

# <sup>131</sup>I-induced changes in rat thyroid gland function

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## Abstract

Therapeutic doses of <sup>131</sup>I administered to thyrotoxic patients may cause thyroid failure. The present study used a rat model to determine thyroid function after the administration of different doses of <sup>131</sup>I (64–277 μCi). Thirty male Fisher rats in the experimental group and 30 in the control group (untreated) were followed for 6 months. The animals were 4 months old at the beginning of the experiment and were sacrificed at an age of 9 months. Hormone concentration was determined before <sup>131</sup>I administration (4-month-old animals) and three times following <sup>131</sup>I administration, when the animals were 7, 8, and 9 months old. The thyroid glands were removed and weighed, their volume was determined and histopathological examination was performed at the end of the experiment. Significant differences in serum triiodothyronine and thyroid-stimulating hormone concentration, measured at the age of 7, 8, and 9 months, were found in the experimental group. During aging of the animals, the concentration of thyroxin fell from 64.8 ± 8.16 to 55.0 ± 6.1 nM in the control group and from 69.4 ± 6.9 to 25.4 ± 3.2 nM in the experimental group. Thyroid gland volume and weight were significantly lower in the experimental than in the control group. Thyroid glands from the experimental group showed hyaline thickness of the blood vessel wall, necrotic follicles, a strong inflammatory reaction, and peeling of necrotic cells in the follicles. In conclusion, significant differences in hormone levels and histopathological findings indicated prolonged hypothyroidism after <sup>131</sup>I administration to rats, which was not <sup>131</sup>I dose dependent.

## Key words

- Thyroid gland
- <sup>131</sup>I
- Hormones
- Histopathology
- T3
- T4
- TSH

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## Introduction

Hyperthyroidism, or thyrotoxicosis, is a clinical state that results from hypersecretion of thyroid hormones, principally triiodothyronine (T3) and thyroxin (T4). The

most common cause of hyperthyroidism is toxic diffuse goiter, or Graves' disease. Less common causes are toxic nodular goiter, or autonomous functioning thyroid nodules, and thyroid cancer. The use of iodine-131 (<sup>131</sup>I) for the treatment of hyperthyroidism dates

back to 1942. Now, after more than 60 years and over a million hyperthyroid patients treated, treatment with  $^{131}\text{I}$  is recognized as the simplest, safest, least expensive, and most effective therapy for most patients, which has largely replaced surgery as the final treatment of this disorder. The biological basis for radioiodine therapy of hyperthyroidism lies in high thyroidal concentration of iodine and in radiation-induced damage that inhibits thyroid follicle cell function (1). The effectiveness of a particular dose of radioiodine in controlling hyperthyroidism depends on several factors: iodine uptake, gland weight, effective half-life of iodine in the gland, tissue distribution of radioactivity, and radiosensitivity of the follicle cells. Since only some of these factors can be easily evaluated, no single formula can be applied in determining the optimal therapeutic dose (1).

Therapeutic doses of  $^{131}\text{I}$  administered to thyrotoxic patients may cause some patients to undergo thyroid failure and some to exhibit recurrent thyrotoxicosis, although most return to a euthyroid state. Evaluation of the functional thyroid state soon after  $^{131}\text{I}$  therapy is often difficult. Correlations between clinical status and various laboratory parameters are variable. Diminished serum T4 does not necessarily signify hypothyroidism because a preferential secretion of T3 may occur. On the other hand, some patients who are clinically hypothyroid may have normal or elevated serum T4 levels. A substantial number of these patients later become hyperthyroid, and they must be under careful surveillance. Only 9% of hyperthyroid patients develop transient hypothyroidism within 8 months of  $^{131}\text{I}$  administration. Although thyroid-stimulating hormone (TSH) is usually a reliable indicator of thyroid hormone production, occasionally it can be found at elevated levels in euthyroid patients, probably because of a reduced thyroid hormone reserve following therapy (1,2).

We used an animal model in order to

determine thyroid function after administering different doses of  $^{131}\text{I}$  (64-277  $\mu\text{Ci}$ ), followed by serum T3, T4, and TSH analyses. We also analyzed the morphological parameters of the thyroid gland 5 months after  $^{131}\text{I}$  administration. The doses of  $^{131}\text{I}$  were calculated according to a known thyroid volume (using thyroid glands of three 4-month-old sacrificed animals) and were in the range from the lowest to the highest therapeutic doses.

In the present study, we followed serum T3, T4, and TSH after  $^{131}\text{I}$  administration in rats during a longer period of time (5 months). This long-term follow-up is important in order to understand the tendency of hormone-status retention following therapy with  $^{131}\text{I}$ . At the same time, we followed the hormone status in a controlled untreated group of animals to evaluate the effect of aging on thyroid activity in rats.

## Material and Methods

Male Fisher rats were kept under controlled conditions (temperature of 22-25°C, a 14-h light and 10-h dark cycle, and 60-70% humidity) receiving food and water without iodine. Three days before testing iodine intake, the animals were not fed. The animals (30 in the experimental group, and 30 in the control untreated group, were 4 months old at the beginning of the experiment and were sacrificed at the age of 9 months.  $^{131}\text{I}$  was given to rats with a gastric tube after intraperitoneal anesthesia with thiopental (50  $\mu\text{g/g}$  animal weight). Due to the small size of the thyroid glands it was not possible to perform determination by ultrasound. Therefore, the volume of the thyroid gland needed to calculate the dose of  $^{131}\text{I}$  to be given to the animals was determined using thyroid glands of three 4-month-old sacrificed animals. Immediately after application, the  $^{131}\text{I}$  activity in the syringe was measured using a Siemens LFOV gamma camera without a collimator (Siemens, Munich, Germany). The syringe was placed on a holder

40 cm above the center of the crystal to reproduce constant geometry. The activity remaining in the syringe after <sup>131</sup>I administration to the animals was also measured. The doses of <sup>131</sup>I were calibrated so as to deliver radiation-absorbed doses to the thyroid of the order of those applied in radioiodine therapy of diffuse toxic goiter in humans. Approximation of the activities to be administered was based on previous measurements of thyroid gland weight and iodine turnover in rats. Control measurements were repeated after 24 h.

Blood samples for hormone analyses were obtained from the jugular vein prior to <sup>131</sup>I administration and three times following <sup>131</sup>I administration, when the animals were 7, 8, and 9 months old. T3, T4, and TSH were determined by radioimmunoassay using the following kits: Cat #3423, Immunotech, Marseille, France, for T3 and T4 (intra-assay coefficient of variation for T3 was 2.5-4%, and for T4 2-4%), and rat TSH Cat #AH R001, Biocode S.A., Salvay, Liege, France (intra-assay coefficient of variation for TSH was 4-5.1%). Body weight was determined at the beginning of the experiment and before each blood collection. The control untreated group was used in order to evaluate thyroid function during aging.

Animals were sacrificed at the age of 9 months. The thyroid gland was removed and weighed, and its volume was determined. Histopathological examination of the thyroid gland was performed by hemalaun-eosin staining.

Hormone measurements and body weight (determined four times during the experiment) are reported as means  $\pm$  SD. Analysis of variance for repeated measurements and the *post hoc* Fisher LSD test were used for statistical evaluation of the data, using the SPSS 12.0 program. Correlation between hormone concentration and animal age, or hormone concentration and the dose of <sup>131</sup>I in combination with animal age, was determined by regression and multiple-regression analysis. Correlation coefficients were

calculated using a linear regression analysis. The weight of the thyroid gland and its volume were compared between treated animals and controls using the *t*-test.

## Results

At the beginning of the experiment, there were no statistically significant differences between the experimental and the control groups regarding hormone levels: T3,  $1.3 \pm 0.14$  vs  $1.26 \pm 0.16$  nM; T4,  $68.5 \pm 6.9$  vs  $64.8 \pm 8.2$  nM; TSH,  $4.78 \pm 2.9$  vs  $3.79 \pm 1.01$  ng/mL) or body weight ( $309.8 \pm 35.2$  vs  $315.4 \pm 28.9$  g).

The volume of the thyroid glands of 4-month-old sacrificed animals ranged from 0.0269 to 0.0282, and that value was used to calculate the <sup>131</sup>I to be given to the experimental group of animals.

The doses of <sup>131</sup>I per animal in the experimental group ranged from 64 to 277  $\mu$ Ci, and in relation to a rat thyroid volume, they were between the lowest and the highest human therapeutic doses (3).

The concentrations of T3, T4, and TSH measured at the ages of 4, 7, 8, and 9 months are shown in Figure 1. Significant differences in serum T3 concentration measured at the ages of 4, 7, 8, and 9 months were found in the experimental group of animals (Figure 1A, T3:  $F = 30.0$ ,  $P = 0.000$ ), while no difference was found in the control group (data not shown). Serum T3 concentration was significantly reduced at the age of 7 months in the experimental group ( $P < 0.05$ ) compared to control animals of the same age (see Figure 1A).

A significant difference in serum T4 concentration measured at the age of 4, 7, 8, and 9 months was found both in the experimental group (Figure 1B, T4:  $F = 430.0$ ,  $P = 0.0000$ ) and in the control group (Figure 2, T4:  $F = 2.45$ ,  $P = 0.000$ ). The concentration of T4 varied in all measurements in both animal groups, tending to decrease with animal age ( $P < 0.05$ ).

Figure 1. Mean values of serum triiodothyronine (T3, nM; panel A), thyroxine (T4, nM; panel B), and thyroid-stimulating hormone (TSH, ng/mL; panel C) concentrations in the experimental group at the age of 4, 7, 8, and 9 months. The animals received  $^{131}\text{I}$  at the age of 4 months. Statistical difference was found among all experimental groups. Asterisk indicates that the values for the animals of the same age differed statistically between experimental and control group ( $P < 0.05$  for the *post hoc* Fisher LSD test performed after analysis of variance for repeated measurements).

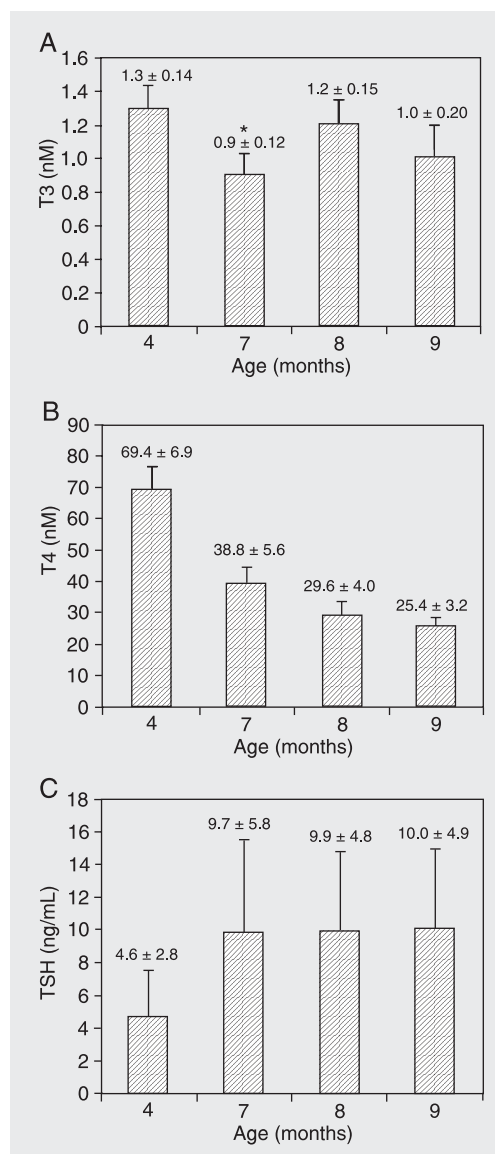
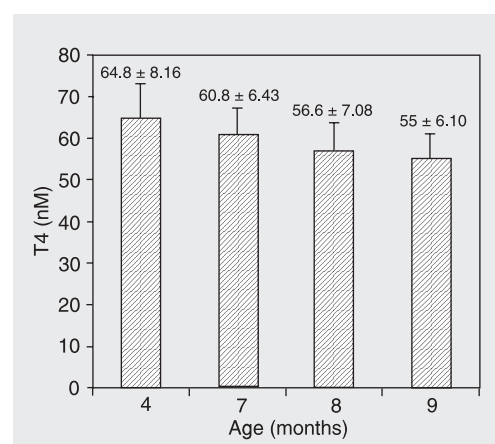


Figure 2. Mean values of serum thyroxine (T4, nM) concentration in the control group at the age of 4, 7, 8, and 9 months. Statistical difference was found among all measurements in control group ( $P = 0.000$ ; analysis of variance for repeated measurements).



A significant difference in serum TSH concentration measured at the age of 4, 7, 8, and 9 months was found in the experimental group (Figure 1C, T3:  $F = 30.0$ ,  $P = 0.000$ , TSH:  $F = 18.9$ ,  $P = 0.0000$ ), while no difference was found in the control group (data not shown). Serum TSH concentration was significantly elevated at the age of 7 months (3 months after  $^{131}\text{I}$  administration) in the experimental group ( $P < 0.05$ ) compared to control animals of the same age.

Regression analysis showed a correlation between serum T3, T4, and TSH concentrations and age in control animals (T3:  $F = 21.9$ ,  $P = 0.000$ , age -  $\beta = -0.40$ ,  $P = 0.000$ ; T4:  $F = 1063$ ,  $P = 0.000$ , age -  $\beta = -0.95$ ,  $P = 0.000$ ; TSH:  $F = 23.9$ ,  $P = 0.000$ , age -  $\beta = 0.42$ ,  $P = 0.000$ ).

Multiple regression analysis showed a correlation between serum T3 and TSH concentrations and  $^{131}\text{I}$  dose, but no correlation with the age in the experimental animals (T3:  $F = 28.6$ ,  $P = 0.000$ , age -  $\beta = 0.12$ ,  $P = 0.34$ ; dose -  $\beta = -0.67$ ,  $P = 0.000$ ; TSH:  $F = 40.6$ ,  $P = 0.000$ , age -  $\beta = 0.196$ ,  $P = 0.089$ ; dose -  $\beta = 0.789$ ,  $P = 0.000$ ). On the other hand, serum T4 concentration showed a correlation with the age of the experimental animals, but not with the dose of  $^{131}\text{I}$  administered ( $F = 551$ ,  $P = 0.000$ , age -  $\beta = -0.87$ ,  $P = 0.000$ , dose -  $\beta = -0.098$ ,  $P = 0.033$ ). The time-course of hormone levels during the follow-up period according to  $^{131}\text{I}$  administration is shown in Table 1. The animals were divided into 6 groups of 5 animals each according to the absorbed doses of  $^{131}\text{I}$ .

#### Volume and weight of the thyroid gland

The thyroid gland volume was significantly lower in the experimental group than in the control group at the age of 9 months, i.e., 5 months after  $^{131}\text{I}$  administration (experimental vs control =  $0.0184 \pm 0.039$  vs  $0.0279 \pm 0.0063$ ,  $t = 6.1$ ,  $P = 0.0000$ ). The weight of the thyroid gland was significantly lower in the experimental group than in the

control group at the age of 9 months (experimental vs control = 0.0173 ± 0.0026 vs 0.020 ± 0.0033, t = 4.0, P = 0.0002).

Regression analysis showed no correlation between the volume and weight of the thyroid gland and the dose of <sup>131</sup>I (volume: F = 0.7, P = 0.41, dose - β = -0.16, P = 0.41; weight: F = 1.4, P = 0.24, dose - β = -0.224, P = 0.24).

### Body weight

Animals of both groups entered the experiment at a similar body weight. At the age of 7 months there was no difference in body weight between the control and the experimental groups (t = 0.79, P = 0.43 (366.6 ± 30.9 vs 372.6 ± 30.0 g)). Statistically significant differences were found at the age of 8 months (t = 2.25, P = 0.03) when the experi-

mental group of animals had a higher body weight than the controls (374.0 ± 29.4 vs 391.5 ± 29.8 g), and disappeared at the age of 9 months (t = 1.8, P = 0.08 (377.8 ± 33.9 vs 391.8 ± 29.0 g)).

### Histopathological analysis of the thyroid gland

A histological image of the thyroid gland of the control group of animals is shown in Figure 3A. Histopathological analysis of thyroid glands from the experimental group of animals showed hyaline thickness of the blood vessel walls, necrotic follicles (Figure 3B), a strong inflammatory reaction, and peeling of necrotic cells in the follicles (Figure 3C). There were no differences between the thyroid glands of animals receiving different doses of <sup>131</sup>I.

Table 1. Effect of <sup>131</sup>I dose on the time-course of serum T3, T4, and TSH in the experimental group of animals.

Animal number	Animal number and <sup>131</sup> I dose (μCi)						Control animals
	1-5	6-10	11-15	16-20	21-25	26-30	
<sup>131</sup> I dose (μCi)	64-98	103-127	137-171	175-197	205-232	233-277	
Before <sup>131</sup> I administration (4-month-old animals)							
T3 (nM)	1.2-1.3	1.1-1.5	1.2-1.3	1.2-1.6	1.2-1.7	1.3-1.6	
T4 (nM)	62.3-77.0	61.0-72.1	53.6-72.0	56.7-72.1	60.1-86.0	60.5-77.1	
TSH (ng/mL)	3.41-9.59	1.80-6.66	2.74-7.91	2.45-8.87	1.12-5.89	2.35-7.19	
3 months after <sup>131</sup> I administration (7-month-old animals)							
T3 (nM)	0.8-1.1	0.9-1.1	0.7-1.1	0.7-1.1	0.8-1.1	0.8-0.9	1.0-1.5
T4 (nM)	37.2-45.1	37.7-45.5	31.2-48.8	40.1-51.0	31.2-45.5	32.5-47.0	45.9-77.8
TSH (ng/mL)	4.22-19.4	3.93-6.62	4.25-11.10	4.10-8.50	6.50-19.40	7.80-19.40	2.05-5.48
4 months after <sup>131</sup> I administration (8-month-old animals)							
T3 (nM)	1.1-1.3	1.2-1.5	1.1-1.2	1.1-1.4	1.1-1.5	0.9-1.5	1.0-1.5
T4 (nM)	32.1-33.2	20.1-38.1	21.4-32.9	27.3-30.6	23.7-32.8	24.2-30.5	45.1-69.1
TSH (ng/mL)	5.22-13.60	5.22-6.66	4.48-11.4	4.66-10.50	7.40-17.0	8.90-17.0	2.27-5.04
5 months after <sup>131</sup> I administration (9-month-old animals)							
T3 (nM)	1.2-1.3	1.2-1.4	0.9-1.3	0.8-1.1	0.7-0.9	0.8-1.0	1.2-1.4
T4 (nM)	22.4-26.9	22.0-25.7	18.9-27.5	19.9-29.1	22.2-31.9	24.7-29.3	37.6-64.5
TSH (ng/mL)	4.99-15.50	5.23-7.20	4.20-11.60	4.88-8.82	7.65-17.0	12.3-17.0	2.04-4.70

Data are reported as range for 5 rats in each group and time-course of serum T3, T4, and TSH in the control group. T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone.

Statistical differences between groups: 7-month-old animals: T3, P < 0.05; T4, P < 0.05; TSH, P < 0.05; 8-month-old animals: T3, non-significant (NS); T4, P < 0.05; TSH, NS; 9-month-old animals: T3, NS; T4, P < 0.05; TSH, NS (analysis of variance for repeated measures and the *post hoc* Fisher LSD test).

## Discussion

In this study rat model was used in order to determine the thyroid function after application of different doses of  $^{131}\text{I}$  (64-277  $\mu\text{Ci}$ ). Concentration of T3, T4 and TSH was determined before  $^{131}\text{I}$  administration (4-month-old animals) and when rats were 7, 8, and 9 months old. Nine-month-old animals were sacrificed, thyroid glands were removed for histopathological examination.

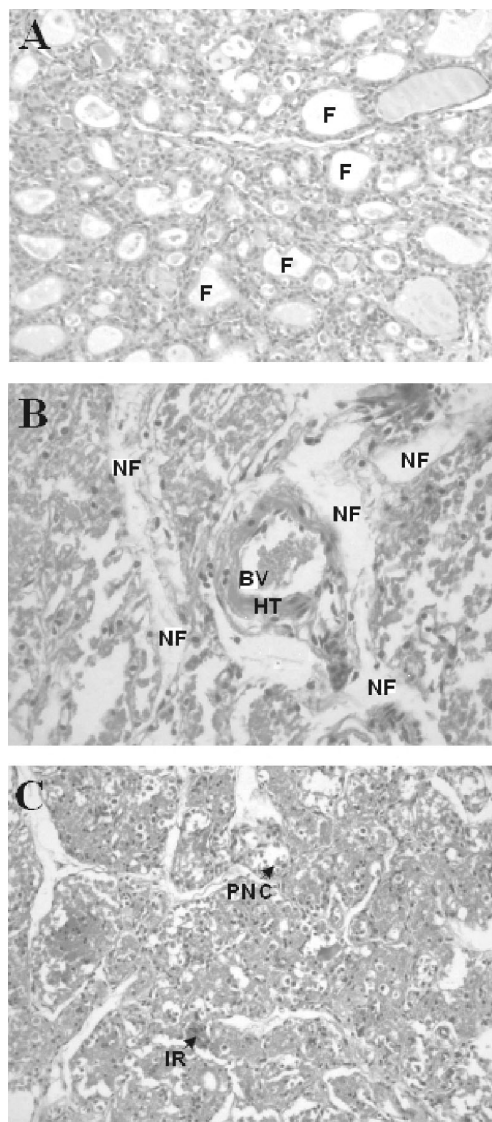
Serum T3, T4, and TSH levels of control animals measured at 4, 7, 8, and 9 months of age agreed with those described in the litera-

ture. We found a decrease in serum T4 concentration between the age of 4 and 8 months, in accordance with the results of da Costa et al. (4). Serum T4 entirely originates from the thyroid gland, while more than 80% of T3 is produced by deiodination of T4 in other tissues, especially the liver and kidneys. Decreased serum T4 concentration stimulates TSH secretion through the pituitary-thyroid feedback mechanism. However, there are only two reports of increased TSH with aging in rats (5,6). Our finding of a constant TSH level corresponds to the most recent studies (4,7-9). No feedback response seems to have occurred, and the serum TSH remained unchanged in old animals despite a decreased serum T4. This may have been due to the unchanged serum T3 levels (4). Furthermore, da Costa et al. (4) explained the increased serum T4 levels by low expression of the gene for thyroperoxidase, the enzyme responsible for thyroglobulin iodination in old males, suggesting that the gene expression of androgens might increase (10, 11). The timing and/or degree of the decline may differ between rat strains (5,10,12). There is also the possibility that the aged murine thyroid becomes less responsive to circulating TSH.

One possible explanation for this lack of response to TSH during thyroid gland aging was given by Studer et al. (13) and Gerber et al. (14). These investigators suggested that a gradual failure of endocytosis in response to normal TSH stimulation would occur in the aging thyroid. At first, thyroglobulin exocytosis and iodination would be unaffected, resulting in gradual distension of the follicular lumen that would impair the normal apical membrane function (13,14).

Since thyroid hormone reserves are exhausted slowly from the follicles, the result of therapy with  $^{131}\text{I}$  should be evaluated two or three months after administration (15). Therefore, we evaluated the thyroid hormone status 3, 4, and 5 months after  $^{131}\text{I}$  administration. We found decreased serum

Figure 3. Histological image of the thyroid gland from a control (panel A) and experimental animals (panels B and C). F = follicle; HT = hyaline thickness; BV = blood vessel; NF = necrotic follicle; IR = inflammatory reaction; PNC = peeling of necrotic cells. Magnification: panels A and B = 40,000X; panel C = 20,000X.



T4 concentrations, while TSH was significantly increased. These findings were in accordance with an earlier report by Reilly et al. (16). However, Raily et al. showed no change in T3 concentration 85 days after administration of <sup>131</sup>I in their Wistar rat model, our Fisher rat model showed a significant decrease of T3 concentration 3 and 5 months after <sup>131</sup>I administration. These differences in behavior of serum T3 are probable the result of the doses applied, the follow-up period, as well as the mechanism of defense against radioactivity in different rat models. Reilly et al. (16) applied a constant dose of 150 µCi, while the doses applied in the present study were between 64 and 277 µCi. In the present study, we showed that serum T3 concentrations significantly decreased as the <sup>131</sup>I dose increased. The lowest concentration of T3 was detected 3 months after administration of <sup>131</sup>I, followed by a slow increase. However, the serum T4 concentration showed a decrease throughout the study period.

It is known that thyroid gland hormones enhance catabolic reactions in the organism. As a result of hormone shortage, catabolism slows down, with a consequent increase in body weight. The latest investigations have shown that the rat thyroid gland expresses receptors of leptin, an adipose tissue-secreted hormone which decreases the caloric intake and increases energy expenditure (17). Therefore, our finding of a significant increase in body weight 4 months after radiotherapy could be caused not only by hypothyroidism, but also by a deficit of leptin receptors in radioiodine-treated animals. The absence of statistically significant differences in body weight between experimental and control animals 5 months after radiotherapy indicates that hypothyroidism became less severe during that period.

The low thyroid weight and volume of the experimental group of animals is in accordance with the findings of Agote et al. (18). The histopathological findings of hya-

line thickness of blood vessels after exposure to radioactivity correspond to changes described after therapy of thyroid hyperfunction by injection of ethanol through the skin (19). The balance between thyroid regeneration and fibrosis appears to determine in part whether hypothyroidism will occur (1). Abnormal distribution of hyaline probably disrupts thyroid hormone secretion, as shown by Li et al. (20). Therefore, despite an increased TSH concentration during the monitoring period, T3 concentration was low but close to the minimum normal level. This finding indicates that T3 occurs in non-thyroid tissues by T4 deiodination and this is the reason why its concentration, contrary to the concentration of T4, does not depend on tissue status.

We have described the state of rats following <sup>131</sup>I administration (the doses they received were in human relations from the lowest to the highest therapeutic doses) which resembles the state clinically observed in some human subjects receiving therapeutic doses of <sup>131</sup>I. There are only few previously published long-term follow-up studies regarding radioiodine treatment of hyperthyroidism in patients (15,21,22). It was observed that hypothyroidism will develop in 82% of patients with Graves' disease and in 32% of patients with multinodular goiter treated with <sup>131</sup>I within 25 years (15). The higher rate of hypothyroidism in patients with Graves' disease than in patients with toxic multinodular goiter might result from protection of the suppressed normal extranodular tissue by its inability to concentrate <sup>131</sup>I in patients with toxic multinodular goiter (15,21). Metso et al. (15) did not find a dose-response relationship between the radioactive dose and the rate of hypothyroidism or a positive correlation between the cure rate and hypothyroidism. Because the development of hypothyroidism seems to be inevitable and unpredictable by any clinical factor, the objective of <sup>131</sup>I treatment should be to minimize the persistence of hyperthyroid-

ism with an easily manageable treatment scheme with minimal costs. Some authors recommend administration of empirical (the same fixed dose for every patient regardless to thyroid volume) doses of  $^{131}\text{I}$  for the treatment of hyperthyroidism (15,23). There are very few data on animal models regarding a prolonged follow-up of thyroid status after administration of different doses of  $^{131}\text{I}$ . The present study clearly showed that hypothy-

roidism develops anyway, when either low or high  $^{131}\text{I}$  doses have been administered, similarly to the findings described for humans. Therefore, our findings support the administration of empirical rather than calculated doses of  $^{131}\text{I}$ . This raises the possibility of avoiding the need to measure the size and the  $^{131}\text{I}$  uptake of the thyroid gland and considerable inconvenience to the patient, as well as additional costs.

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