02 | 0.62 ± 0.02 |
| Spleen | g% | 0.19 ± 0.01 | 0.21 ± 0.01 | 0.19 ± 0.01 | 0.15 ± 0.01** | 0.20 ± 0.02 | 0.23 ± 0.01** |
| Testis | g% | 1.13 ± 0.01 | 1.16 ± 0.05 | 1.14 ± 0.04 | 1.57 ± 0.03** | 0.99 ± 0.03 | 1.34 ± 0.07** |
| Pituitary | mg% | 3.3 ± 0.6 | 3.1 ± 0.6 | 3.8 ± 0.2 | 5.0 ± 0.7** | 2.9 ± 0.4 | 3.7 ± 1.2 |
| Thyroid | mg% | 4.2 ± 0.5 | 5.2 ± 0.6 | 17.9 ± 2.2* | 53.0 ± 7.6** | 4.2 ± 0.6 | 25.7 ± 5.1** |
| Adrenal | mg% | 14.2 ± 1.0 | 14.7 ± 1.6 | 14.4 ± 1.3 | 20.7 ± 1.8** | 11.5 ± 1.0 | 15.2 ± 2.0** |
| Submaxillary G. | g% | 0.16 ± 0.01 | 0.17 ± 0.02 | 0.15 ± 0.02 | 0.13 ± 0.03* | 0.17 ± 0.02 | 0.15 ± 0.02* |
| Thymus | g% | 0.15 ± 0.02 | 0.12 ± 0.01* | 0.11 ± 0.02** | 0.09 ± 0.01** | 0.12 ± 0.01 | 0.10 ± 0.01** |

Data are mean ± S.D. values

*,**: Significantly different from the relevant control at $p<0.05$, $p<0.01$, respectively

**Table 5.** Organ weights for female rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| Groups(Dose) | | Treatment | | | | Recovery | |
|---|---|---|---|---|---|---|---|
| | | Control | 2 mg/kg | 10 mg/kg | 50 mg/kg | Control | 50 mg/kg |
| No of animals | | 5 | 5 | 5 | 5 | 5 | 5 |
| Body weight | g | 155 ± 7 | 150 ± 10 | 155 ± 7 | 115 ± 2** | 168 ± 11 | 129 ± 5** |
| Brain | g | 1.69 ± 0.04 | 1.71 ± 0.04 | 1.66 ± 0.02 | 1.54 ±0.02* | 1.72 ± 0.06 | 1.63 ± 0.07* |
| Heart | g | 0.50 ± 0.03 | 0.48 ± 0.02 | 0.49 ± 0.02 | 0.33 ± 0.01** | 0.50 ± 0.04 | 0.42 ± 0.02** |
| Lung | g | 0.71 ± 0.06 | 0.69 ± 0.03 | 0.71 ± 0.05 | 0.57 ± 0.02** | 0.65 ± 0.04 | 0.63 ± 0.04 |
| Liver | g | 4.52 ± 0.18 | 4.26 ± 0.39 | 5.06 ± 0.31* | 5.01 ± 0.26** | 4.24 ± 0.42 | 3.94 ± 0.22 |
| Kidney | g | 1.08 ± 0.04 | 1.05 ± 0.06 | 1.18 ± 0.06** | 0.91 ± 0.03** | 1.05 ± 0.09 | 0.92 ± 0.06* |
| Spleen | g | 0.35 ± 0.03 | 0.33 ± 0.02 | 0.35 ± 0.01 | 0.18 ± 0.01** | 0.34 ± 0.04 | 0.35 ± 0.04 |
| Ovary | mg | 57.1 ± 5.5 | 55.8 ± 8.8 | 61.2 ± 4.7 | 23.8 ± 1.6* | 51.7 ± 7.2 | 37.7 ± 0.9** |
| Pituitary | mg | 9.6 ± 1.3 | 9.6 ± 1.1 | 8.1 ± 0.6 | 7.5 ± 1.5* | 10.3 ± 2.1 | 9.4 ± 1.4 |
| Thyroid | mg | 8.8 ± 1.1 | 8.8 ± 1.6 | 29.2 ± 5.7** | 73.6 ± 3.6** | 10.6 ± 1.1 | 48.2 ± 13.4** |
| Adrenal | mg | 42.2 ± 2.3 | 39.5 ± 1.8 | 38.3 ± 2.4* | 35.3 ± 3.1** | 39.3 ± 5.7 | 31.4 ± 2.2* |
| Submaxillary G. | g | 0.30 ± 0.03 | 0.28 ± 0.02 | 0.30 ± 0.02 | 0.19 ± 0.01** | 0.31 ± 0.02 | 0.25 ± 0.02** |
| Thymus | g | 0.31 ± 0.04 | 0.25 ± 0.02** | 0.22 ± 0.01** | 0.12 ± 0.02** | 0.27 ± 0.03 | 0.19 ± 0.02** |
| Brain | g% | 1.10 ± 0.04 | 1.15 ± 0.08 | 1.08 ± 0.05 | 1.34 ± 0.03** | 1.02 ± 0.04 | 1.26 ± 0.04** |
| Heart | g% | 0.32 ± 0.01 | 0.32 ± 0.02 | 0.32 ± 0.02 | 0.28 ± 0.01** | 0.30 ± 0.02 | 0.33 ± 0.01** |
| Lung | g% | 0.46 ± 0.02 | 0.46 ± 0.03 | 0.46 ± 0.02 | 0.50 ± 0.03* | 0.39 ± 0.02 | 0.49 ± 0.03** |
| Liver | g% | 2.92 ± 0.05 | 2.84 ± 0.20 | 3.27 ± 0.14** | 4.35 ± 0.18** | 2.51 ± 0.10 | 3.04 ± 0.11** |
| Kidney | g% | 0.70 ± 0.04 | 0.70 ± 0.02 | 0.76 ± 0.03** | 0.79 ± 0.02** | 0.62 ± 0.03 | 0.71 ± 0.03** |
| Spleen | g% | 0.23 ± 0.02 | 0.22 ± 0.01 | 0.23 ± 0.01 | 0.16 ± 0.01** | 0.21 ± 0.01 | 0.27 ± 0.03** |
| Ovary | mg% | 36.9 ± 2.7 | 37.4 ± 6.3 | 39.7 ± 3.2 | 20.6 ± 1.2* | 30.3 ± 3.0 | 29.1 ± 1.0 |
| Pituitary | mg% | 6.2 ± 1.0 | 6.4 ± 0.7 | 5.3 ± 0.6 | 6.5 ± 1.2 | 6.1 ± 1.0 | 7.2 ± 1.1 |
| Thyroid | mg% | 5.7 ± 0.8 | 5.9 ± 1.1 | 18.9 ± 3.6* | 63.9 ± 2.4** | 6.3 ± 0.9 | 37.3 ± 10.2* |
| Adrenal | mg% | 27.3 ± 0.6 | 26.5 ± 1.4 | 24.8 ± 0.7* | 30.7 ± 2.6 | 23.2 ± 1.9 | 24.8 ± 1.8 |
| Submaxillary G. | g% | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.16 ± 0.1** | 0.18 ± 0.01 | 0.19 ± 0.01 |
| Thymus | g% | 0.20 ± 0.02 | 0.16 ± 0.01** | 0.14 ± 0.01** | 0.10 ± 0.02** | 0.16 ± 0.01 | 0.15 ± 0.01 |

Data are mean ± S.D. values

*,**: Significantly different from the relevant control at $p<0.05$, $p<0.01$, respectively

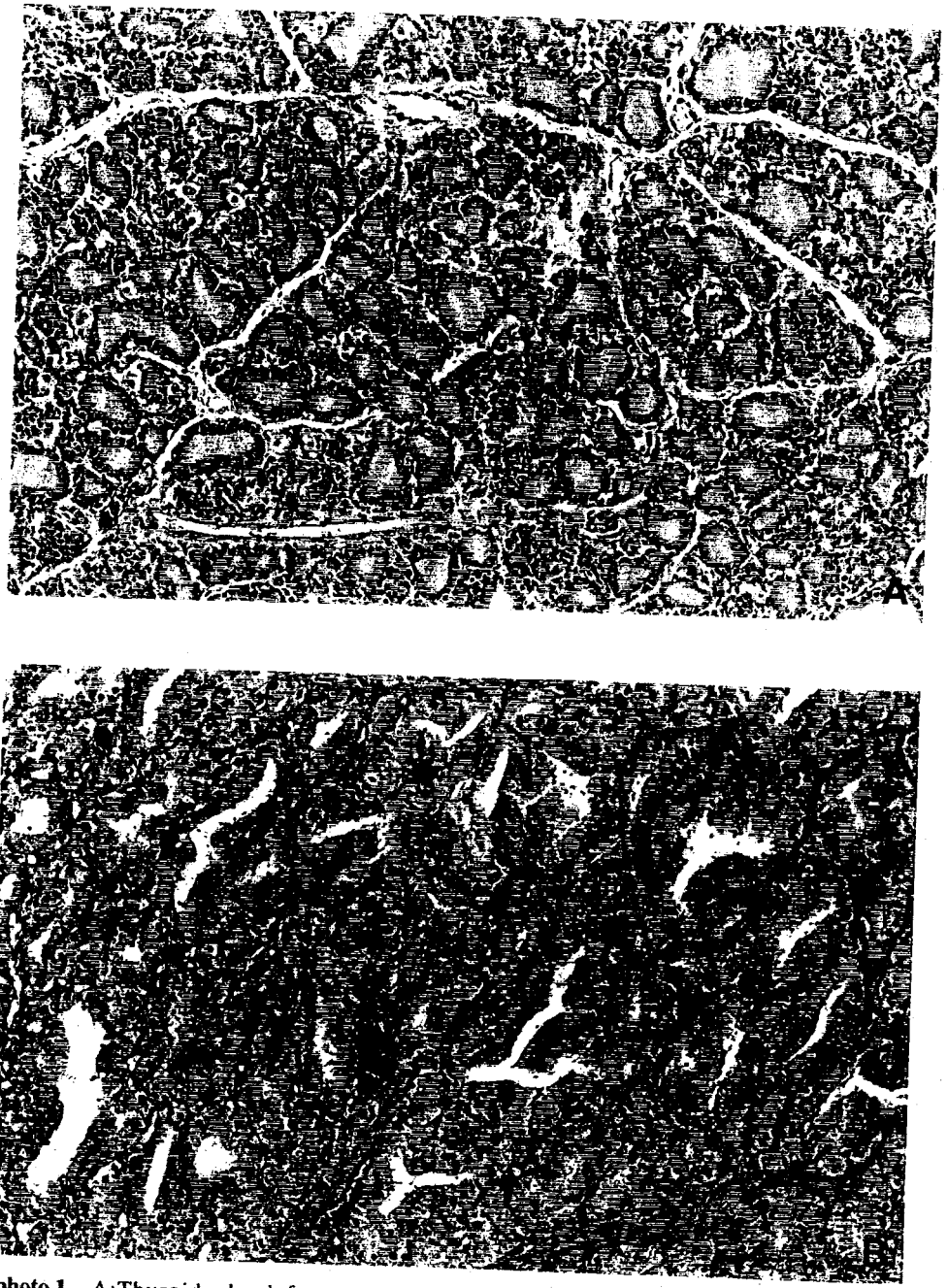

**photo 1.** A: Thyroid gland from a control rat. Note normal glands with follicles containing abundant colloid and lined by low cuboidal cells. HE ×48
B: Thyroid gland from a rat treated for 28 days with repeated doses of 2-MBI (50 mg/kg). Note diffuse follicular hyperplasia characterized by tall columnar epithelial cells and markedly decreased colloid. HE ×48

**Table 6.** Histopathological findings for rats after 28 days of treatment with 2-mercaptobenzimidazole and a 2-week recovery period.

| | | Treatment | | | | | | | | Recovery | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | | Male | | | | Female | | | | Male | | Female | |
| Groups(Dose mg/kg) | | 0 | 2 | 10 | 50 | 0 | 2 | 10 | 50 | 0 | 50 | 0 | 50 |
| No. of animals examined | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| **Heart** | | | | | | | | | | | | | |
| Intramyocardial cell infiltration | ± | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| Fibrosis | ± | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Myocardial calcification | ± | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Lung** | | | | | | | | | | | | | |
| Alveolar wall thickening | ± | 0 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| Perivascular cell infiltration | ± | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Perivascular edema | ± | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 2 | 1 |
| Interstitial cell infiltration | ± | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **Liver** | | | | | | | | | | | | | |
| Microgranuloma formation | ± | 0 | 1 | 0 | 1 | 3 | 0 | 2 | 1 | 1 | 1 | 1 | 0 |
| Interstitial cell infiltration | ± | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| **Kidney** | | | | | | | | | | | | | |
| Eosinophilic bodies | ± | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| Calcification of collecting tubules | ± | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| **Testis** | | | | | | | | | | | | | |
| Interstitial edema | ± | 1 | 0 | 2 | 2 | - | - | - | - | 1 | 0 | - | - |
| **Spleen** | | | | | | | | | | | | | |
| Hemosiderin deposition | ± | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| **Pancreas** | | | | | | | | | | | | | |
| Acinar cell vacuolation | ± | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Acinar cell necrotic degeneration | ± | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| **Thyroid gl.** | | | | | | | | | | | | | |
| Diffuse hyperplasia of follicles | ± | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 |
| Decrease of colloid | ± | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Fibrous capsule thickening | ± | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | ++ | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| **Pituitary gl.** | | | | | | | | | | | | | |
| Increase of thyroidectomized cells | ± | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| **Adrenal cortex** | | | | | | | | | | | | | |
| Hyperplasia of lipid-laden cells | ± | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |