Effects of selenium deficiency on thyroid necrosis, fibrosis and proliferation: a possible role in myxoedematous cretinism

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It has been suggested that selenium deficiency is a co-factor to iodine deficiency in the pathogenesis of myxoedematous cretinism. The mechanism proposed is that the generation of hydrogen peroxide is greatly increased in iodine-deficient thyroid glands, and that selenium is involved in the control of hydrogen peroxide and its derived free radicals. This study was carried out to investigate the effect of the possibly impaired cellular defence mechanism associated with selenium deficiency on thyroid necrosis and tissue repair. For this purpose, we studied thyroid tissue from selenium- (SE−) and/or iodine-deficient (1−) rats before and after acute toxic iodine overload. In 1− thyroid glands, necrotic cells were numerous. Acute iodine administration increased this effect. Necrosis was associated with transient infiltration of inflammatory cells. In I−SE+ thyroid glands the tissue resumed its normal appearance. In I−SE− thyroid glands, the iodide toxicity was stronger, with greater necrosis and inflammatory reaction. The inflammation resolved but was replaced by fibrotic tissue. Fifteen days after the toxic overload, the connective tissue volume was twice the control value. Before iodide overload, the proportion of dividing cells was equal in I−SE+ and I−SE− thyroid glands. Three days after the iodide overload, this proportion was increased in I−SE+ thyroid but reduced in the I−SE− thyroid glands. Overall, the I−SE− thyroid glands had four times fewer dividing cells than the I−SE+ thyroid glands. In summary, selenium deficiency coupled to iodine deficiency increased necrosis, induced fibrosis and impeded compensatory epithelial cell proliferation. These results are compatible with histological and functional descriptions of thyroid tissue from myxoedematous cretins.

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In some iodine-deficient areas, long-standing hypothyroidism is accompanied by thyroid gland destruction instead of goitre formation (1). These subjects develop overt and irreversible hypothyroidism associated with a clinical picture of myxoedema, dwarfism and mental retardation (2). Although they are similar clinically to sporadic cretins, myxoedematous cretins do not have thyroid dysplasia or metabolic defects in thyroid hormone synthesis or metabolism (3). Moreover, hypothyroidism has been shown to be reversible in young myxoedematous cretins (4–6), suggesting that the destruction of the gland is a slow process (4).

In 1908, McCarrison (7) described the thyroid gland of a myxoedematous cretin from the Himalayan region. It consisted of large and uniform fibrous strands with little remaining functional thyroid tissue. In 1936, DeQuervain and Wegelin (8) described a very similar picture in Switzerland. The thyroid was atrophied, with loss of epithelial tissue, extensive fibrosis and lymphocytic infiltration. Since then, iodine metabolism studies in Africa showed that the thyroids of myxoedematous cretins have a very reduced uptake and fast turnover of iodine (3). This suggests that the amount of functional tissue is reduced and is performing at the maximum of its capacity (3, 9). The scattered aspect of the thyroids shown by scanning suggests a fibroed gland (10).

The prevalence of myxoedematous cretinism varies widely from one goitre-endemic area to another, and is not always associated with thyroid destruction (11). To explain this discrepancy, the involvement of co-factors to iodine deficiency, such as goitrogens (e.g. thiocyanate or flavonoids), and of autoimmunity (12, 13) has been suggested. More recently, selenium deficiency has been described in areas where the prevalence of myxoedematous cretinism is very high. Although the association is not reported everywhere (13), it has been proposed that selenium deficiency may play a role as a co-factor to iodine deficiency in thyroid destruction (14, 15). Indeed, most of the identified selenoproteins (i.e. glutathione peroxidase (GPX) (16, 17), phospholipid hydroperoxide (Ph-GPX) (18, 19) and selenoprotein-p (20)) and also organic selenium compounds like ebselen (21) are involved in antioxidant defences through their ability to reduce either H2O2 or other H2O2-derived free...
radicals and lipid peroxides (22). Hydrogen peroxide is produced in large amounts in stimulated dog and human thyroids (23), and therefore presumably also in iodine-deficient glands. Overproduction of H₂O₂ could also occur around birth, when, even in iodine-sufficient areas, the thyroid is stimulated.

Excessive free-radical generation has also been suggested to occur during iodine-induced goitre involution (24). To investigate further the association between selenium and thyroid gland destruction, the known toxic effect of iodine overload on the iodine-deficient thyroid gland (24–29) was combined with selenium deficiency in this study. An earlier study had enabled us to demonstrate that, in rats, selenium deficiency dramatically enhances the necrotizing effect of iodide (30). Investigating those results further, we looked in more detail at the influence of selenium deficiency on both the inflammatory reaction and the thyroid repair process. The results further support the hypothesis that selenium deficiency may be a co-factor in the pathogenesis of myxoedematous cretinism.

Methods

Animals and treatments (see Fig. 1)

Young female Wistar rats, 21 days old, weighing approximately 40 g at the onset of the study, were used. They were housed in a light- and temperature-controlled room and had free access to food and water. They were fed either a semi-synthetic diet containing 0.005 mg selenium/kg with torula yeast as protein source (SE– groups) (Hope Farms, Woerden, The Netherlands) or the same diet supplemented with 0.180 mg selenium/kg (SE+ groups). The diet contained 0.380 mg iodine/kg.

Goitre was induced by giving 1% sodium perchlorate in tapwater for 5 weeks (I–, goitrous rats), while (I+, control rats) received tapwater only. At the end of this period, the perchlorate was withdrawn to allow goitre involution. Twelve hours after perchlorate weaning, the rats were injected once ip with 1 mg of sodium iodide (Nal) diluted in 0.5 ml of saline. In the I– group, one further group was created (I–SE–MMI). In this group, perchlorate in drinking water was replaced by 0.02% methimazole (MMI) for 3 days. The animals (five rats/group) were killed before iodide administration or 3, 15 and 26 days later, under Nembutal anaesthesia. Three rats of each group were submitted to cardiac puncture: plasma was separated and stored at −20°C for biochemical determinations. Thyroid glands were removed quickly and weighed. One lobe was fixed in Bouin’s liquid, and the other was frozen in isopentane cooled in liquid nitrogen. Two rats per group were perfused through the heart for 1 min with saline and for 5 min with glutaraldehyde (31), and their thyroid was processed further for light microscopy (32, 33).

Thyroid hormones and glutathione peroxidase assays

Thyroid hormone determinations were made in duplicate by radioimmunoassay, with commercially available kits (Amersham, UK, for T₄ and T₃). Glutathione peroxidase activity was measured in the plasma with a commercial kit (Randox, Ransel Kit, Grumblin, Ireland), with cumene hydroperoxide as substrate.

Morphological analysis and stereological analysis

The thyroid fragments from perfused rats were immersed in a solution of 2.5% glutaraldehyde (Taab, Reading, UK) in 0.1 mol/l cacodylate buffer (Taab, pH 7.4), postfixed for 1 h in 1% osmium tetroxide, dehydrated in alcohol solutions of increasing strength and embedded in LX112 resin (Ladd Research Industries, Burlington, USA). Sections 0.5 μm thick were cut from the centre of each fragment and stained with toluidine blue. All measurements and thyroid analyses were made blind. The extent of cell necrosis was quantified by counting the number of necrotic cells (i.e. with pyknotic or caryolytic nuclei) on semi-thin sections at a magnification of 400×. Counting was performed on 100 follicles per section and on 10 sections per experimental group. Results are expressed

Plate 1. (A) Thyroid from a control rat (I+SE+) (×400): the gland has normal morphology. (B) Thyroid from a selenium-deficient control rat (I+SE–) (×400): the glandular morphology is similar to that of control rats.

Plate 2. (A) Thyroid from a goitrous rat (I–SE+) (×400). The hyperplastic goitre is characterized by reduction of colloid, hypertrophy of epithelial cells and vasodilatation. The epithelial layer contains necrotic cells (arrows) recognizable by their swollen aspect, light cytoplasm and karyolytic nucleus. (B) Thyroid from a selenium-deficient goitrous rat (I–SE–) (×400). The hyperplastic goitre is also typical. Necrotic cells are numerous (arrows).
as the mean percentage of necrotic cells. The relative volumes of the glandular components (epithelium, follicular lumen, connective tissue, blood vessels), and the epithelial height were determined in each group by stereological methods described previously (32).

**Aldehyde detection**

Aldehyde detection was performed as described by Taper et al. (34) by Schiff staining on unfixed frozen thyroid sections from rats of the different experimental groups. The staining intensity was measured by cytodensitometry, at a wavelength of 560 nm, according to the method of Rahier et al. (35).

**Proliferating cell nuclear antigen labelling**

Proliferating cell nuclear antigen (PCNA), a marker for cell proliferation (36), was detected in Bouin's fixed, paraffin-embedded thyroids from goitrous rats treated or not with iodide for 3 days. The PCNA was detected using a double-antibody immunohistochemical method: a mouse monoclonal antibody (clone PC 10, dilution 1:200, Boeringher Mannheim, Germany) and an anti-mouse antibody coupled to peroxidase. This was revealed by diaminobenzidine. Endogenous peroxidase was inhibited by treatment with 0.3% H₂O₂ for 30 min. The PCNA index was estimated by counting the number of positive cells per 1000 follicular cells in each section and then expressing this as a percentage (%).

**Statistical analysis**

Comparison of the means between the various groups was made by one-way analysis of variance or by a Kruskall–Wallis non-parametric test. Means are expressed ± SD in Table 1 and in the Results section and ± SEM in the figures. In Table 1 and the figures, significant differences are expressed in the I−SE−MMI group vs I−SE− group, in I−SE− vs I−SE+ groups, in I−SE+ vs I+SE+ groups and in I+SE− vs I+SE+ groups at the same moment of the study; or for days 15 and 26 post iodide in the I−SE+ group vs day 3 post iodide in the I+SE+ group.

**Results**

**Control group I+SE+**

In the selenium-sufficient control group (I+SE+), the thyroids had a normal morphology (Plate 1A). The follicular lumina were lined by a cuboidal epithelium. The connective tissue was loose and represented 16.8±1.2% of the glandular volume. After iodide administration for 3 days, the gland morphology was not modified. No significant difference was observed in the various glandular parameters: thyroid weight, epithelial height (Fig. 2), relative volumes of epithelium, follicular lumen, connective tissue and vascular tissue (Fig. 3A–D). Iodide injection did not affect the number of necrotic cells and aldehyde staining (Fig. 4), and plasma T₄ and T₃ levels were unchanged (Table 1). The PCNA index was very low, close to zero.

**Control group I+SE−**

In selenium-deficient control rats (I+SE−), plasma GPX as well as plasma T₃ were reduced as compared to I+SE+ rats (Table 1). The glandular morphology was not modified strongly (Plate 1B). However, the mean number of necrotic cells (Fig. 4A) and the relative volume of colloid (Fig. 3C) were significantly higher than in the controls (p < 0.05). Three days after iodide administration, the number of dead cells was increased further. As in the I+SE+ group, the percentage of PCNA-positive epithelial cells was very low.
Fig. 3. Changes in the mean ± sem percentages of the relative volumes of the various components of the thyroid gland—(A) follicular epithelium tissue, (B) connective tissue, (C) colloid, (D) vascular tissue—in the various groups before iodide administration (T0) and 3 days, 15 days and 26 days after acute iodide administration: (▲) I+SE+ group; (△) I+SE− group; (●) I−SE+ group; (○) I−SE− group; (▲) I−SE− group. Significant differences are expressed in the I−SE− MMI group vs I−SE− group, in the I−SE− vs I−SE+ groups, in the I−SE− vs I+SE+ groups and in the I+SE− vs I+SE+ groups at the same moment of the study or for days 15 and 26 post iodide in the I−SE+ group vs day 3 post iodide in the I+SE+ group; *p < 0.05 and **p < 0.01 levels of significance.

Table 1. Mean ± so plasma T4, T3 and plasma glutathione peroxidase (GPX) in the various groups before iodide overload and 3 days and 15 days after acute iodide administration.*

<table>
<thead>
<tr>
<th></th>
<th>Control I+SE+</th>
<th>Control I+SE−</th>
<th>Goitrous I−SE+</th>
<th>Goitrous I−SE−</th>
<th>Goitrous I−SE− MMI</th>
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<tr>
<td>T4 (μg/dl)</td>
<td></td>
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<tr>
<td>Before iodide</td>
<td>3.0 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>1.1 ± 0.12**</td>
<td>1.0 ± 0.21</td>
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<tr>
<td>3 Days post iodide</td>
<td>2.4 ± 0.01</td>
<td>3.4 ± 0.16*</td>
<td>5.2 ± 1.20**</td>
<td>5.3 ± 0.75</td>
<td>3.0 ± 0.28</td>
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<tr>
<td>15 Days post iodide</td>
<td></td>
<td></td>
<td>2.9 ± 0.29</td>
<td>2.4 ± 0.58</td>
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<tr>
<td>T3 (μg/dl)</td>
<td></td>
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<tr>
<td>Before iodide</td>
<td>72 ± 22</td>
<td>45 ± 9**</td>
<td>9 ± 0.5**</td>
<td>17 ± 3.9</td>
<td>14 ± 3**</td>
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<tr>
<td>3 Days post iodide</td>
<td>41 ± 8</td>
<td>48 ± 19</td>
<td>97 ± 19**</td>
<td>54 ± 16**</td>
<td>39 ± 7</td>
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<tr>
<td>15 Days post iodide</td>
<td></td>
<td></td>
<td>53 ± 9</td>
<td>39 ± 7</td>
<td>39 ± 10</td>
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<tr>
<td>GPX (U/l)</td>
<td></td>
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<tr>
<td>Before iodide</td>
<td>13500 ± 1000</td>
<td>900 ± 300**</td>
<td>12050 ± 550</td>
<td>1890 ± 95**</td>
<td>2220 ± 900</td>
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<tr>
<td>3 Days post iodide</td>
<td>13150 ± 620</td>
<td>1100 ± 230**</td>
<td>12010 ± 1700</td>
<td>1710 ± 300**</td>
<td>1160 ± 65**</td>
</tr>
<tr>
<td>15 Days post iodide</td>
<td></td>
<td></td>
<td>12500 ± 220</td>
<td>1160 ± 65**</td>
<td>1320 ± 170</td>
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*Significant differences are expressed in the I−SE− MMI group versus I−SE− group, in the I−SE− vs I−SE+ groups, in the I−SE+ vs I+SE+ groups and in the I+SE− vs I+SE+ groups at the same moment of the study or for days 15 and 26 post iodide in the I−SE+ group versus day 3 post iodide in the I+SE+ group; *p < 0.05 and **p < 0.01 levels of significance.
Three days after the administration of a high iodide dose to the goitrous rats, plasma T₄ and T₃ levels were increased strongly and reached the control values after day 15. Morphologically, iodide induced a progressive involution of the hyperplastic goitre, but also had an early toxic effect. At day 3, the follicular lumina were enlarged slightly and contained necrotic debris. The epithelium comprised numerous necrotic cells, which were swollen, had a light cytoplasm and caryolytic nucleus. The interstitium was infiltrated with inflammatory cells, mainly macrophages (Plate 3A). The increase in the mean number of necrotic cells was associated with an increased aldehyde content (Fig. 4). While follicular cells had died, others seemed to have replicated actively, the PCNA index reaching 5.7 ± 0.4% after 3 days.

The toxic effect of iodide was transient. The number of necrotic cells, as well as the aldehyde content, decreased (Fig. 4) and the inflammatory reaction resolved. Fifteen days after acute iodide administration, the thyroid resumed a normal aspect (Plate 4A) and the various glandular parameters (Fig. 3A–D) reached control values, except for the remaining dead cells counted in the lumen (Fig. 4).

**Goitrous group I—SE—**

In selenium-deficient goitrous rats, hyperplastic goitre was also typical (Plate 2B) and associated with severe hypothyroidism (Table 1). However, as compared to I—SE+ rats, the thyroid gland weight (Fig. 2A), the relative volume of connective tissue (Fig. 3B), the number of necrotic cells and the aldehyde content (Fig. 4) were increased significantly (p < 0.01) while the PCNA index (1.9 ± 0.03% in SE— vs 2.1 ± 0.4% in SE+) was similar. Iodide administration had a very strong toxic effect. At day 3 after iodide, necrotic cells were very common in the epithelial layer, and the lumen contained much necrotic debris (Plate 3B). Their number had more than doubled as compared to that observed before iodide administration, and tripled as compared to the goitrous selenium group (I—SE+) (Fig. 4A). Increased necrosis was concomitant with increased aldehyde staining (Fig. 4B). The interstitium was modified by massive infiltration of inflammatory cells, mainly macrophages (Plate 3B). Contrary to what was observed in the I—SE+ group, iodide administration was not associated with an increase of the PCNA index at day 3. Three days after iodide it was 1.4 ± 0.2% in this SE— group, which is even less

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**Plate 3.** (A) Thyroid from goitrous rat (I—SE+) 3 days after acute iodide administration (×400). The slightly enlarged follicular lumen contains cell debris. Necrotic cells are numerous in the epithelial layer (arrows). (B) Thyroid from a selenium-deficient goitrous (I—SE—) rat 3 days after iodide treatment (×400). Necrotic thyocytes are common (arrows). The interstitium is infiltrated by numerous inflammatory cells, mainly macrophages (arrow heads).

**Plate 4.** (A) Thyroid from a goitrous (I—SE+) rat 15 days after acute iodide treatment (×150). The gland has a normal aspect, except for the remaining dead cells in the colloid. (B) Thyroid from a selenium-deficient goitrous (I—SE—) rat 15 days after iodide administration (×150). The gland has not resumed a normal aspect. Fibrous tissue is present, which surrounds clusters of follicles.

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Goitrous group I—SE—

After 5 weeks of perchlorate treatment, a typical hyperplastic goitre was obtained (Plate 2A). It was characterized by increased thyroid weight (Fig. 2A), hypertrophy of the follicular epithelium, reduction of colloid, vasodilatation (Fig. 3A, C, D) and severely reduced plasma T₄ and T₃ levels (Table 1). In the goitrous glands, the number of necrotic cells was increased significantly as compared to controls (Fig. 4A) (p < 0.01). The mean number of PCNA-positive epithelial cells reached 2.1 ± 0.4%, suggesting active multiplication.
(\( p < 0.05 \)) than before iodide, and only 25% of the value in the I–SE+ group, but similar to the value in the I–SE–MMI group.

Iodide administration, as observed in the I–SE+ group, induced goitre involution, and after 15 days the thyroid weight reached control values. The amount of necrotic cells as well as the aldehyde content were very high after 3 days (Fig. 4). Necrotic cells decreased thereafter, but remained significantly elevated as compared with other groups; the aldehyde content remained elevated (Fig. 4). However, the selenium-deficient thyroids showed peculiar changes during goitre involution. In contrast to the selenium-sufficient goitrous group (I–SE+), SE– thyroids did not resume a morphological aspect comparable with control groups at all. Fibrous tissue developed, surrounding clusters of follicles (Plate 4B). This corresponded to an important increase of its relative volume (Fig. 3B), from day 3 up to day 26, at the expense of epithelium (Fig. 3A) and colloid (Fig. 3C), which were both reduced significantly in volume as compared to the control thyroids.

Goitrous group I–SE–MMI

Methimazole administered to the iodide- and selenium-deficient rats (I–SE–) blocked the acute iodide-induced cell necrosis and decreased aldehyde staining (\( p < 0.05 \)) 3 days after iodide overload. The PCNA index at day 3 after iodide administration was 1.5 ± 0.3%, which is similar to the value in the I–SE– group before iodide administration and also to the I–SE+ value at day 3 after iodide. Whilst the appearance and morphology of the thyroids were comparable to that in the I–SE+ rats after day 15 (Figs 2–4), the aldehyde concentration was raised.

Discussion

The thyroid gland is one of the organs protected against selenium deficiency (37). In the selenium-deficient condition, both the total selenium content and the activity of the type I deiodinase (which is one of the selenium-containing enzymes (38–40)) are preserved more in the thyroid than in other organs, e.g. the liver and the kidney (41–43). However, selenium metabolism is also regulated at the cellular level. When this trace element is scarce, selenium is supposed to be incorporated preferentially in type I deiodinase to the detriment of glutathione peroxidase and the selenium-mediated cell defences (37, 43–47). The present data show that the thyroid gland remains sensitive to selenium deficiency despite its relative protection. They also show that selenium deficiency leads to some thyroid changes, even without iodine deficiency. When combined with iodine deficiency, it increases the amount of dead cells, aldehyde staining and fibrosis. The combined deficiencies render the gland much more sensitive to the toxic effect of iodine re-feeding. Moreover, the gland does not show a similar compensatory epithelial cell proliferation, nor does restoration of the lost thyroid tissue occur. The gland does not resume a normal appearance and is altered irreversibly.

Thyroid cell necrosis

The acute necrotic effect of iodine has been demonstrated in hypothyroid mice (25–27) and rats (28, 29) and in human thyroid follicles (24). The iodine-induced necrosis occurs early after iodine administration and is transient.

The iodine-induced toxicity hypothesis (24) (Fig. 5) is based on: the presence of thyroperoxidase activity (TPO); \( \text{H}_2\text{O}_2 \) being both oxidant and reductant; and an excess of iodine as compared to tyrosyl residues. In this situation, iodinium (\( I^+ \)) is formed by iodide (I–) oxidation in the presence of \( \text{H}_2\text{O}_2 \) and TPO. Then, instead of being organised on tyrosyl residues for further thyroid hormone synthesis, \( I^+ \) can react again with the excess iodide to form molecular iodine (I2), which in turn could react with \( \text{H}_2\text{O}_2 \) to form free radicals. These free radicals are responsible for DNA and membrane damage, lipid peroxidation and eventually cell necrosis (24, 48). Without iodine in excess, free radicals could also be produced. Indeed, by being alternatively electron acceptor and donor, \( \text{H}_2\text{O}_2 \) alone can react in cascade with TPO and then with the superoxide anion produced from this cascade, finally producing oxygen derived free radicals (48, 49) (Fig. 5).

As judged by the morphology, iodine deficiency alone does not stimulate the production of enough free radicals (through, presumably, increased \( \text{H}_2\text{O}_2 \) generation) to overcome the cell defences and induce massive...
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Thyroid necrosis. However, when iodine-deficient glands are exposed to either iodine overload or selenium deficiency, the cell defences are overwhelmed by the free radicals generated. Together, these factors have a cumulative effect. The two mechanisms both involve TPO, but at different steps. Blocking TPO through MMI had two effects: it inhibited the iodide-induced necrosis, which supports the iodine toxicity hypothesis; and it also decreased the aldehyde staining, instead of just keeping it unchanged. This supports the theory of free-radical generation by TPO and H₂O₂ involvement without iodide overload. Furthermore, aldehyde staining was seen mainly around the apical membrane, where TPO is situated (not shown). This means that neither the presence of a metal—and a Fenton reaction (22)—nor iodine overload are required for free-radical generation and subsequent thyroid damage in the selenium-deficient hypothyroid rat.

Selenium deficiency alone or iodine overload alone seem to affect the iodine-deficient epithelial cell with a similar degree of severity with respect to thyroid necrosis, the amount of aldehyde and the appearance of the epithelial cells. Indeed, the number of necrotic cells and the aldehyde staining are in the same range in the I−SE− group before iodide and in the I−SE+ group 3 days after acute iodide administration. However, an important difference is that iodine overload exerts an acute and transient effect, while the selenium effect is persistent. Therefore, selenium deficiency could damage the iodinedeficient thyroid more than acute iodine administration. As shown previously (30), we also observed that the iodide-induced necrosis in the I−SE− group is three times more severe than in the I−SE+ group.

Necrosis seems to involve only a small fraction of the epithelial cells (28). It is probably not a major event in the process of goitre involution or in the rapid decrease in thyroid weight observed after iodine refeeding. It has been suggested that this weight decrease could be due to a decreased epithelial cell size (28). The data from the present MMI-treated group support this hypothesis. In the iodine-deficient condition with or without selenium, both thyroid weight (50) and cell size are increased. After iodine administration, the thyroid weight decreased in both I− groups but also in the MMI group, while these MMI-treated thyroids escaped the iodide-induced necrosis. Therefore, the weight decrease observed early after iodine treatment is not related to the loss of thyroid cells through necrosis.

Inflammation and fibrosis

Cell necrosis is known to induce a transient inflammatory reaction (25, 26, 51, 52), whereas apoptosis does not seem to do this (25, 53).

In this experimental protocol, whatever the selenium supply, the inflammatory reaction after iodine overload was transient and in the I−SE+ group the thyroids resumed a normal appearance. The accentuated inflammatory reaction in the I−SE− group as compared to the I−SE+ group might be related to the severity of the necrosis. However, what has not been described before is that goitre involution in the selenium-deficient thyroids leads to thyroid fibrosis.

These data suggest that the fibrosis present in the I−SE− thyroids was not the simple consequence of necrosis. Inflammation and connective tissue accumulation seemed poorly regulated in selenium deficiency. The considerable amount of fibrotic tissue surrounding the thyroid lobules could impede cell proliferation and epithelial thyroid tissue restoration.

Proliferation

Independently of TSH stimulation (54, 55), cell necrosis and wounding (56, 57) induce a burst of epithelial cell proliferation (28, 32). It is suggested that this is capable of compensating for the iodine-induced necrosis of epithelial cells. If proliferation, like inflammation, is proportional to the degree of necrosis, it should be more significant in the I−SE− group than in the I−SE+ group both before and after acute iodide administration. However, we found quite the opposite. The data suggest that SE− thyroids did not proliferate when necrosis was the stimulus but did proliferate when TSH was the stimulus, as shown by comparison of the PCNA indexes in the different groups. Indeed, when TSH was assumed to be the main stimulus to proliferation, i.e. when the groups had similar hypothyroid status, the various groups had a similar PCNA index (in the I−SE+, I−SE− before iodide administration and the I−SE−MMI 3 days after iodide); and this in spite of the already substantial degree of necrosis in the I−SE− group. Moreover, the goitres were at least as large in the I−SE− group as in the I−SE+ group. When necrosis was assumed to be the main stimulus to proliferation, i.e. 3 days after acute iodide administration, the SE+ thyroids showed the expected proliferation burst while the SE− thyroids showed a surprisingly decreased PCNA index as compared to before iodide administration. This is in complete contrast with the level of necrosis in this group.

Concluding remarks

The results suggest that selenium deficiency alone leads to thyroid damage through free-radical generation and that selenium deficiency associated with iodine deficiency has a persistent damaging effect on the thyroid, which then becomes fibrotic if exposed acutely to a toxic agent like iodide.

The increased thyroid necrosis demonstrated previously (30) in selenium deficiency is complemented by the findings of fibrosis and proliferation. This further suggests the involvement of selenium deficiency in both the observed inflammatory fibrotic process and in the decreased epithelial cell proliferation. Together, these
observations fit well with the initial hypothesis linking selenium deficiency and myxoedematous cretinism (14). Indeed, the histological pictures obtained in this experiment seem consistent not only with the few histological descriptions available from myxoedematous cretins from Switzerland and the Himalayas, but also with the conclusions drawn from the thyroid function tests and scans carried out in myxoedematous cretins from Africa and examination of thyroid fragments from endemic goitre patients. These results further support the theory that selenium deficiency is one of the co-factors of iodine deficiency in the thyroid destruction that is observed in most myxoedematous cretins.

Despite the very severe experimental conditions used, the present observations, if transposed to the human situation, not only suggest practical applications with respect to iodine and selenium prophylaxis, but also reveal the paradoxical relationship between iodine and selenium. Acute iodine administration to subjects deficient in both selenium and iodine could be harmful for the most severely affected subjects. However, iodine supplementation programmes have suppressed the occurrence of new cases of myxoedematous cretinism, and so far no case of ensuing thyroid destruction has been described. Moreover, in some, but not all cretins, massive iodine administration may improve or even restore thyroid function to normal (4, 6). To date, it is not known if a more physiological way of administering iodine would have allowed better recovery of thyroid function. It remains debatable whether subjects who did not improve their thyroid function already had a totally destroyed thyroid gland before iodine supplementation, or if the massive iodine administration destroyed the little remaining thyroid tissue. Boyages et al. (5) describe not only irreversibility of hypothyroidism in old myxoedematous cretins, but also a worsening of thyroid function after iodine administration. They suggest that iodine supplementation in these subjects is potentially harmful. Elimination of endemic cretinism requires the elimination of its main cause, i.e. iodine deficiency, and only following that, the elimination of co-factors. However, taking into account the iodide toxicity, exacerbated in selenium deficiency, it would seem advisable to avoid repeated administration of high doses of iodine in hypothyroid patients.

In selenium- and iodine- deficient populations, selenium supplementation and restoration of cell defences might diminish thyroid destruction and thereby be beneficial. However, it must not be forgotten that selenium is a constituent of type I deiodinase enzyme (39, 40). Supplementation with selenium alone worsens iodine deficiency and hypothyroidism, presumably through increasing type I deiodinase activity (6, 58), thereby decreasing T₄, which is the precursor of the brain T₃, and thus possibly increasing neurological damage. These conclusions warn us against selenium supplementation alone in areas of combined iodine and selenium deficiency.

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