

# Radiation-Induced Thyroid Carcinogenesis as a Function of Time and Dietary Iodine Supply: An *in Vivo* Model of Tumorigenesis in the Rat

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It is believed that a combination of environmental factors with mutagens induces carcinomas derived from thyroid follicular cells. In this study we tried to ascertain whether a single short-term exposure to external radiation is sufficient to induce thyroid carcinomas in rats under long-term high or low dietary iodine intake.

Rats were tested over a period of 110 wk under high (~10-fold of normal), normal, and low (~0.1-fold of normal) daily iodine intake. Forty-day-old animals were subjected to single external radiation of 4 Gy or sham radiation. Thyroid function was tested weekly, and thyroid morphology was determined after 15, 35, 55, and 110 wk.

Iodine deficiency, but not high iodine intake, led to a decrease in T<sub>3</sub> and T<sub>4</sub> plasma levels, but to an increase in TSH, which

became significant after 9 and 11 wk of treatment, respectively. Both high and low iodine treatment significantly increased the proliferation rate and induced thyroid adenomas, but no malignancies after 55 and 110 wk. Radiation with 4 Gy resulted in a significant destruction of the follicular structure. Under high and low iodine intakes (50–80% of animals), but not under normal iodine supply, thyroid carcinomas were observed in irradiated rats. Thus, the increased proliferation rate induced under the experimental conditions described in this study is apparently not sufficient to cause thyroid carcinomas, but the presence of a mutagen-like radiation is required. This model may help to define genetic alterations long before histological changes are detectable. (*Endocrinology* 143: 2584–2592, 2002)

THYROID NODULES affect approximately 20–45% of the population during their lifetime, but only a minority of nodular goiters bear a clinically relevant malignant potential (1). A simple diagnostic approach to solving this problem does not exist. Cytological evaluation after fine needle aspiration obtained from thyroid nodules allows only for the detection of thyroid carcinoma in 80–90% of the cases (2). Thus, better methods predicting the malignant potential of thyroid nodules and/or diagnosing existing malignancies are urgently needed.

Four types of thyroid cancer comprise more than 98% of all thyroid malignancies: papillary thyroid carcinoma (PTC; 40–70%), follicular thyroid carcinoma (FTC; 10–40%), undifferentiated (anaplastic) thyroid carcinoma (5–10%) and medullary thyroid carcinoma (MTC; 5–10%) (3). About 25% of patients with MTC are hereditary and subclassified as familial MTC, multiple endocrine neoplasia type 2A or type 2B (4). Germline mutations (almost exclusively point mutations) of the protooncogene *RET* are found in more than 95% of patients with this prototype of a familial form of carcinoma. In contrast, PTC, FTC, and undifferentiated (anaplastic) thyroid carcinoma are generally sporadic, but familial occurrence has been described in 3–7% of these carcinomas (5). A worldwide search has revealed only a few families with nonmedullary thyroid cancer (6).

Abbreviations: FTC, Follicular thyroid carcinoma; I+, high iodine diet; I-, low iodine diet; In, normal iodine diet; InR, normal diet, irradiated; I+R, high iodine diet, irradiated; I-R, low iodine diet, irradiated; MTC, medullary thyroid carcinoma; PTC, papillary thyroid carcinoma.

Although the etiology of the more common sporadic forms of thyroid cancer remains speculative, it is believed that environmental factors explain the development of carcinomas derived from thyroid follicular cells. Data obtained from follow-up studies of patients subjected to external radiation and to the fall-out of nuclear bombs or, more recently, the results achieved by examining victims of the Chernobyl accident clearly demonstrate that internal and/or external radiation play an important role in thyroid carcinogenesis (7, 8). The effects of radiation were studied more systematically in animal models back in the 1950s through 1970s. Lindsay *et al.* (9, 10) and Doniach (11, 12) report that both papillary and follicular carcinomas occur more frequently after external radiation or application of radioactive iodine, with no comparable increase in the frequency of medullary carcinomas. In all of their cases investigated, thyroid adenomas were much more frequent and were detectable earlier than carcinomas, supporting the hypothesis proposed by Wynford-Thomas that thyroid carcinomas originate from benign thyroid tumors in a multistep fashion (13, 14).

From the cell kinetic point of view, adult thyroid follicular cells are classified as a stable cell population (15), characterized by a very low rate of both cell proliferation and cell death, but in which a significant proliferative response can occur once the appropriate stimulus has been given. Based on several studies dealing with signal transduction pathways (16, 17), a major role of TSH in proliferation control of the thyroid was suggested. TSH is connected with multiple intracellular signal transduction pathways linked to prolifer-

ation and differentiation of thyroid follicular cells (18, 19). In thyroids, as in other organs, unregulated growth and proliferation signals acting on cells with multiple transforming lesions result in malignancy. The development of thyroid cancer is mostly associated with special environmental factors, *i.e.* long-term iodine deficiency and ionizing radiation. In this respect, an imbalance of intracellular TSH-dependent pathways caused by mutational, *i.e.* postradiation, events may explain shifts in proliferation-*vs.* differentiation-associated pathways. Iodide uptake to the thyrocytes is mediated by the recently cloned sodium-iodide symporter (20), and sodium-iodide symporter expression is directly regulated by TSH (21). Numerous studies have addressed the question of a predominant iodide-dependent regulation in the development of hyperplasia and proliferation of thyroid follicle cells *in vivo* (22, 23) or have reported a major contribution of TSH

in this respect (24, 25). However, most of these studies, based on severe iodide depletion, used a short observation period to define iodide-dependent effects. Only a few reports have dealt with the ontogenesis of morphological and hormonal changes during moderate long-term iodine deficiency, which more closely parallels the situation of humans in an iodine-deficient area (26–28). The long-term effects of iodine excess in humans have not been studied in detail, but recent reports suggest that iodine excess also induces goitrogenesis and benign thyroid tumors (29).

The present study aimed at systematically monitoring the influence of a long-term increase or decrease in the daily iodine supply in rats on the morphology of the thyroid. To develop a reproducible model of thyroid tumorigenesis, we combined this treatment with a short-term external radiation of the thyroid using known environmental hazards. It is expected that such a model helps to define the relevant genetic alterations causing thyroid tumor formation in a subsequent step and may thus contribute to the diagnosis of thyroid adenomas.

## Materials and Methods

Ethical approval was given by the regional board in Dessau, Saxony-Anhalt, Germany, as forwarded in our application dated November 11, 1998.

### Animals and housing conditions

Male Sprague Dawley (Han:SPRD) rats with an initial mean body weight of  $80 \pm 5$  g (28 d old) were maintained under pathogen-free conditions in individual cages in a temperature-controlled ( $23 \pm 1$  C, 50–70% relative humidity) and light-controlled (illuminated from 0600–1800 h) room. None of the animals died unexpectedly.

### Dietary iodine intake

Diet started on the 28th d of life, on the first day after separating the offspring from the mother. Three groups of 80 animals, each differing in daily iodine supply, were investigated: group 1, normal iodine supply equivalent to a daily intake of 7000 ng iodine/100 g body weight-d using a standard chow (Altromin 1324, Altromin, Lage, Germany; iodine content, 1 mg/kg; In); group 2, low iodine diet equivalent to 420 ng iodine/100 g body weight-d using a dietary chow (Altromin C 1042; iodine content, 0.06 mg/kg; I-); and group 3, high iodine diet equivalent to 72,000 ng iodine/100 g body weight-d by adding a defined admixture

**TABLE 1.** Time table of experimental design

Age (weeks)	Procedure
0	Date of birth
4	240 28-d-old rats were admitted to our lab; 80 rats each started with a normal, a low or a high iodine diet (3 groups); first measurement of body weight; continued at weekly intervals
5	First measurement of thyroid hormones; continued at weekly intervals
6	On d 40 a single 4-Gy external radiation was performed, with 40 animals belonging to each diet group; the other 40 were sham-irradiated
15	20 animals of each diet group were killed, including 10 radiated and 10 unirradiated rats (killed, 60; alive, 180)
35	20 other animals of each diet group were killed, including 10 radiated and 10 unirradiated rats (killed, 60; alive, 120)
55	20 other animals of each diet group were killed, including 10 radiated and 10 unirradiated rats (killed, 60; alive, 60)
110	20 other animals of each diet group were killed, including 10 radiated and 10 unirradiated rats (killed, 60; alive, 0); end of study

**TABLE 2.** Thyroid weights after 15, 35, 55, and 110 wk under normal nutrition iodine conditions (In), iodine deficiency (I-), and iodine supplement (I+; n = 10) without radiation (A) and after radiation (B)

A) Age (wk)	In (mg)	I-		I+	
		mg	% vs. In	mg	% vs. In
15	25 ± 3	42 ± 2	+68	40 ± 2	+64
35	25 ± 3	56 ± 7	+113	39 ± 2	+62
55	20 ± 3	65 ± 8	+225	37 ± 2	+85
110	20 ± 3	67 ± 3	+235	37 ± 3	+85
<i>P vs. In</i>			<0.001		<0.005

B) Age (wk)	InR		I-R		I+R	
	mg	% vs. In	mg	% vs. I-	mg	% vs. I
15	30 ± 3	+12	35 ± 2	-17	32 ± 2	-20
35	28 ± 2	+8	43 ± 2	-23	35 ± 2	-10
55	22 ± 2	+5	55 ± 2	-15	37 ± 2	±0
110	21 ± 2	+2	60 ± 3	-10	40 ± 3	+8
<i>P value vs. UR</i>		0.71		0.54		0.70

Values are the mean ± SEM. UR, Unradiated.

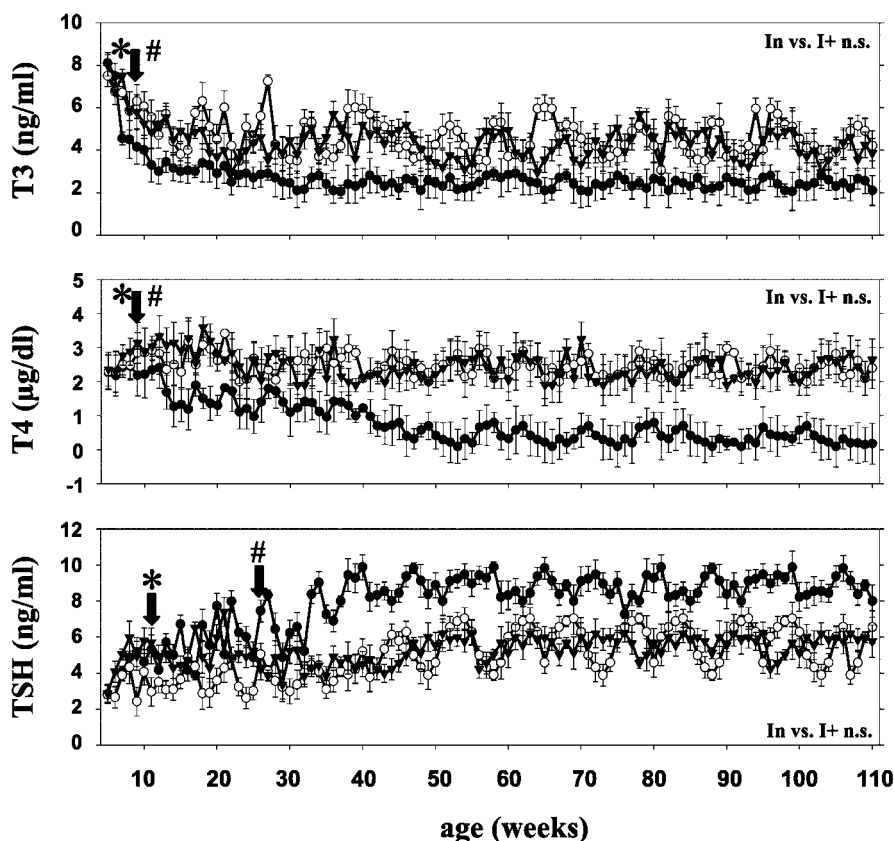


FIG. 1. Development of  $T_3$ ,  $T_4$ , and TSH plasma concentrations in rats under normal iodine conditions (In;  $\circ$ ), iodine deficiency (I $-$ ;  $\bullet$ ), and iodine supplementation (I $+$ ;  $\blacktriangledown$ ;  $n = 10$ ; mean  $\pm$  SEM). Arrow, First point of continuous statistical significant differences: \*, In vs. I $-$ ; #, I $+$  vs. I $-$ ,  $P < 0.05$ . n.s., Nonsignificant.

of potassium iodine to drinking water (I $+$ ). Distilled water was available to all animals *ad libitum* (Table 1).

#### Radiation

On d 40 the animals were anesthetized with pentobarbital (1  $\mu\text{g/g}$  body weight, ip injection). Using the data found in Refs. 7–10, the thyroid region of half of each group was externally irradiated with a single dose of 4 Gy x-rays (InR, I $-$ R, I $+$ R). During this procedure, the rats were protected by a whole body lead shield with a circular opening (diameter, 1.3 cm) over the thyroid gland. Control animals (In, I $-$ , I $+$ ) were sham irradiated. All animals survived this procedure (Table 1).

#### Samples and tissues

Between the 5th and 110th wk of life the animals' body weights were monitored every week, and blood samples were obtained weekly from a retroorbital vein during the morning hours (0800–1000 h). In each of the 6 groups, 10 animals were killed at wk 15, 35, 55, and 110; the thyroid was removed, weighed, and stored for further studies (Table 1).

Thyroid tissue was embedded in Histowax (Leica, Nussloch, Germany) and cut at 5- $\mu\text{m}$  thickness 20 times each. The sections were stained with hematoxylin-eosin for histological examination. Using a morphometrical ocular screen system (Carl Zeiss, Jena, Germany), follicles per square millimeter and the colloid diameter were measured in the central, middle, and peripheral high power fields in all 20 sections of each thyroid gland. To evaluate the area covered by follicles and interstitial tissue, a product of the main follicular diameter and the number of follicles per square millimeter was formed; the interstitial tissue was calculated by subtracting the follicle area from the total area (index of fibrosis). In addition, neoplasias were classified and counted in every section.

#### Proliferation studies

For immunohistochemistry, a monoclonal mouse antibody to Ki-67 (clone MIB-5, 1:30 dilution; Dianova, Hamburg, Germany) and antigen

retrieval using microwave heating (three times for 5 min each time) were used after inhibition of endogenous peroxidase activity. The primary antibody was incubated for 1 h at 37 C. The slides were subsequently incubated with a 1:10 dilution of normal swine serum (Vector Laboratories, Inc., Burlingame, CA). After washing in PBS (pH 7.4), the samples were incubated with a 1:200 dilution of biotinylated antigoat secondary antibody (Vector Laboratories, Inc.) for 30 min at room temperature. The detection of bound antibody was accomplished using the avidin-biotin complex method (Dianova UniTect A.B.C. System XHC1). A 0.1% solution of 3,3'-diaminobenzidine (5 min) was used as a chromogen. The specificity of the immunostaining was checked by omitting single steps in the immunochemical protocol and replacing the primary antibody with nonimmune serum. Activated rat lymph nodes served as an external positive control for MIB-5. The number of MIB-5-positive cells was counted and indicated as a percentage of cells.

#### Hormone determinations

Plasma levels of the TSH were measured as previously described using TSH RIA materials provided by Dr. Parlow, NIDDK (30). The lower limit of sensitivity was 360 pg TSH/ml. Depending on the concentrations used, the intra- and interassay coefficients of variation ranged from 3.7–7.1% and 11.7–12.2%, respectively.

$T_3$  and  $T_4$  were measured with commercial kits for use in human serum (enzyme immunoassay, Roche Molecular Biochemicals, Mannheim, Germany) with maximal intra- and interassay coefficients of variation of 7.4%. The usage of these assays and the compatibility in rats have previously been described in detail by Hoang-Vu *et al.* (22) and Stubner *et al.* (31).

#### Statistics

Hormonal measurements were made each week and over the whole experimental period by repeated measurement variance analysis (F test). We coupled the variance analysis with a *post hoc* test. The differences in histomorphological data were tested for statistical significance using the

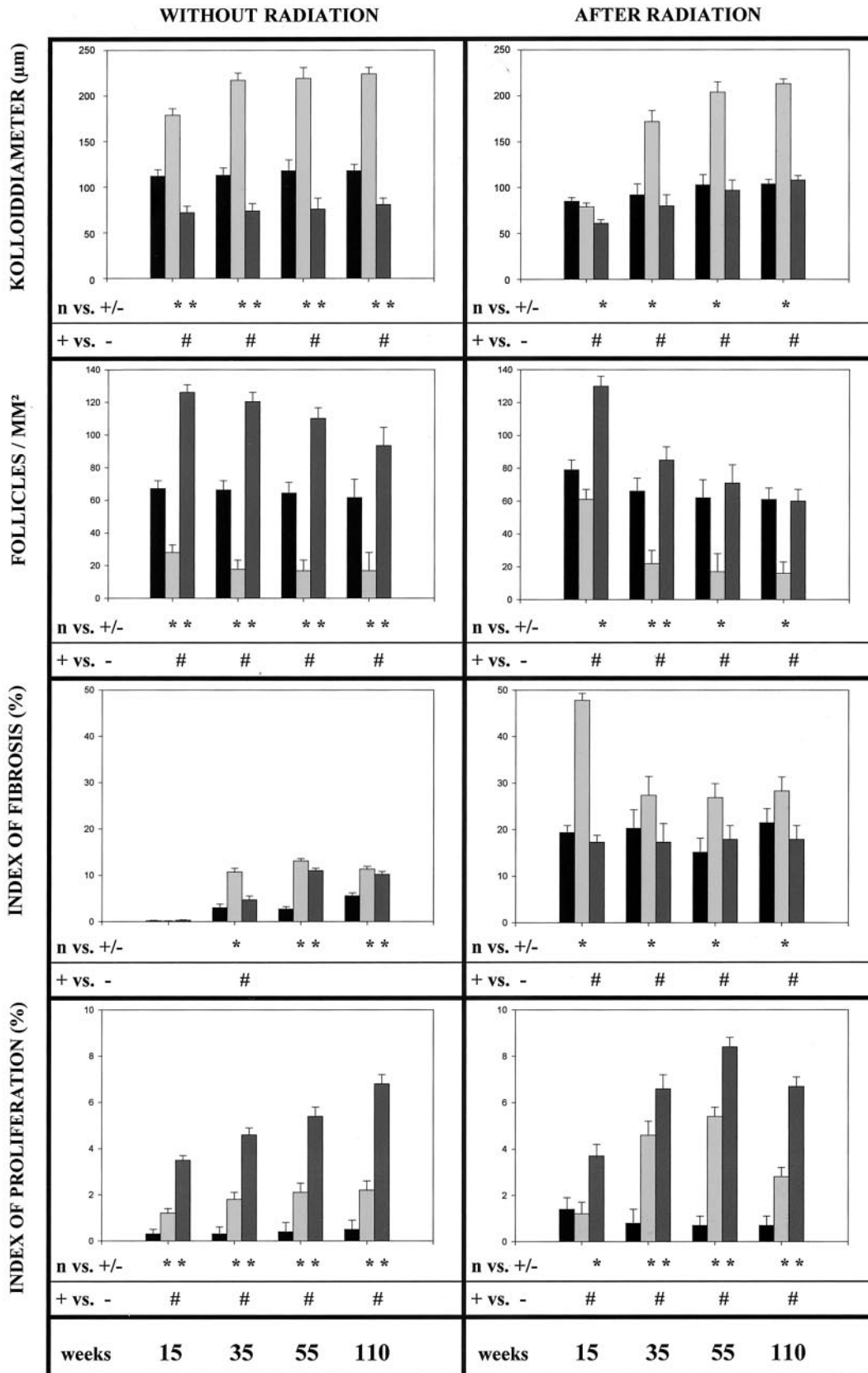


FIG. 2. Histological data of the thyroids after 15, 35, 55, and 110 wk under normal iodine conditions (In; ■), iodine deficiency (I-; □), and iodine supplement (I+; ▒) with or without irradiation (n = 10; mean ± SEM). \*, In vs. I- or I+,  $P < 0.05$ ; #, statistical significance I+ vs. I-,  $P < 0.05$ .



proportional *t* test.  $P < 0.05$  was considered statistically significant. All data are given as the mean  $\pm$  SEM.

## Results

### Iodine-dependent changes without radiation

Iodine deficiency led to lower daily growth rates and a significantly lower final mean body weight of  $429.9 \pm 16$  g (I<sup>-</sup>;  $P < 0.005$ ) vs.  $500.6 \pm 13$  g (In) vs.  $474.9 \pm 14$  g (I<sup>+</sup>;  $P < 0.01$ ). The growth process was finished after 18 wk in I<sup>+</sup> and after 21 wk in In and I<sup>-</sup>.

As expected, thyroid weight was higher with the low iodine diet than with the normal iodine supply, but was also increased with the high iodine diet (Table 2A).

As shown in Fig. 1, long-term iodine deficiency significantly decreased plasma T<sub>3</sub> and T<sub>4</sub> concentrations after wk 9. During the previous period (between the fifth and eight week), thyroid hormone levels were slightly decreased: T<sub>3</sub>, 6.12 ng/ml (I<sup>-</sup>;  $P = 0.78$ ) vs. 6.68 ng/ml (In); T<sub>4</sub>, 2.39  $\mu$ g/dl (I<sup>-</sup>;  $P = 0.98$ ) vs. 2.42  $\mu$ g/dl (In). TSH increased significantly after wk 11, whereas the increase was low at wk 5–10: 3.87 ng/ml (I<sup>-</sup>;  $P = 0.66$ ) vs. 3.34 ng/ml (In; Fig. 1). In contrast, the high iodine diet did not change thyroid function during the experimental period: T<sub>3</sub>, 4.58 ng/ml (I<sup>+</sup>;  $P = 0.71$ ) vs. 4.91 ng/ml (In); T<sub>4</sub>, 2.56  $\mu$ g/dl (I<sup>+</sup> and In equally); TSH, 4.96 ng/ml (I<sup>+</sup>;  $P = 0.58$ ) vs. 4.19 ng/ml (In).

All changes manifested themselves in alterations in thyroid morphology. Iodine deficiency was associated with significantly large, but fewer, follicles, whereas the high iodine

diet significantly decreased the diameter, but increased the number, of follicles (Figs. 2 and 3, A, C, and E).

The mitotic activity of thyrocytes was very low under normal iodine intake conditions ( $<1 \pm 0.2\%$ ). Not only iodine deficiency, but also higher iodine intake, significantly increased proliferation rates (Fig. 2). At wk 55 and 110, all nonirradiated animal groups were free of malignant tumors, and benign tumors were not detected until wk 55 (Table 3 and Fig. 3, B, D, and E).

### Iodine-dependent changes after radiation

Figure 4 shows the changes in thyroid hormone and TSH levels during the experimental period (between the 5th and 110th wk); there were increases in T<sub>3</sub> [3.41 ng/ml (I<sup>-</sup>R;  $P = 0.56$ ) vs. 2.97 ng/ml (I<sup>-</sup>)] and T<sub>4</sub> [1.80  $\mu$ g/dl (I<sup>-</sup>R;  $P < 0.05$ ) vs. 1.16  $\mu$ g/dl (I<sup>-</sup>)] and a significant decrease in TSH [4.92 ng/ml (I<sup>-</sup>R;  $P < 0.05$ ) vs. 7.23 ng/ml (I<sup>-</sup>)] in the group with iodine deficiency after radiation. The hormone concentrations of the normal iodine and high iodine groups were not significantly altered. In all groups, thyroid weight was not significantly influenced by radiation (Table 2B).

Figures 2 and 5 show the alterations in proliferation, follicular structure, and the ratio of follicles to the interstitium induced by radiation treatment. The most important finding in irradiated low iodine diet thyroids was the total destruction of follicles observed at wk 15 after radiation (Fig. 5C). After this destruction, a short-term increase in T<sub>3</sub> (7th wk)

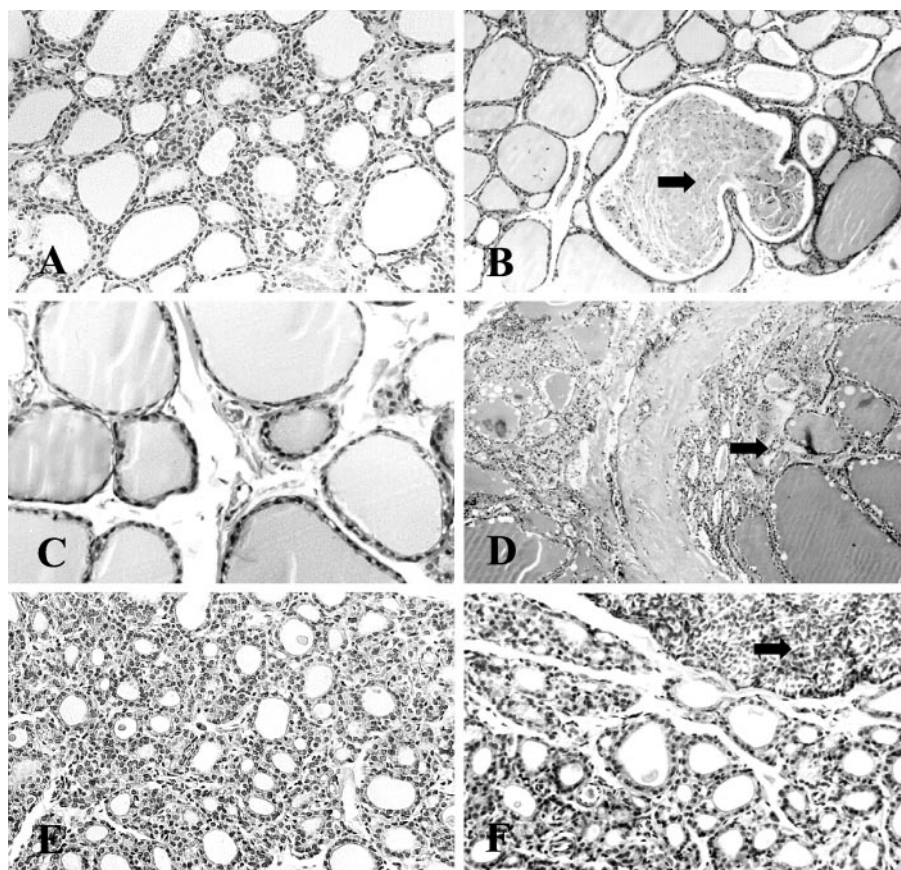


FIG. 3. Histological examples of rat thyroids after 55 wk of normal nutrition iodine conditions (A and B), iodine deficiency (C and D), and iodine supplement (E and F) without radiation (magnification,  $\times 100$ ). A, Colloid-filled follicles with normal dimensions. Rare fibrosis. B, Dermoid cyst (arrow). C, Extended follicle diameters. Increased interstitial tissue. D, Follicular adenoma (arrow). E, Decreased follicle diameters and increased follicle number. Increased interstitial tissue. F, Hyperplastic parathyroid gland (arrow).

**TABLE 3.** Number of benign and malignant thyroid tumors after and without nutrition pretreatment and radiation (n = 10)

Group and tumors	55th wk	110th wk
Normal iodine		
In		
BT	2 dermoid cysts	3 dermoid cysts
TC	0	0
OM	0	0
InR		
BT	7 adenomas	8 adenomas
TC	0	0
OM	0	1 squamous cell carcinoma
Iodine deficiency		
I–		
BT	5 adenomas	9 adenomas
TC	0	0
OM	0	0
I–R		
BT	7 adenomas	9 adenomas
TC	0	2 FTC/3 PTC
OM	1 adenocarcinoma	1 adenocarcinoma
Iodine supplementation		
I+		
BT	5 adenomas	9 adenomas
TC	0	0
OM	0	0
I+R		
BT	9 adenomas	10 adenomas
TC	0	3 FTC/5 PTC
OM	0	1 squamous cell carcinoma

BT, Benign thyroid tumors; PTC, papillary thyroid carcinoma; TC, thyroid carcinoma; FTC, follicular thyroid carcinoma. Parathyroid carcinomas (OM) were also induced: squamous cell carcinomas of the cervical soft tissue and adenocarcinomas of salivary glands.

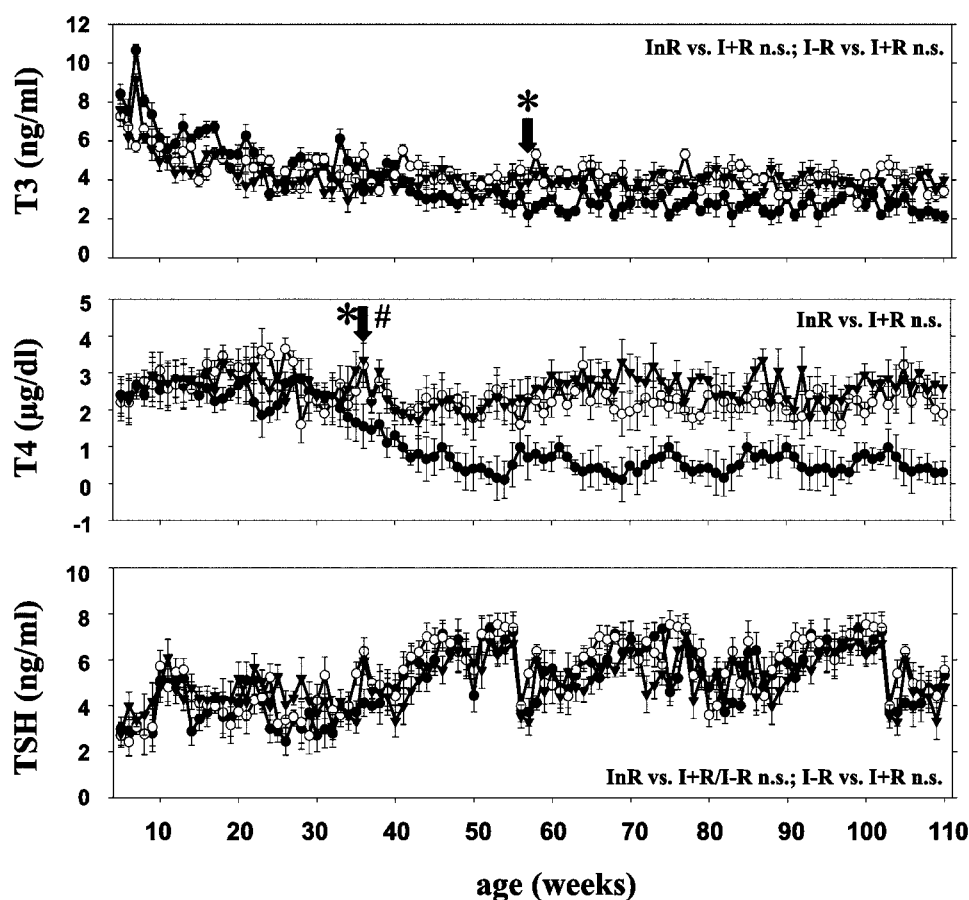


FIG. 4. Development of  $T_3$ ,  $T_4$ , and TSH plasma concentrations in rats after radiation with 4 Gy under normal nutrition iodine conditions (InR; ○), iodine deficiency (I–R; ●), and iodine supplementation (I+R; ▼; n = 10; mean  $\pm$  SEM). Arrow, First point of continuous statistical significant differences: \*, InR vs. I–R; #, I+R vs. I–R,  $P < 0.05$ . n.s., Nonsignificant.



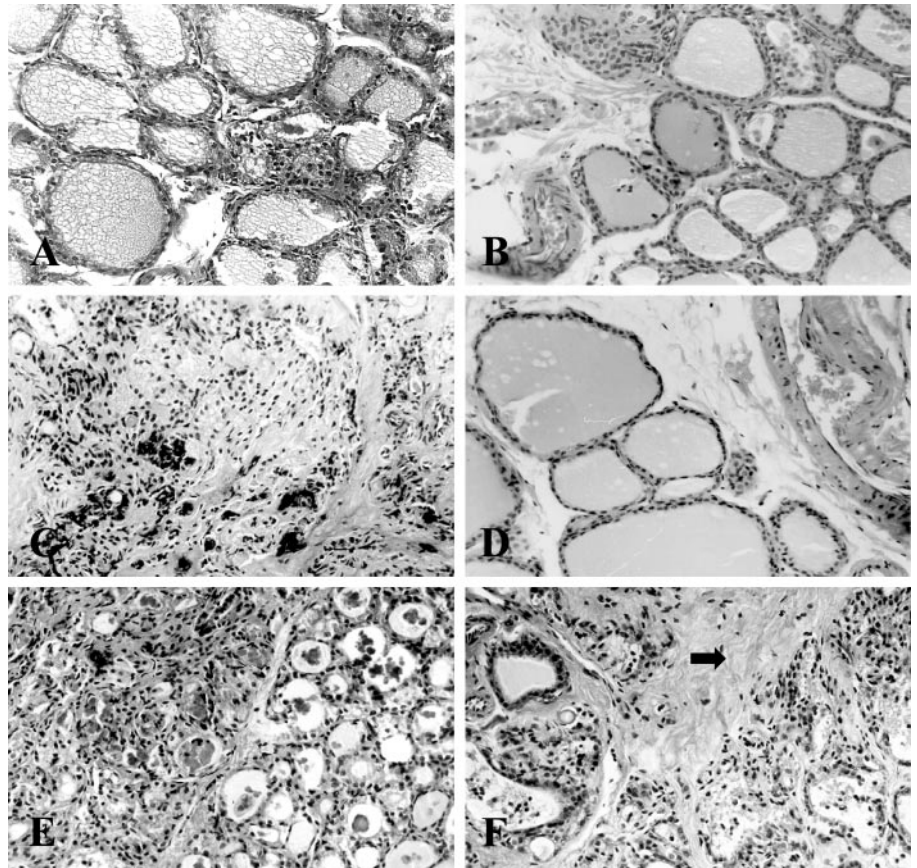


FIG. 5. Histological examples of thyroids of under normal nutrition iodine conditions (A and B), iodine deficiency (C and D), and iodine supplementation (E and F) after 4 Gy radiation after 15 (A, C, and E) and 55 wk (B, D, and F; magnification,  $\times 100$ ). A, Colloid-filled follicles with normal dimensions. Rare fibrosis. B, Increase in fibrosis, but continuity of other histomorphological parameters. C, Destruction of follicle structure and decrease in follicle number. D, Removed follicle structure. Extended follicle diameters. Increased interstitial tissue. E, Decreased follicle diameters and increased follicle number. F, Increased interstitial tissue. Breadth fibrotic septae (arrow).

was measured (Fig. 4). After the 55th wk, a complete restitution of the follicle structure was observed (Fig. 5D).

In contrast to the sole manipulation of iodine intake, radiation treatment led to a higher number of benign tumors, starting 55 wk after having changed the nutritional iodine supply (Fig. 6A), and to malignant tumors after 110 wk (Table 3 and Fig. 6, D–F). Parathyroid carcinomas were also induced: squamous cell carcinomas of the cervical soft tissue and adenocarcinomas of the salivary glands (Table 3 and Fig. 6, B and C). The thyroid carcinomas were solitary tumors; their size ranged between 0.1 and 1.5 mm. Neither local lymph node metastasis nor distant metastasis was found.

### Discussion

In this long-term study we investigated the influence of manipulations caused by dietary iodine supply with and without external radiation on thyroid function, proliferation, and differentiation and thyroid tumorigenesis.

Between the 1950s and 1970s, the morphological effects of radiation on the thyroid gland were systematically studied in various animal models (9–12), but long-term data are lacking. Most of these studies were performed under conditions of extreme iodine deficiency enhanced by the application of goitrogens or under conditions of excessive iodine dosage. In this experiment we investigated the conditions closely related to iodine supply in iodine deficiency or excess areas (27–29, 32).

Iodine deficiency induced by a long-term iodine intake of approximately 10% of the normal dietary supply resulted in

a significant drop in  $T_3$  and  $T_4$  plasma levels after 9 wk. Plasma TSH levels significantly increased after 11 wk, but consistently doubled later, *i.e.* after approximately 32 wk, implicating a borderline adaptive capacity of the thyroid. These changes were combined with the expected development of follicular hypertrophy and subsequently with follicular epithelial cell hyperplasia and organ hypertrophy, starting after 15 wk and reaching a maximum after 110 wk. The proliferation rate was doubled between the 15th and 110th wk.

Apart from a slight decrease in serum  $T_3$  levels, iodine supplementation to approximately 16 times the normal concentration did not alter thyroid function. However, follicular growth changed dramatically, leading to hyperplasia of follicular cells, organ hypertrophy, and a massively increased proliferation rate. Interestingly, the high proliferation rates did not induce any malignant changes. The risk that a benign thyroid tumor develops after iodine deficiency or excess was 2- to 3-fold higher than in the controls. This suggests that under our experimental conditions both long-term iodine deficiency and excess are insufficient to stimulate carcinogenesis, whereas benign tumors, detectable in approximately 90% after 110 wk of treatment, are clearly more frequent than in the control group with normal iodine supply. It seems that previous studies contradict our data (33, 34). In those and other experiments, thyroid carcinomas were reported to have arisen after low or high iodine diets. Our experiments were performed under conditions of milder iodine deficiency and iodine supplement, using very young rats. This may explain

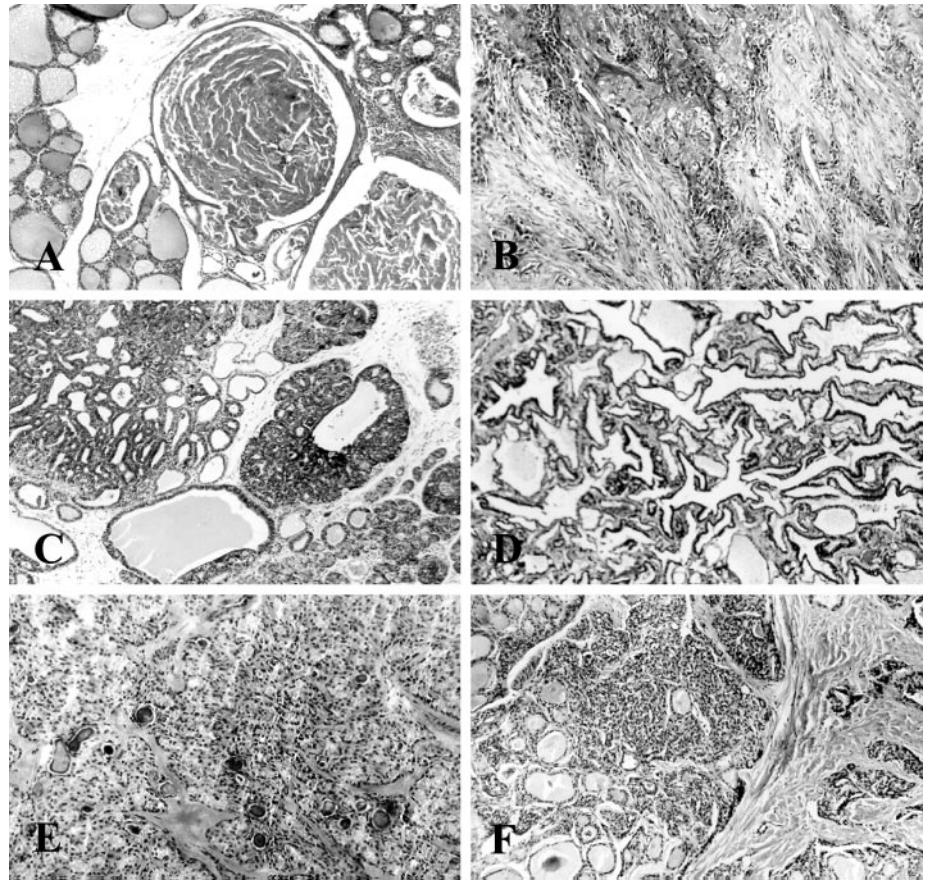


FIG. 6. Histological examples of tumors induced after 4 Gy radiation of the thyroid region under normal nutrition iodine conditions (A and B), iodine deficiency (C and D), and iodine supplementation (E and F; magnification,  $\times 100$ ). A, Metaplasia of squamous epithelium with dermoid cysts (55 wk). B, Squamous cell carcinoma of the cervical soft tissue beside the thyroid gland (55 wk). C, Adenocarcinoma of a salivary gland (glandula submandibularis; 55 wk). D, Follicular thyroid carcinoma with a bizarre pattern (110 wk). E, Follicular thyroid carcinoma, small follicular/insular type (110 wk). F, Papillary thyroid carcinoma, predominant follicular pattern, sporadic papillae (110 wk).

why carcinomas did not develop. Circulating plasma TSH concentrations do not appear to be a determining factor for adenoma formation, because no difference was found between increased TSH plasma levels in iodine-deficient animals and normal TSH associated with high iodine intake.

In contrast, 50% or 80% of animals receiving a single shot of radiation on d 40 developed thyroid carcinomas when living under conditions of iodine deficiency or excess, respectively, whereas normal iodine intake prevented any radiation-induced carcinogenesis.

In the last few years the molecular basis of thyroid cancer has been widely studied. *RAS* oncogene activation in various thyroid tumors has frequently been reported and has been related to radiation exposure (35, 36). On the other hand, a low frequency of *RAS* alterations was found in both benign and malignant tumors from children exposed to radiation after the Chernobyl nuclear accident (37). This may be due to the special characteristics of these childhood neoplasms or, probably more importantly, to the papillary histological pattern, as *RAS* mutations have been linked to FTC (38). In PTC, rearrangements involving the *RET* protooncogene represent the most common somatic changes. At least eight types of *RET* rearrangements (inversions and translocations, named *RET/PTC1–8*) have been described to date (5). It has been shown that irradiation is able to induce these rearrangements, perhaps because of the proximity of chromosomal loci participating in the rearrangement process (39). Under our experimental conditions, radiation induced the development of FTC (five cases) and PTC (eight cases). To find these

neoplasias and to measure tumor size, it was absolutely necessary to completely investigate the thyroids histologically. Therefore, we failed to discover any alteration of the known key target genes in the thyroid tissue. This study aimed at creating a basic animal model of thyroid carcinogenesis with an intensive and detailed description of changes in thyroid morphology and functions. Further investigations based on this model will certainly find the molecular mechanisms involved.

Our model clearly supports the very long latency observed in thyroid carcinogenesis between the mutational event and the development of malignant changes. This contradicts previous studies using a higher stimulation of thyrocyte proliferation by iodine deficiency, where malignancies were detected after much shorter time intervals (33). Large doses of iodine may induce thyroid carcinomas (34). Here we show that mild iodine excess is not necessarily associated with the formation of thyroid malignant neoplasms, but when combined with a mutagen, carcinomas arise with high frequency. These data on mild forms of high iodine intake thus put a note of caution to a long-term use of high iodine.

This model raised the question of whether there exists a cancer protection effect of hormone substitution after radiation exposure. In our opinion, the proliferation rate of follicular epithelium cells before exposure of environmental factors is the main factor responsible for development of thyroid carcinoma. Iodine deficiency or excess led to a 5- to 30-fold increase in the number of proliferating cells. We believe that these activated cells accumulate a higher number



of genetic alterations after radiation. We found carcinomas after radiation in these two groups, but not in controls with normal iodine intake. Supplementation of thyroid hormones after radiation may not be helpful, because the normal iodine group and the iodine-supplemented group had the same hormone levels after radiation, but carcinomas were found in only I+R. Substitution therapy with potassium iodide possibly constitutes a more favorable approach after inhalation of radioactive iodine, as shown in a Polish field experience after the Chernobyl accident (40), whereas after external radiation, the same effect is more questionable. Our experience has shown that euthyrosis is the best protection against thyroid cancer before environmental hazards are effective.

The well defined setting in our experiments clearly demonstrates that mutational lesions acquired by radiation are clinically silent over a long period of time. It is tempting to use such a model to search for candidate genes altered by mutagens, but which are not changed in thyroid adenomas found under control conditions. The definition of such changes may then have important implications for the characterization of the malignant potential of a given adenoma well before cytological or histological changes occur.

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