Hypertrophy and hyperplasia during goitre growth and involution in rats – separate bioeffects of TSH and iodine

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Abstract. Goitre growth was investigated in rats receiving a low iodine diet (&lt; 0.1 μg iodine/g) and either 1 g/l KClO4 or 1 g/l propylthiouracil (PTU), or a combination of KClO4 or PTU with 50.82 nmol/l T3 in tap water for 3 weeks. To investigate goitre involution, rats with iodine-deficient goitres were treated for 3 weeks either with T3 (0.5 μg T3/day = 0.768 nmol/day), iodide (0.5 or 2.7 μg KI/day) or a combination of T3 with both iodide doses. Histology together with total DNA distinguished between hypertrophy and hyperplasia of the gland. During goitre growth there was a highly significant correlation between goitre weight and TSH serum level (r = 0.93, P &lt; 0.001). Thyroid total DNA, however, was only weakly correlated to TSH but was inversely related to the degree of iodine deficiency. During goitre regression, TSH levels were normalized, histological signs of hypertrophy had disappeared, and thyroid weight was nearly normalized in all therapy groups. Total DNA, however, was normalized only with 2.7 μg KI/day (95 ± 18 μg DNA/gland), and still elevated in all other groups. The highest DNA levels were found under T3 therapy (143 ± 21 μg DNA/gland) and under 0.5 μg KI/day (161 ± 19 μg DNA/gland). Reduction of total DNA was independent of TSH, but followed replenishment of the iodine content of the glands. We conclude that TSH mainly induces hypertrophy, whereas thyroid hyperplasia is mainly regulated by the intracellular iodine content.

It is well accepted that iodine deficiency is the cause of endemic goitre. From in vivo studies it has been concluded that TSH regulates goitre size not only by increasing cell volume, but also by stimulating thyroid cell proliferation (Studer & Gebel 1986; Wynford-Thomas et al. 1982a; Denef et al. 1980).

There are, however, controversial data about the role of TSH as a growth factor for thyroid cells. In vitro, a stimulatory effect of TSH on thyroid cell growth has been found (Roger & Dumont 1984), as well as no or even an inhibitory effect of TSH on cell proliferation (Westmark et al. 1979; Eggo et al. 1984; Gärtner et al. 1985). Growth factors like EGF (epidermal growth factor) have consistently been found to stimulate thyroid cell growth in vitro (Roger & Dumont 1984; Eggo et al. 1984; Gärtner et al. 1985). This growth promoting effect of EGF on thyroid follicles was shown to be regulated by iodide (Gärterner et al. 1985).

The conflicting in vitro data encouraged us to re-investigate the role of TSH and iodine in goitre genesis and therapy. Goitre growth in rats was studied under different goitrogens with or with-
out T₃ substitution to modify the effect of TSH on goitre growth. Goitre involution was investigated during different iodide substitutions, T₃ therapy or a combination of T₃ with iodide. To differentiate between hypertrophy and hyperplasia, histological evaluation together with determination of total DNA of the thyroid gland was performed.

From our data we conclude that the iodine content of the thyroid cells is by itself the major autoregulator of cell proliferation in iodine-depleted thyroid glands and the important factor for reduction of cell number during goitre treatment. TSH, however, stimulates only hypertrophy of thyroid cells by functional activation, and affects cell number only via further iodine depletion.

**Materials and Methods**

**Animals**

The animals used in the study were young Sprague Dawley rats weighing between 100 and 120 g. The experimental groups consisted of 5 males and 5 females, housed in separate cages. In the therapy group only females in groups of 5 to 10 animals were used.

**Goitre genesis**

In the goitre genesis group all rats were fed a moderately low iodine diet (LID C1042, Altromin, Lage, FRG; < 0.1 µg iodine/g). In addition they received either normal drinking water or drinking water with 1 g/l (= 7.22 mol/l) KClO₄, 1 g/l (= 5.87 mol/l) propylthiouracil (PTU), or KClO₄ or PTU in combination with 33 µg/l (= 50.82 nmol/l) T₃ for 3 weeks. Control animals had access to water without additives and received a normal diet (NID 1324, Altromin; 0.9 µg iodine/g).

**Goitre therapy**

Iodine-deficient goitres, weighing approximately twice as much as control thyroids, were induced in female rats during 6 weeks, by feeding 2 weeks only LID, 3 weeks LID in combination with 1 g/l KClO₄ in tap water (= 13 mg ClO₄⁻/day), and again 1 week LID alone. For treatment either T₃ (0.5 µg/day = 768 pmol/day), iodide (0.5 or 2.7 µg KI/day) or a combination of T₃ with both iodide doses was given in tap water for 3 weeks under continued LID.

At the end of the experiment, the animals were anaesthetized with CO₂ and decapitated. Thyroid glands were prepared, weighed and frozen at -80°C until further processing.

**Analytical methods**

**Serum.** Serum T₃ and T₄ levels in rat sera were measured by double antibody radioimmunoassay as described previously, using a protein independent technique (Gärtnert et al. 1980a).

TSH was measured by radioimmunoassay using the reagents supplied by the NIADDK Rat Pituitary Hormone Distribution Program. Data are expressed as mg rat TSH per 1 referring the results obtained with the NIADDK Rat TSH Reference Preparation 2 to the Reference Preparation 1 by multiplication with 176.

**Thyroid.** The thyroid glands were homogenized with glass potters in 600 µl 0.05 mol/l sodium-phosphate buffer, pH 7.4 with 0.15 mol/l NaCl, 0.003 mol/l NaN₃, and 0.001 mol/l phenylmethyl-sulfonyl-fluoride, to block proteolytic activity.

DNA was measured by a modification of the method of Wollman & Breitman (1970). In brief, DNA and RNA of homogenized tissue were precipitated with 0.3 mol/l trichloroacetic acid (TCA), washed once with 0.5 mol 0.03 mol/l TCA, and solubilized in 1N NaOH overnight at 37°C. After cleavage of DNA with 1N HClO₄ at 70°C for 15 min, deoxyribosides were estimated with the diphenylamine reagent at 600 nm according to Burton (1956) with calf thymus DNA (Boehringer Mannheim, FRG) as standard. Thyroglobulin up to 10 µg/tube did not influence the determination of DNA.

Iodine was measured in the supernatant of the homogenized, centrifuged tissue by a modification of the Ger-Arsenit-method according to Sandell & Kolthoff (1957) using a Technicon Autoanalyzer. The lower limit of detection was 0.03 µg iodine per gram.

Thyroglobulin (Tg) was determined in the supernatant, using a double antibody radioimmunoassay, developed for rat Tg, corresponding to the method described for human Tg (Gärtnert et al. 1980b).

**Histology**

Two thyroids from each group were fixed in 40 g/l buffered formalin, embedded in paraffin, cut into 4-µm sections and stained with haematoxylin-eosin for light microscopy. In the goitre therapy groups, cellular and nuclear areas (at magnification ×1640) were determined with an image analyzing system (Videoplan, Kontron Electronic, Munich, FRG). Four hundred cells and nuclei on two representative histological sections from one animal of each group were measured.

**Data presentation and statistics**

Data are presented either as means ± SD or as means with range. Significant differences between the groups were evaluated by the unpaired Wilcoxon test. Differences between distributions of cellular and nuclear areas were tested with the Kolmogorov-Smirnov two-sample test. Correlations were tested by Fisher’s t-test.

Because of numerous significant sex differences, males and females were looked at separately, except in correlations.
Results

Goitre genesis

Effects of different goitrogens. LID alone, as well as KCIO4 together with LID, did not change body weight or activity of the animals compared with control rats on NID. Rats on PTU and LID, however, gained less body weight than controls (PTU: males 157 ± 14 g, females 132 ± 7 g; control: males 234 ± 18 g, females 183 ± 13 g; P < 0.01 for males and females). T3 serum levels in the PTU group (0.443 ± 0.081 nmol/l) were similar to those in the KCIO4 group (0.520 ± 0.05 nmol/l). The T4 levels in both groups were below the lower limit of detection (<5 nmol/l).

Compared with control rats, thyroid weight was significantly elevated with each of the goitrogens used (Table 1). Under LID, however, the DNA content of the glands remained normal. Administration of KCIO4 or PTU together with LID led to a significant increase in thyroid DNA. Under PTU, DNA was on a 99% level significantly higher (236 ± 46 µg DNA/gland) than under KCIO4 (157 ± 23 µg DNA/gland). The TSH levels in these groups were identical. The iodine content of the glands decreased with increasing thyroid weights and DNA contents (Table 1). The thyroglobulin content of the glands roughly followed the changes in iodine content.

Total DNA did not increase to the same extent as thyroid weight: compared with control rats, mean thyroid weight increased up to 126% for LID, 302% for KCIO4 and 716% for PTU rats; mean total DNA content increased up to 102% for LID, 181% for KCIO4 and 272% for PTU rats (males and females taken together).

Addition of T3 to goitrogens. The T3 supply was not sufficient to suppress TSH below the lower limit of detection. TSH levels ranged from 0.06 (lower limit of detection) to 4.43 mg/l (median 1.21 mg/l) in the KCIO4/T3 group and were from 0.16 to 7.46 mg/l (median 3.53 mg/l) in the PTU/T3 group.

T4 levels of all KCIO4/T3 and PTU/T3 treated animals were below the lower limit of detection (<5 nmol/l). T3 levels in the combination group KCIO4/T3 were between 0.753 and 2.468 nmol/l and in the rats with PTU/T3 between 0.609 and 1.671 nmol/l (controls: 1.812 ± 0.252 nmol/l).

Table 1.

Goitre genesis: Effects of different goitrogens. Rats in groups of 5 animals were fed different goitrogens for 3 weeks: a low iodine diet (LID), LID with 1 g/l KCIO4 or LID with 1 g/l PTU in tap water. Controls received a normal diet (NID). Thyroid weight and total DNA content, iodine and thyroglobulin content of the thyroids, as well as TSH serum levels are listed separately for males and females. Animals given PTU and KCIO4 (males and females taken together) showed significantly different weights (P < 0.001) and DNA content of their thyroids (P < 0.01), as well as significantly different iodine (P < 0.001) and thyroglobulin content (P < 0.05) of the glands, but identical TSH levels.

<table>
<thead>
<tr>
<th></th>
<th>Thyroid weight mg/100g body weight</th>
<th>TSH mg/l</th>
<th>DNA µg/gland</th>
<th>Iodine ng/mg thyroid weight</th>
<th>Tg µg/mg thyroid weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.9 ± 0.7</td>
<td>1.00 ± 0.16</td>
<td>84.0 ± 10.7</td>
<td>717 ± 79</td>
<td>81.3 ± 16.4</td>
</tr>
<tr>
<td>LID</td>
<td>7.2 ± 0.6*</td>
<td>0.56 ± 0.11**</td>
<td>92.3 ± 6.7 n.s</td>
<td>184 ± 30*</td>
<td>42.3 ± 4.6*</td>
</tr>
<tr>
<td>LID/KCIO4</td>
<td>18.4 ± 1.2**</td>
<td>5.98 ± 0.36**</td>
<td>173.3 ± 19.8*</td>
<td>0.95 ± 0.22*</td>
<td>4.7 ± 0.3*</td>
</tr>
<tr>
<td>LID/PTU</td>
<td>46.5 ± 2.2**</td>
<td>5.77 ± 0.36**</td>
<td>204.8 ± 27.7*</td>
<td>0.43 ± 0.00*</td>
<td>3.8 ± 0.5*</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.4 ± 0.8</td>
<td>0.58 ± 0.15</td>
<td>89.0 ± 4.0</td>
<td>765 ± 124</td>
<td>91.3 ± 20.4</td>
</tr>
<tr>
<td>LID</td>
<td>9.6 ± 1.5*</td>
<td>0.58 ± 0.26 n.s.</td>
<td>83.3 ± 6.7 n.s</td>
<td>141 ± 12*</td>
<td>36.4 ± 1.2*</td>
</tr>
<tr>
<td>LID/KCIO4</td>
<td>21.9 ± 0.7**</td>
<td>5.25 ± 0.41**</td>
<td>140.3 ± 13.1*</td>
<td>0.97 ± 0.12*</td>
<td>4.3 ± 0.2*</td>
</tr>
<tr>
<td>LID/PTU</td>
<td>49.1 ± 5.8**</td>
<td>5.83 ± 0.47**</td>
<td>266.3 ± 41.8*</td>
<td>0.43 ± 0.00*</td>
<td>3.6 ± 1.0*</td>
</tr>
</tbody>
</table>

Results are given as mean ± sd. * P < 0.05, ** P < 0.01, n.s: not significant compared with controls.
Correlation of TSH serum levels to thyroid weight during goitre genesis (males and females). Rats were given different goitrogens for 3 weeks: a low iodine diet (+), LID with 1 g/l KCIO₄ (■), LID with a combination of KCIO₄ and 50.82 nmol/l T₃ (▲), LID with 1 g/l PTU (□) or LID with a combination of PTU and 50.82 nmol/l T₃ (Δ) in tap water. Controls (×) received a normal diet. Dashed lines give the interval of two standard deviations from the mean for TSH levels of control animals (x = 0.79 ± 0.54 mg/l) and the upper normal limit for thyroid weight (two SD from the mean of controls: x = 6.7 + 2.0 mg/100 g body weight).

**Fig. 1a.**

Correlation of TSH serum levels to DNA content of the glands during goitre genesis (males and females). Dashed lines give the interval of two standard deviations from the mean for TSH levels of control animals (x = 0.79 ± 0.54 mg/l) and the upper normal limit for thyroid DNA content (two SD from the mean of controls: x = 86.3 + 15.6 µg DNA/gland).

**Fig. 1b.**

**TSH and thyroid weight.** The correlation coefficient between TSH and thyroid weight for 38 animals on PTU was calculated as 0.93 (P < 0.001; regression Y = 5.6 + 7.3 X) and for 40 animals on KCIO₄ as 0.92 (P < 0.001; regression Y = 5.2 + 2.7 X). Thus, both groups showed an excellent correlation between TSH and thyroid weight (Fig. 1a), however, the regression line had a greater slope for the PTU animals than for the KCIO₄ animals. This additional PTU effect is independent of the TSH level.

**TSH and DNA content.** When we compared serum TSH with the thyroidal DNA, a large scatter of values was found (Fig. 1b). The correlation coefficient calculated for the PTU group was 0.76 (P < 0.001, N = 27) and for the KCIO₄ group 0.55 (P < 0.01, N = 32). Animals on PTU tend to have a higher DNA content than rats on KCIO₄ with comparable TSH levels. A normal thyroid DNA content was only found in animals with TSH serum levels below 1.5 mg/l. However, also elevated DNA with TSH levels below this value was found (Fig. 1b).

**Fig. 1c.**

Correlation of iodine content to DNA content of the thyroid during goitre genesis (males and females). Dashed lines give the lower normal limit for thyroid iodine content (two SD from the mean of controls: x = 0.741–0.20 µg iodine/mg thyroid weight) and the upper normal limit for thyroid DNA content (two SD from the mean of controls: x = 86.3 + 15.6 µg DNA/gland).

Note the logarithmic scale for the iodine content.
Fig. 2.
Goitre genesis. Rat thyroids (4 μm thick sections; haematoxylin-eosin staining; × 1280). a) Control thyroid. b) Goitre after 3 weeks of LID treatment. c) Goitre after 3 weeks of LID plus PTU treatment. d) Goitre after 3 weeks of LID plus KClO₄ treatment.
Goitre therapy: Female goitrous rats on LID were treated for 3 weeks with either T₃ (0.5 µg T₃/day), iodine (0.5 or 2.7 µg KI/day) or a combination of T₃ with both iodine doses. Control animals received either NID or LID. Thyroid weight and total DNA content, iodine and thyroglobulin content of the glands, as well as TSH levels are shown.

<table>
<thead>
<tr>
<th>Females</th>
<th>Thyroid weight mg/100g body weight</th>
<th>TSH µg/l</th>
<th>DNA µg/gland</th>
<th>Iodine ng/mg thyroid weight</th>
<th>Tg µg/mg thyroid weight</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.4 ± 0.8</td>
<td>0.58 ± 0.15</td>
<td>89.0 ± 4.0</td>
<td>765 ± 124</td>
<td>91.3 ± 20.4</td>
</tr>
<tr>
<td>Goitre</td>
<td>16.8 ± 2.2**</td>
<td>1.00 ± 0.22*</td>
<td>191.0 ± 30.0*</td>
<td>13 ± 1*</td>
<td>6.8 ± 0.8*</td>
</tr>
<tr>
<td>LID</td>
<td>12.8 ± 1.3**</td>
<td>0.82 ± 0.28ns*</td>
<td>148.5 ± 11.3**</td>
<td>61 ± 6**</td>
<td>19.3 ± 2.2**</td>
</tr>
<tr>
<td>T₃</td>
<td>8.1 ± 1.6ns*</td>
<td>0.51 ± 0.24ns*</td>
<td>143.0 ± 21.0**</td>
<td>133 ± 14**</td>
<td>33.9 ± 3.5**</td>
</tr>
<tr>
<td>KI (0.5 µg)</td>
<td>9.0 ± 1.1*</td>
<td>0.31 ± 0.08*</td>
<td>161.0 ± 19.0*</td>
<td>131 ± 25*</td>
<td>28.9 ± 5.0*</td>
</tr>
<tr>
<td>KI (2.7 µg)</td>
<td>7.6 ± 1.6ns*</td>
<td>0.48 ± 0.22ns*</td>
<td>95.0 ± 18.0ns*</td>
<td>451 ± 28*</td>
<td>64.6 ± 5.5ns*</td>
</tr>
<tr>
<td>T₃ + KI (0.5 µg)</td>
<td>8.9 ± 0.9*</td>
<td>0.52 ± 0.12ns*</td>
<td>107.0 ± 16.0*</td>
<td>193 ± 31*</td>
<td>36.9 ± 7.9*</td>
</tr>
<tr>
<td>T₃ + KI (2.7 µg)</td>
<td>7.8 ± 0.4ns*</td>
<td>0.52 ± 0.11ns*</td>
<td>108.0 ± 15.0*</td>
<td>484 ± 37*</td>
<td>69.6 ± 8.4ns*</td>
</tr>
</tbody>
</table>

Results are given as mean ± sd. *P < 0.05, **P < 0.01, n.s.: not significant compared with controls.

Iodine and DNA content. There was an inverse relationship between iodine and DNA (Fig. 1c). The iodine content had to fall from 0.741 ± 0.1 µg iodine/mg thyroid weight in normal rats to about 0.2 µg iodine/mg thyroid weight before growth occurred. The increase in DNA followed the severity of iodine depletion, showing approximately an exponential course. Together with the iodine content, the thyroglobulin content of the thyroids decreased (results not shown).

Histology. Thyroids of the animals on LID alone revealed signs of enhanced functional stimulation when compared with normal rats (Fig. 2a,b). The follicles were more irregular in size, but still colloid-filled. The epithelium was markedly heightened, cuboid to cylindric, and the nuclei enlarged and rounded.

Thyroids of the rats on LID and PTU (Fig. 2c) had follicles with high cylindric epithelium and contained only small amounts of colloid. Nuclei were also increased in size, rounded and had a prominent nucleolus.

Thyroids of the rats on LID and KClO₄ appeared similar to those on LID and PTU (Fig. 2d). In all groups, neither nodular proliferation nor enlarged connective tissue or lymphoid infiltrates was detected.

Goitre therapy

Effect of LID. Before therapy was started, the goitrous rats received LID without KClO₄ for one week. The amount of iodine in the diet was already sufficient to raise the iodine content of the glands significantly (13 ± 1 ng iodine/mg thyroid weight) compared with the KClO₄ rats in the goitre genesis group (0.97 ± 0.12 ng iodine/mg thyroid weight).

The TSH levels in the goitrous rats before therapy were therefore only slightly elevated (1.00 ± 0.22 mg/l) compared with the highly elevated TSH levels in the KClO₄ rats of the goitre genesis group (5.25 ± 0.41 mg/l).

Thyroid weight of the goitrous animals before therapy was lower (16.8 ± 2.2 mg/100 g body weight) than in the KClO₄ rats of the goitre genesis group (21.9 ± 0.7 mg/100 g body weight). Goitre size was reduced, whereas the total DNA content of the glands (191 ± 30 µg DNA/gland) still remained on its high level.

However, when LID was given for another 3 weeks, the DNA decreased to 148.5 ± 11.3 µg DNA/gland (Table 2). Thyroid weight fell to 12.8 ± 1.3 mg/100 g body weight and TSH level to 0.82 ± 0.28 mg/l. These changes were accompanied by a further considerable increase in the iodine content of the thyroid glands to 61 ± 6 ng/mg (Table 2).

Comparison of different therapies. As shown in Table 2, thyroid weight, which was more than twice the control values before therapy, was effectively reduced with all treatments. TSH levels under therapy were not significantly different from control
Goitre therapy. Rat thyroids (4 \( \mu \text{m} \) thick sections; haematoxylin-eosin staining; \( \times 1280 \)).

a) Goitre before therapy after 6 weeks on LID, half of the time combined with KClO\(_4\).

b) Thyroid after 3 weeks with T\(_3\) therapy.

c) Thyroid after 3 weeks of therapy with 2.7 \( \mu \text{g} \) KI/day.

d) Thyroid after 3 weeks of therapy with T\(_3\) combined with 2.7 \( \mu \text{g} \) KI/day. For comparison with control thyroid return to Fig. 2a.
Goitre therapy: For explanation of experiment return to Table 2. Cellular and nuclear areas measured in two representative histological sections from one animal of each group are shown together with the calculated nucleocytoplasmic ratios.

<table>
<thead>
<tr>
<th>Females</th>
<th>Cellular area µm²</th>
<th>Nuclear area µm²</th>
<th>Nucleocytoplasmic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.9 ± 10.1</td>
<td>7.5 ± 2.8</td>
<td>0.28</td>
</tr>
<tr>
<td>Goitre</td>
<td>77.3 ± 23.4*</td>
<td>16.9 ± 5.3*</td>
<td>0.22</td>
</tr>
<tr>
<td>LID</td>
<td>48.1 ± 14.7*</td>
<td>12.9 ± 3.3*</td>
<td>0.27</td>
</tr>
<tr>
<td>T₃</td>
<td>23.4 ± 8.2</td>
<td>9.1 ± 3.2*</td>
<td>0.39</td>
</tr>
<tr>
<td>KI (0.5 µg)</td>
<td>40.7 ± 15.8*</td>
<td>12.6 ± 3.7*</td>
<td>0.31</td>
</tr>
<tr>
<td>KI (2.7 µg)</td>
<td>31.0 ± 10.3</td>
<td>10.7 ± 3.3</td>
<td>0.34</td>
</tr>
<tr>
<td>T₃ + KI (0.5 µg)</td>
<td>30.6 ± 10.0</td>
<td>10.9 ± 3.9*</td>
<td>0.36</td>
</tr>
<tr>
<td>T₃ + KI (2.7 µg)</td>
<td>30.9 ± 8.9</td>
<td>9.9 ± 3.3</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of 400 cells and nuclei.

* Significant difference in the shape of the distribution of the sample compared with controls, tested with the Kolmogorov-Smirnov test at the 90%-level.

T₄ levels were closest to normal values in the group on 2.7 µg KI/day (T₄ = 30.96 ± 7.74 nmol/l, controls: T₄ = 46.44 ± 7.74 nmol/l). Both combination groups had significantly lower T₄ levels (T₃ + 0.5 µg KI/day: T₄ = 19.35 ± 5.16 nmol/l and T₃ + 2.7 µg KI/day: T₄ = 19.35 ± 3.87 nmol/l).

Histology. Histological evaluation of the goitres before therapy revealed follicles lined by a high cylindric epithelium (Fig. 3a). Measurement of the cellular area showed a mean increase by 187% in goitrous animals before therapy compared with normal controls (Table 3). This difference was further confirmed by analysing the histograms of the measured cell areas. A broader variation in cell size together with a shift to higher values was demonstrated in the goitre group (Fig. 4). The nuclei were also enlarged, rounded and had prominent nucleoli, the nucleocytoplasmic ratio was decreased (Table 3). Colloid was present only in few follicles. Hyperaemia occurred, however, no increased connective tissue nor nodular proliferation or lymphocellular infiltrates could be detected.

After therapy, all thyroids showed colloid-filled follicles lined by a cuboid or flat epithelium (Fig. 3b–d). Hyperaemia was no longer present. Necrosis or lymphocytic infiltrates were never found.
The nucleocytoplasmic ratio increased in all therapy groups, most prominently under T3 therapy (Table 3). Histograms of the groups on T3 therapy, on 2.7 µg KI/day and on the combination of T3 and 2.7 µg KI/day were comparable to those of the controls (Fig. 4). However, the mean cellular area under T3 therapy was 15% smaller than in the controls, whereas on 2.7 µg KI/day and in the two combination groups it was still 15% larger than in the controls (Table 3). These four groups were uniformly near-to-normal on histological examination, whereas in the group on 0.5 µg KI/day, signs of persistent functional stimulation were clearly visible. Mean cellular area in this group was still elevated by 51% over control (Table 3).

Discussion

The prominent finding in our study is the inverse relationship between iodine and total DNA content of the thyroid glands, indicating an autoregulatory effect of iodine on cell proliferation. However, a general difference in this relationship was observed between goitre growth and involution. In the goitre genesis group we found an approximately exponential course, with a progressively elevated DNA only below an iodine content of 0.007 µg/mg thyroid weight. In the goitre therapy group, a linear correlation with normalization of the elevated DNA occurred only above an iodine content of 0.2 µg iodine/mg thyroid weight. The cause for this discrepancy is probably a different distribution of iodine in different compartments of the thyroid during goitre induction and during goitre regression (Many et al. 1985).

In the present study, total DNA content of the thyroids was used as an indicator of hyperplasia, which means changes in cell number. It has been shown that polypliod cells produce an additional increase in total DNA content by maximally 10 to 20% in severely iodine-deficient goitres (Al-Saadi & Beierwalters 1966). The major part of this additional increase in DNA content not accompanied by a change in cell number is, however, caused by tetraploid cells in the S- or G2-phase. This is also the reason why in normal thyroids the overall increase induced by polyploidy decreases to 2–4% of the total DNA content, because normal thyroids contain a much lower amount of proliferating cells, i.e. tetraploids. The minor contribution of extranuclear DNA content can be disregarded (Wollman & Breitman 1970). In vivo it has been shown that normal thyroid cells have a lifespan of more than 3 weeks (Doniach & Williams 1987). In high iodine treated animals, DNA

![Cell Area](image1)

**Fig. 4.** Goitre therapy. For explanation of experiment see legend to Table 2. Four hundred cell areas were measured in two representative histological sections from one animal of each group. Histograms of the values are shown for control animals on NID, goitrous animals before therapy, and treated animals on T3, 2.7 µg KI/day and T3 combined with 2.7 µg KI/day. Dashed lines indicate the mean of each sample.
content was reduced to maximal 50%, indicating a shorter lifespan of the cells during iodine treatment.

Goitre genesis

The correlation between TSH serum levels and thyroid weight was excellent in KCIO₄- as well as in PTU-treated animals. However, the regression line had a greater slope for PTU animals than for KCIO₄ animals. PTU apparently produced an additional TSH-independent enhancing effect on thyroid weight. An explanation of this effect of PTU compared with KCIO₄ could be a more effective depletion of iodinated compounds within the cells. Low iodine diet (LID) alone stimulated only thyroid weight, but not the DNA content of the whole gland, indicating hypertrophy but not hyperplasia. The iodine content in these glands were about 200-fold higher compared with KCIO₄- and 400-fold compared with PTU-treated animals where both hyperplasia and hypertrophy occurred. Although TSH levels were not significantly different in LID-treated and normal animals, the hypertrophy may be related to TSH stimulation, because low iodine supplemented thyroid cells are more sensitive to TSH (Bray 1968).

The thyroid has been shown to iodinate fatty acids (Boeynaems & Hubbard 1980; Chaz'enbalk et al. 1985). Chemically synthesized iodinated derivatives of arachidonic acid were able to inhibit thyroid enlargement in methimazole-treated rats without affecting the TSH level (Pisarev et al. 1986), indicating that follicular epithelial cell growth may be autoregulated by these iodocompounds.

In agreement with Bray (1968) we too can postulate an increased sensitivity of PTU-treated, severely iodine-depleted thyroid glands to TSH stimulation as compared with KCIO₄-induced goitres if only thyroid weight is considered.

However, TSH exerts its influence on thyroid weight predominantly by stimulating hypertrophy of the cells. Differences in total DNA content of the glands cannot be convincingly explained by TSH. Rats with different TSH levels had identically elevated total DNA, the iodine content of these glands, however was always correspondingly low. In contrast, some of the rats on KCIO₄ without T₃ had an unexpected low total DNA with high TSH levels and a degree of iodine depletion identical to that in animals on KCIO₄ with T₃ which had a higher DNA content associated with lower TSH levels. This could suggest an inhibitory effect of high TSH levels on DNA synthesis in the glands. Inhibitory effects of TSH on [¹⁴C] thymidine incorporation of human thyroid cells and porcine thyroid follicles have been observed in vitro (Westermark et al. 1979; Eggo et al. 1984; Gärnter et al. 1985). A direct growth inhibiting effect of TSH could explain the observed ‘desensitization’ of the thyroids towards the ‘growth-stimulating action of TSH during prolonged goitrogen administration’ (Wynford-Thomas et al. 1982a,b). This ‘growth-stimulating effect’ could be mediated by an initial acceleration of iodine depletion. Also the observed weak correlation between TSH levels and total DNA content in the goitre genesis group can be explained by the different degrees of functional stimulation, with subsequent acceleration of iodine depletion and therefore indirect growth stimulation.

Besides the kidney and pancreas, the thyroid gland contains the highest concentrations of EGF in humans (Hirata & Orth 1979) and EGF is a specific growth factor for thyroid cells in vitro (Roger & Dumont 1984; Eggo et al. 1984). In addition, it has been shown in vitro that the growth promoting effect of EGF is under negative control of high iodide supplementation of thyroid cells as well as it is inhibited by TSH (Gärnter et al. 1985). These in vitro data are in agreement with our now in vivo observed effects and we conclude that the iodine content of the thyroid follicular cells is the major autoregulatory factor for cell proliferation, whereas TSH mainly induces hypertrophy of the cells.

In contrast, it has been shown in other in vitro systems that TSH stimulates thyroid cell growth (Tramontano et al. 1986; Dere & Rapoport 1986). However, this was only shown in thyroid cell monolayer cultures, and in both studies together with foetal calf serum or a growth stimulating agent like insulin. Thyroid cell monolayers are depleted in iodine, do not store iodine, and are not comparable to intact thyroid follicles (Gärnter et al. 1985). Furthermore, most of the recent data, showing that TSH stimulates thyroid cell growth, were obtained with the FRTL 5 cell line, a clone selected by the criterion of ability to grow only in the presence of TSH (Ambesi-Impiombato et al. 1980). Therefore FRTL 5 cells are not a valid model for answering the question whether TSH is a growth factor for normal thyroid cells.
Goitre therapy

The criteria for rational goitre therapy are not only that it abolishes hypertrophy and decreases the thyroid weight, but also that it normalizes the hyperplasia and restores the iodine and thyroglobulin content.

Reduction of elevated TSH to normal values in all therapy groups led to normalization of the functional status of the cells with subsequent shrinkage of the hypertrophic glands, as indicated by reduction of cellular and nuclear size, and normalization of thyroid weight. Hyperplasia, however, persisted until the iodine content in the glands was restored over the critical value of 0.2 µg iodine/mg thyroid weight. T₃ therapy was able to reduce mean cell area size, but it could not abolish hyperplasia and could not restore the iodine content over the critical point. After 3 weeks on 2.7 µg KI/day, we found a nearly normal value for mean cell area, a normal value for thyroid size, total DNA content and thyroglobulin content, together with an almost normalized iodine content of the glands. In our experiments, administration of T₃ together with iodide had no advantages in comparison to treatment with iodide alone.

These data indicate that iodine supplementation is the causal therapy for iodine-deficient goitre, because it abolishes not only hypertrophy, but also hyperplasia of the glands and restores normal function and regulation. However, the use of this therapy in man is limited by thyroid autonomous, which develop during long-standing iodine deficiency in the thyroid (Bährle 1987). The only possible way of solving this problem effectively is to start with early iodine prophylaxis.

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References


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