0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(2):994–1002

doi: 10.1210/en.2003-0886

Thiocyanate Induces Cell Necrosis and Fibrosis in Selenium- and Iodine-Deficient Rat Thyroids: A Potential Experimental Model for Myxedematous Endemic Cretinism in Central Africa

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Thyroid destruction leading to endemic myxoedematous cretinism is highly prevalent in central Africa, where iodine (I) and selenium (SE) deficiencies as well as thiocyanate (SCN) overload are combined. All three factors have been studied experimentally in the etiology of the disease, but they have never been studied in combination. In a model using rats, we have previously shown that combining I and SE deficiencies increases the sensitivity of the thyroid to necrosis after iodide overload, an event unlikely to occur in the African situation. To develop a model that would more closely fit with the epidemiological findings, we have determined whether an SCN overload would also result in thyroid necrosis as does the I overload. The combination of the three factors increased by 3.5 times the amount of necrotic cells, from 5.5 ± 0.3% in the I^-SE^+ thyroids to 18.9 ± 1.6% in the I^-SE^-SCN-overloaded ones. Methimazole administration prevented the SCN-induced necrosis. SE^- thyroids evolved to fibrosis, whereas SE^+ thyroids did not. TGFβ was prominent in macrophages present in SE^- glands. Thyroid destruction in central Africa might therefore originate from the interaction of three factors: I and SE deficiencies by increasing H_2O_2 accumulation, SE deficiency by decreasing cell defense and promoting fibrosis, and SCN overload by triggering follicular cell necrosis. (*Endocrinology* 145: 994–1002, 2004)

ENDEMIC CRETINISM, the most severe manifestation of iodine (I) deficiency, affects or has affected many endemic goiter areas around the world (1). However, the clinical presentation of cretinism differs much between endemias, and the reasons for these clinical variations are not yet clear. Quantitative differences in the severity of I deficiency alone cannot account for the clinical variations of cretinism (2).

The vast majority of cretins in central Africa are myxedematous, a feature that is not found in endemias where neurological cretinism prevails (3, 4). Impaired thyroid function in the central African endemias is caused by progressive destruction of the thyroid gland, a slow process affecting the population on a large scale (5, 6). Thyroid damage impairs the adaptive mechanisms to I deficiency (7, 8). Therefore, people progressively tend to settle into hypothyroidism, and when damage is severe enough, long-standing hypothyroidism sets in, and myxedema develops. Finally, this may lead to complete thyroid tissue atrophy, and some myxedematous cretins no longer respond to I supplementation (5, 6). Moreover, the thyroids become fibrotic with time, and this is likely to contribute to the destruction process (2). In the most severe cases, thyroid damage starts *in utero*, and most of the damage is believed to occur around birth and during the first years of life (1).

The etiology of the elevated prevalence of thyroid destruction in African areas of endemic goiter is unknown. Factors other than I deficiency are known to interfere with thyroid metabolism. In this peculiar situation, people are exposed to thiocyanate (SCN) overload caused by consumption of cassava roots, which are used as a staple food and contain the cyanogenic glucoside linamarin. Linamarin metabolism produces SCN, a well known goitrogen (9). SCN competes with iodide (I) trapping as well as with I for its oxidation and I binding to thyroglobulin at the level of the thyroperoxidase enzyme (10, 11). Hence, SCN induces both a release of I from the thyroid cell and a loss from the body. As a consequence, SCN ultimately results in an I-deficient thyroid and a decrease in thyroid hormone synthesis. Experimental and epidemiological studies have shown that SCN overload aggravates the severity of I deficiency and worsens its outcomes (12). However, the association of these two factors, *i.e.* SCN overload and nutritional I deficiency, is not sufficient to explain thyroid destruction in central Africa and hence the elevated prevalence of myxedematous cretinism.

Selenium (SE) also interferes with thyroid function, and its deficiency has been documented in central Africa (13–15). Among the complex SE thyroid interactions (16–18), SE deficiency has been implicated in the etiology of thyroid destruction and of myxedematous cretinism (13). SE is involved in cell defense, *e.g.* as an active component of the family of

Abbreviations: ClO_4, Perchlorate; GPX, glutathione peroxidase; H_2O_2, hydrogen peroxide; I, iodide; MMI, methimazole; $OSCN^-$, hypothyocyanite; O_2SCN, cyanosulfurous acid; O_3SCN, cyanosulfuric acid; SCN, thiocyanate, SE, selenium; TPO, thyroperoxidase.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

glutathione peroxidase enzymes (19), selenoprotein P (20, 21), and thioredoxin reductase (22). Its deficiency favors cell destruction in tissues exposed to free radical damage, including tissues that synthesize large amounts of hydrogen peroxide (H_2O_2). Such increased amounts of H_2O_2 are present in the I-deficient thyroids as the result of their increased TSH level (23) or by the lack of NADPH oxidase inhibition by I (24). In agreement with this hypothesis, experiments have repeatedly demonstrated increased necrosis in various SE-deficient tissues exposed to free radical damage (21, 25–27).

Experiments in rats have shown a high sensitivity of the I- and SE-deficient thyroid gland to cell necrosis and suggest a defective thyroid repair, through an impaired proliferation of thyrocytes, and a fast evolution to fibrosis (25–27). In these experiments both cell necrosis and the cascade of events leading to thyroid fibrosis were dramatically increased when necrosis was elicited by I overload. However, I supplementation in humans, even at a high dose, is evidently not involved in the etiology of myxedematous cretinism in I-deficient human endemias (28). Therefore, the experimental association of only two environmental factors, *i.e.* severe I deficiency combined with SE deficiency, would not be sufficient to induce the considerable thyroid necrosis suggested by the thyroid atrophy observed in central Africa. Nevertheless, the use of I overload to elicit thyroid necrosis in the rat suggested that an SCN overload might have a similar and yet unexplored effect on thyroid function. Indeed, SCN and I share some common physicochemical properties (29) (*i.e.* SCN is a pseudo-halide and has the same molecular volume and comparable oxido-redox potential as I), and both are oxidized by the peroxidase enzymes. It therefore appeared important to test whether a SCN overload would also be capable of triggering thyroid cell necrosis in I- and SE-deficient glands. It is shown here that SCN triggers thyroid cell necrosis in rats made deficient in both I and SE. It is also shown that with this combination of the three environmental factors, which indeed coexist in central Africa, thyroid glands evolve to fibrosis.

Materials and Methods

Animals and treatments

Young female Wistar rats, 21 d old, weighing approximately 40 g at the onset of the study, were used (four or five rats per group). They were housed in a light- and temperature-controlled room and had free access to food and water. They were fed either a semisynthetic diet containing 0.005 mg SE/kg with Torula yeast as protein source (SE^- groups; Hope Farms, Woerden, The Netherlands) or the diet supplemented with 0.180 mg SE/kg (SE^+ groups). The diets contained 0.380 mg I/kg.

Because of the difficulty in obtaining an SE-deficient diet that is also I deficient, depletion of the I content of the thyroid was induced by giving 1% sodium perchlorate in tap water [goitrous (I^-) rats], while control (I^+) rats received tap water only, as described in the previous experiments showing the necrotic effects of an I overload (26, 27). Perchlorate is known to compete with I^- trapping by the thyroid, resulting in severe thyroidal I deficiency and high TSH stimulation of the gland. After 5 wk, perchlorate was withdrawn, as it would interfere with the thyroidal uptake of the SCN or I overloads. Twelve hours after perchlorate weaning, I^+SE^+, I^+SE^-, I^-SE^+, or I^-SE^- groups of rats were injected ip with 1 mg sodium I diluted in 0.5 ml saline.

Other I^-SE^+ or I^-SE^- groups of rats were injected ip with 20 mg sodium SCN (Na-SCN; Sigma-Aldrich, Bornem, Belgium) diluted in 0.5 ml saline. The same dose of SCN was injected again 24 and 48 h after the first SCN injection. The SCN-treated groups of rats received 1% SCN in tap water starting after the first ip injection. In addition, to test the possible role of the thyroperoxidase (TPO) enzyme in the hypothesis of SCN toxicity, TPO was blocked in I^-SE^+ and I^-SE^- groups of rats by adding 0.02% methimazole (MMI) to the water of the SCN-treated rats, starting the evening before the SCN overload was administered.

The animals were killed, under Nembutal anesthesia, at different times of the study. I-deficient rats (both SE^+ and SE^-) were killed after 5 wk of perchlorate treatment, before the I or SCN overloads. Rats were also killed 3 and 15 d after the acute I overload or the onset of the SCN treatment.

As previously described (26), in each rat one lobe of the thyroid was quickly removed and weighed. The lobe was cut into two pieces; the first half-lobe was fixed in Bouin's liquid, and the other half was frozen in isopentane, cooled in liquid nitrogen. The rats were then submitted to cardiac puncture; plasma was separated and stored at −20 C for biochemical determinations. The rats were perfused through the heart for 1 min with saline and for 5 min with glutaraldehyde. The thyroids were further processed for morphological and stereological analyses. Aliquots measurements and analyses were made in a blinded manner.

Morphological analysis and stereological analysis

The thyroid fragments after glutaraldehyde perfusion were immersed for 1.5 h in a solution of 2.5% glutaraldehyde (Taab, Reading, UK) in 0.1 M 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, VT).

Sections (0.5 μm) were cut from the center of each fragment and stained with toluidine blue. They were used for counting the number of necrotic thyroid cells (*i.e.* with pycnotic or karyolitic nuclei). Counting was performed at a magnification of ×400 on 100 follicles/section and on 10 sections/experimental group. Results are expressed as the mean percentage of necrotic cells ± SD. The relative volumes of the glandular components (epithelium, follicular lumen, connective tissue, and blood vessels) were determined in each group by stereological methods previously described (26).

Immunohistochemical analysis

The immunohistochemical analysis was performed on frozen sections in which endogenous peroxidase had been inhibited by treatment with periodic acid (0.228%) for 45 sec. Two different antibodies were used as first antibodies. A mouse antibody against rat macrophages and dendritic cells (MCA 275, Serotec, Abingdon, UK), and a rabbit anti TGFβ antibody (AB-20-PB, R&D Systems, Abingdon, UK). The method followed has been previously described (27). The thyroid sections were incubated with the first antibody (MCA 275 for 1 h and anti-TGFβ antibody for 12 h). For the anti-TGFβ antibody, the time of incubation used was chosen to allow clear labeling of inflammatory cells without background staining, as shown previously (27). Thereafter, the sections were incubated for 1 h with a second immunoperoxidase-conjugated antibody. Antibody binding was revealed using diaminobenzidine tetrachloride in the presence of H_2O_2.

Thyroid hormones, thiocyanate, and glutathione peroxidase assays

Thyroid hormone determinations were made by chemiluminescence with commercially available kits for T_4 and T_3 (Chiron Diagnostics, Fernwald, Germany). Thyroid stimulation after perchlorate treatment was assessed by RIA measurement of TSH (Amersham, Little Chalfont, UK). Plasma glutathione peroxidase activity was measured with a commercial kit (Ransel kit, Randox, Crumlin, UK) using cumene hydroperoxide as substrate. Serum SCN measurement was made by spectrophotometry, adapted on a Cobas Mira-S, with phenylenediamine replacing benzidine (30).

Statistical analyses

Comparison of the means between the various groups was made by one-way ANOVA after logarithmic transformation of data, or Kruskal-

Wallis and Mann-Whitney nonparametric tests. Results are expressed as the means ± SD, and as the median and 95% confidence interval for plasma SCN.

Results

SE deficiency

Selenium deficiency decreased the mean plasma glutathione peroxidase to the 1/10th of the value in the SE control groups. The mean GPX was 1450 ± 371 IU/liter in the SE^- rats *vs.* 13749 ± 778 IU/liter in the SE^+ rats. No significant differences in plasma GPX were observed between the various SE-deficient groups.

SCN administration

The median circulating SCN value was 5.7 mg/liter (95% confidence interval, 5.2–7.3 mg/liter) in non-SCN-treated rats. Treatment with SCN increased this concentration significantly ($P < 0.01$) to a median SCN value of 32.6 mg/liter (20.9–86.6 mg/liter) in SE^+ groups of rats and to 18.5 mg/liter (12.9–53.0 mg/liter) in SE^- groups of rats. Plasma SCN showed highly variable individual values among SCN-treated rats. These tended to be lower in SE^- rats compared with SE^+ rats (not significant).

I deficiency and thyroid stimulation after perchlorate administration

The severe I deficiency that accompanies inhibition of thyroidal I trapping by perchlorate resulted in undetectable circulating T_4 and T_3 (Table 1) and in the expected thyroid stimulation, as evidenced by elevated plasma TSH (139 ± 47 ng/ml in perchlorate-treated rats; 16 ± 2 ng/ml in euthyroid rats), and by goiter formation. One lobe of the thyroid weighed 7.4 ± 0.5 mg in the euthyroid (I^+) condition (Table 1) and 17.3 ± 0.2 and 21.0 ± 1.2 mg in the I^-SE^+ and I^-SE^- groups, respectively ($P < 0.05$).

As described in previous experiments (25, 26) using the same protocol, the stimulation of the thyroid observed in the perchlorate-treated, I-deficient rats significantly increased the amount of necrotic epithelial cells compared with that in the euthyroid group (<0.3% in the I^+SE^+ euthyroid group and 5.5 ± 0.4% in the I^-SE^+ hypothyroid group; $P < 0.01$).

TABLE 1. T_4 and T_3 concentrations and weight of one thyroid lobe

	T_4 (μg/dl)	T_3 (ng/dl)	Thyroid weight (mg)
$I+SE^+$	3.0 ± 0.5	72 ± 22	7.9 ± 1.1[a]
$I+SE^-$	3.2 ± 0.5	45 ± 9	9.1 ± 2.1[a]
$I-SE^+$	ND	ND	17.3 ± 0.2
$I-SE^-$	ND	ND	21.0 ± 1.2
$I-SE^+$ NaI, 3 d	3.4 ± 1.2	68 ± 26	20.2 ± 2.6[a]
$I-SE^-$ NaI, 3 d	3.2 ± 0.5	65 ± 9	27.6 ± 4.3[a]
$I-SE^+$ SCN, 3 d	ND	45 ± 25	20.6 ± 1.7[a]
$I-SE^-$ SCN, 3 d	ND	77 ± 26	25.0 ± 6.3[a]
$I-SE^+$ SCN MMI, 3 d	ND	ND	29.1 ± 0.9
$I-SE^-$ SCN MMI, 3 d	ND	9 ± 7	24.2 ± 2.8
$I-SE^+$ NaI, 15 d	2.4 ± 0.3	32 ± 3	12.4 ± 1.8
$I-SE^-$ NaI, 15 d	2.0 ± 0.5	33 ± 4	12.03 ± 0.81
$I-SE^+$ SCN, 15 d	ND	35 ± 18	15.1 ± 7.3
$I-SE^-$ SCN, 15 d	ND	39 ± 10	15.6 ± 2.0

ND, Nondetectable.
[a] $P < 0.05$ (ANOVA analysis after logarithmic transformation).

SE deficiency increased the percentage of necrotic epithelial cells in the glands of hypothyroid rats (9.9 ± 0.6% in the hypothyroid SE-deficient (I^-SE^-) group; $P < 0.01$; Fig. 1). However, this was not associated with any modification of the relative volume of the interstitium (Fig. 2).

SCN overload compared with I overload after 3 d

Three days after the onset of SCN treatment, mean plasma T_3 increased to 45 ± 25 and 77 ± 26 ng/dl in I^-SE^+ and I^-SE^- rats, respectively; the increase was significant ($P < 0.01$) with respect to the concentrations observed before SCN treatment in both groups, whereas T_4 remained undetectable. This increase is likely to be the consequence of the withdrawal of perchlorate treatment just before the onset of SCN overload. T_3 was undetectable in the I^-SE^+ rats treated with MMI and SCN and 9 ± 7 ng/dl in the I^-SE^- rats receiving MMI and SCN. Three days after SCN administration, one thyroid lobe weighed 20.6 ± 1.7 and 25.0 ± 6.3 mg in the I^-SE^+ and I^-SE^- groups, respectively ($P < 0.05$ between I^-SE^+ and I^-SE^-). In the groups treated with SCN and MMI, one thyroid lobe weighed 29.1 ± 0.9 and 24.2 ± 2.8 mg in the I^-SE^+ and I^-SE^- groups, respectively (P = NS between these groups).

The acute I overload was also accompanied after 3 d by increased plasma T_3 concentrations, to 68 ± 26 and 65 ± 9 ng/dl for I^-SE^+ and I^-SE^- groups, respectively. Circulating T_4 also increased to 3.4 ± 1.2 and 3.2 ± 0.5 μg/dl, respectively, in the same two groups ($P < 0.01$ compared with before I for T_3 and T_4, in both I^-SE^+ and I^-SE^- groups). One thyroid lobe weighed 20.2 ± 2.6 and 27.6 ± 4.3 mg in the I^-SE^+ and I^-SE^- groups, respectively ($P < 0.01$).

Cell necrosis

Three days after its onset, SCN treatment significantly increased the proportion of necrotic follicular cells from 5.5 ± 0.4% before SCN to 7.8 ± 0.15% after SCN in the I^-SE^+ groups ($P < 0.001$) and from 9.9 ± 0.6% before SCN to 18.9 ± 1.6% in the I^-SE^- group ($P < 0.001$; Fig. 1).

Concomitant MMI administration significantly reduced the SCN-induced necrosis to 4.0 ± 0.2% in the I^-SE^+ group and 6.8 ± 0.3% in the I^-SE^- group ($P < 0.01$ compared with values in groups not receiving MMI). These values after MMI administration are in the same range as those in I-deficient goitrous rats before SCN administration (Fig. 1).

Three days after acute I administration, necrotic cells were 9.0 ± 0.5% in the I^-SE^+ rats and 20.2 ± 0.3% in the I^-SE^- group ($P < 0.001$; Fig. 1). The necrotic effects, assessed after 3 d of treatment, were multiplied by 2.4 by SCN and by 2.2 by I.

Inflammatory reaction

Thiocyanate administration for 3 d allowed some goiter involution, compared with that of rats before perchlorate withdrawal. The follicular lumina were slightly enlarged, and they contained cell debris. (Fig. 3, A and B). In the SE deficient rats, the colloid was much denser and necrotic cells were more numerous in the epithelial layer (Fig. 3B). Apical

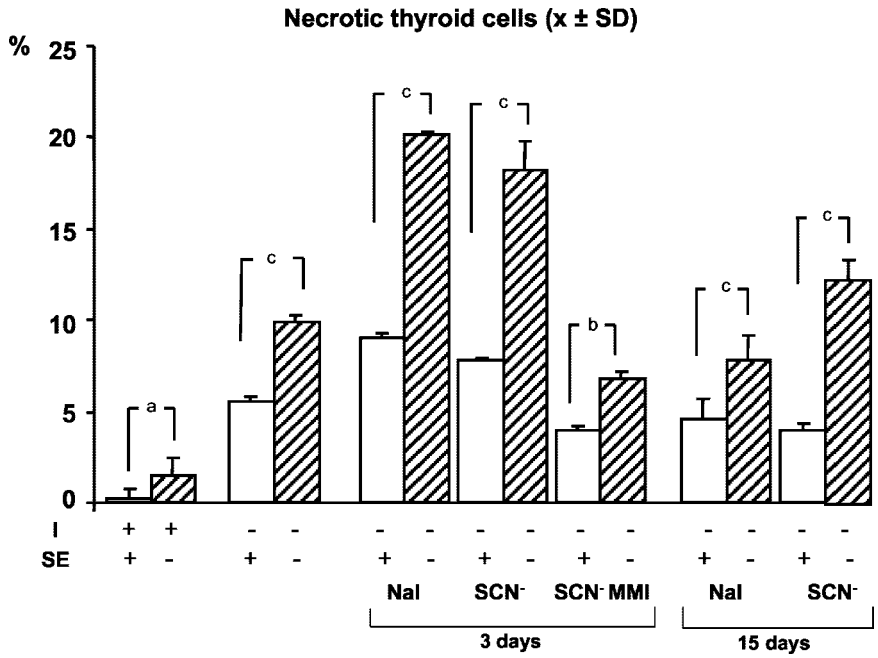

FIG. 1. Mean percentages ± SD of necrotic cells in SE-sufficient and SE-deficient rats. SCN was administered for up to 15 d, whereas the I overload was acute. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$ (by ANOVA after logarithmic transformation).

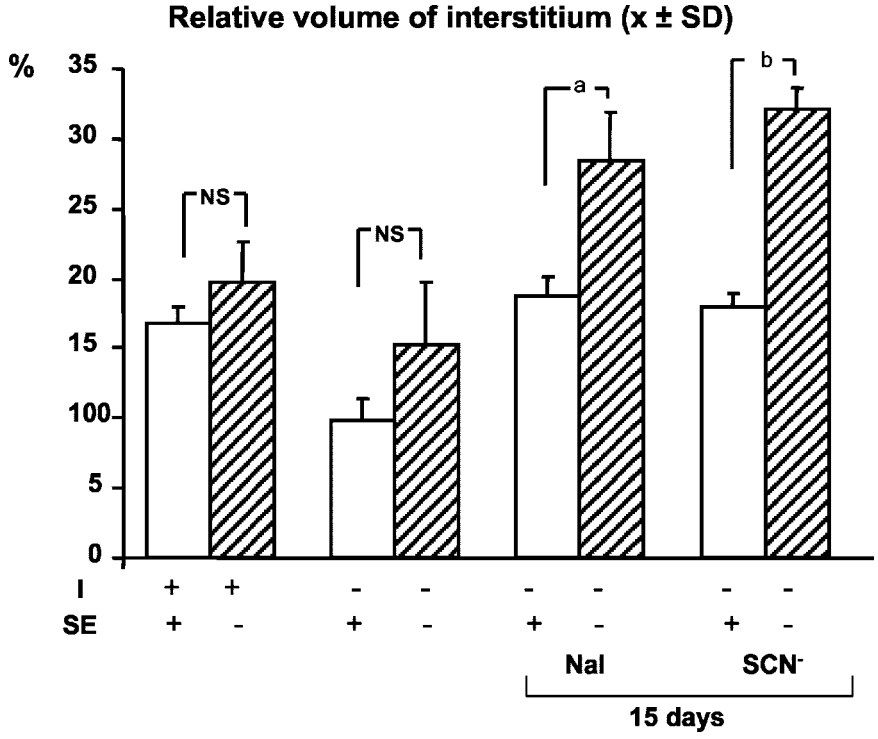

FIG. 2. Mean (±SD) percentage of the surface occupied by the connective tissue in SE-sufficient and SE-deficient rats. a, $P < 0.01$; b, $P < 0.001$ (by ANOVA after logarithmic transformation).

blebs were frequently observed, and they were often large including damaged organelles (Fig. 3, C and D). This suggests that the apical pole is the first site of lesion. The interstitium was infiltrated by mononuclear cells. These mononuclear cells were mainly macrophages immunostained with MCA 275 monoclonal antibody. They were much more numerous in the SE deficient rats (Fig. 4, A and B). In these SE deficient rats, cells producing TGFβ were also frequently observed in the thyroid interstitium (Fig. 4C). No labeling was observed when replacing the first anti-TGFβ antibody by PBS buffer (Fig. 4D).

Necrosis and fibrosis 15 d after the onset of SCN overload

At this time point, mean plasma T_3 was 35 ± 18 and 39 ± 10 ng/dl in the I^-SE^+ and I^-SE^- groups, respectively. T_4 remained undetectable in both groups. The mean weight of one thyroid lobe was 15.1 ± 7.3 and 15.6 ± 2.0 mg in the I^-SE^+ and I^-SE^- groups, respectively (P = NS). Necrotic follicular cells were still 4.0 ± 0.4% and 12.3 ± 1.1% in I^-SE^+ and I^-SE^- groups, respectively (Fig. 1). This last value in the I^-SE^- group was significantly higher ($P < 0.01$) than that in this group in the I-deficient goitrous condition before SCN

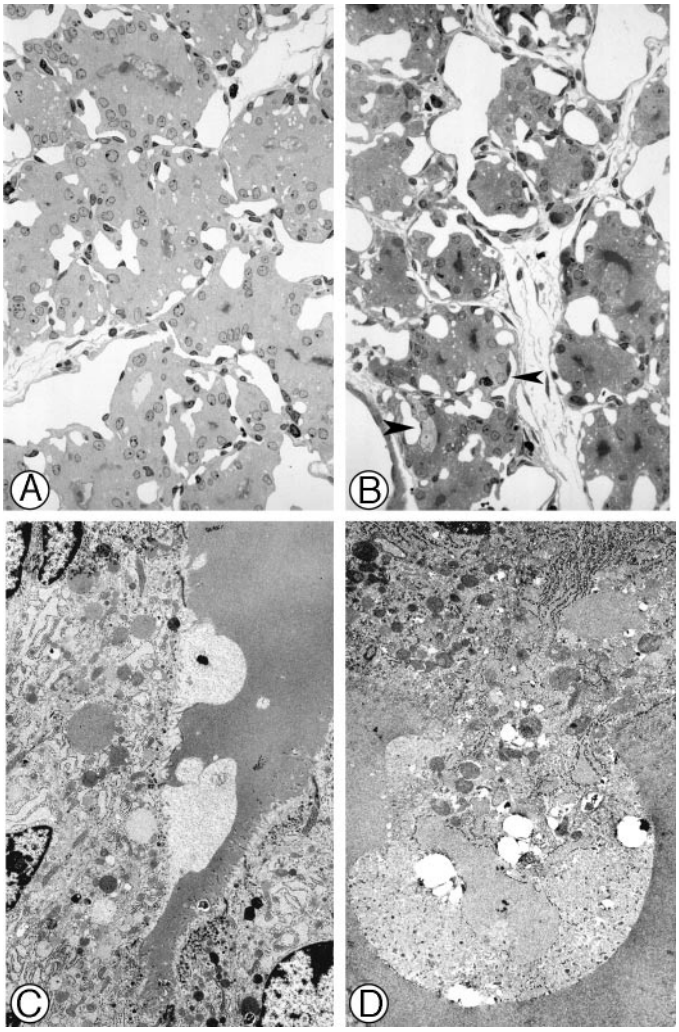

FIG. 3. A and B, Semithin sections of thyroids from I^-SE^+ (A, ×350) and I^-SE^- (B, ×350) rats receiving SCN overload for 3 d. In I^-SE^+ rats, the slightly enlarged follicular lumina contain cell debris. In I^-SE^- rats, the colloid is dense. Necrotic cells are observed in the epithelial layer (*arrows*). C and D, Ultrathin sections of thyroids from I^-SE^- rats (C, ×8500; D, ×7700). Apical lesions are frequent with the formation of blebs (C), sometimes including damaged organelles (D).

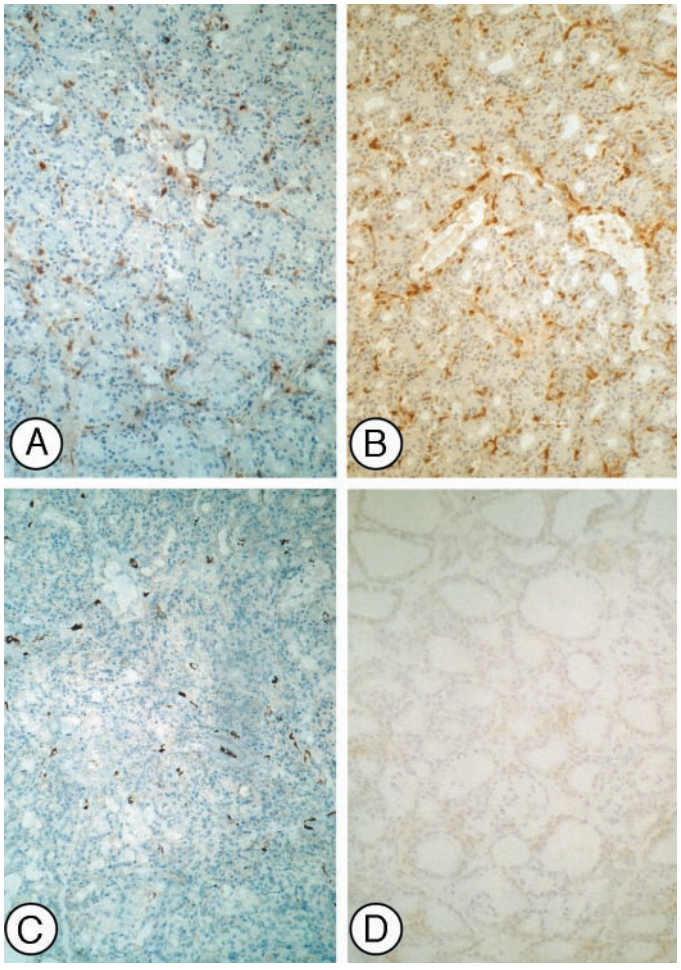

FIG. 4. Immunoperoxidase staining using MCA 275 antibody specific for rat macrophage (A and B) and anti-TGFβ antibody (C) on frozen sections of thyroids from I^-SE^+ (A) and I^-SE^- rats (B and C) receiving SCN for 3 d (×125). Macrophages are more numerous in SE-deficient rats, in which TGFβ-producing cells are detected. No labeling is observed when the first anti-TGFβ antibody was replaced by PBS buffer (D).

and was significantly higher ($P < 0.01$) than the value in this group 15 d after acute I administration.

At 15 d after acute I administration, plasma T_3 levels were 32 ± 3 and 33 ± 4 ng/dl in the I^-SE^+ and I^-SE^- groups of rats; the corresponding plasma T_4 values were 2.4 ± 0.3 and 2.0 ± 0.5 μg/dl. The percentage of necrotic cells was 4.6 ± 0.3% in the I^-SE^+ and 7.6 ± 0.6% in the I^-SE^- thyroids.

After SCN treatment or I overload, the SE-deficient thyroids evolved to fibrosis, while thyroids of SE-supplemented rats did not (Fig. 5). The relative volume of connective tissue 15 d after SCN was 18.0 ± 1.1% and 32.1 ± 1.7% in I^-SE^+ and I^-SE^- thyroids, respectively, compared with 17 ± 1.2% in control I^+SE^+ thyroids. The relative volume of connective tissue 15 d after the acute I overload was 18.7 ± 1.5% and 28.5 ± 3.5% in the I^-SE^+ and I^-SE^- thyroids, respectively (Fig. 2).

Discussion

The present experimental association of three environmental factors: I and SE deficiencies plus SCN excess, represents a short-term nutritional combination akin to the chronic nutritional situation in central Africa. Together, these factors are sufficient to produce thyroid necrosis and progressive fibrosis. A similar chronic interaction of nutriments may therefore explain the progressive thyroid destruction and the elevated prevalence of myxedematous cretinism that characterizes that area of the world. Thereby, it may also explain some of the discrepancies that exist between the various forms of endemic cretinism world-wide.

First, this observation is a new insight into the relationship that exists between SCN and thyroid function. Indeed, the results show that SCN may trigger thyroid cell necrosis if a few conditions are satisfied. These conditions are 1) thyroid stimulation, and 2) SCN oxidation. Although necrosis is present if these two conditions are fulfilled, SE deficiency markedly increases this necrosis.

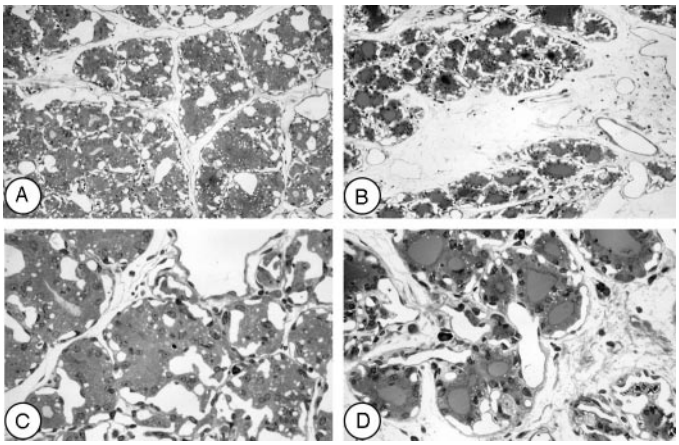

FIG. 5. Semithin sections of thyroids from I^-SE^+ (A, ×120; C, ×400) and I^-SE^- (B, ×120; D, ×400) rats receiving SCN overload for 15 d. In I^-SE^- rats, the gland is dissociated by an extensively developed fibrous tissue made of collagen fibers and fibroblasts.

Thyroid stimulation

Indeed, the necrotic effects of I or SCN are only observed in the experimental condition leading to intense thyroid stimulation, namely in the I^- groups. According to the epidemiological I/SCN ratio findings in the central African endemics, *i.e.* a low I/SCN ratio is required for a significant goitrogenic effect of SCN to be observed, I deficiency should be the cause of high TSH and thyroidal stimulation. Starting from a urinary I/SCN ratio of 7 μg/mg corresponding to normal populations, goiter appears when the ratio is 3, whereas at a ratio of 2, TSH rises markedly, and cretinism become prevalent (9, 10).

The restriction of the necrotic effect of SCN on the I-deficient thyroids could be explained by the influence of thyroid status on SCN metabolism. Indeed, as it occurs with I trapping, SCN trapping by the thyroid cell is increased by TSH (31). The intrathyroidal metabolism of SCN also increases with TSH stimulation, and SCN is rapidly oxidized by the TPO enzyme in the presence of H_2O_2. Therefore, SCN does not accumulate in the thyrocyte, and the thyroid/serum SCN ratio remains low (29, 31). Therefore, the high sensitivity of the I-deficient stimulated thyroid to SCN may be explained by its increased trapping accompanied by an increased metabolism into a toxic metabolite, as postulated below.

SCN has first to be oxidized by TPO, because MMI administration to rats could block the SCN-induced necrosis

This condition is not necessary for the goitrogenic effects of SCN to occur (9). Therefore, it appears that an oxidized metabolite of SCN, rather than SCN itself, is toxic for the thyroid cell. It is likely that MMI blocking of the SCN-induced necrotic effect was not the consequence of decreased SCN trapping at the basal membrane level. Indeed, MMI transport into the thyroid cell differs from the transport of I, and MMI does not inhibit I trapping (32). Presumably, therefore, MMI does not inhibit SCN trapping.

The present description of a necrotic effect of SCN has to be dissociated from its already known goitrogenic effect, *i.e.* competition as a substrate with I for its trapping and for its metabolism at the TPO level. As a goitrogen, SCN is a weaker inhibitor of I trapping than perchlorate (33). Both goitrogens were used in the present experiment. Perchlorate was used first to induce thyroidal I deficiency and intense thyroid stimulation. Its substitution with SCN could not sustain a comparable level of I deficiency, and SCN administration allowed a rise in plasma T_3, goiter involution, and some colloid accumulation.

Which SCN metabolite is toxic for the thyroid and why? SCN metabolism in the thyroid produces at least sulfate (31, 34) and an ill-identified protein-bound sulfur. It is not known whether one of these two end products interferes with thyroid cell regulation and might take part in the observed necrotic effect. However, SCN metabolism has been studied in other systems. From these studies, it is known that sialo-, myelo-, lacto-, and thyroperoxidase enzymes (35) have similar activities, and that hypothiocyanite ($OSCN^-$) is produced from the oxidation of SCN by peroxidase enzymes in presence of H_2O_2 (36–38). $OSCN^-$ is a potent bacteriostatic agent that inhibits bacterial metabolism and growth (39), although $OSCN^-$ itself does not seem to be toxic for the mammalian cell (40, 41).

However, in certain conditions, *i.e.* when H_2O_2 is in excess compared with SCN or $OSCN^-$, further $OSCN^-$ oxidation produces cyanosulfurous acid (O_2SCN) and cyanosulfuric acid (O_3SCN) (42). These oxidized forms of $OSCN^-$ are indeed reported to be more bactericidal than $OSCN^-$, to be toxic for the mammalian cell, and to be more toxic for the cell than H_2O_2 alone (40). In this same condition of H_2O_2 in excess, cyanide (CN^-) formation may also occur, which might take part in the toxic effects of SCN metabolism (43). The first step of SCN metabolism, the oxidation of SCN to $OSCN^-$, appears to be rate limiting in the metabolism of SCN (34). It requires the peroxidase enzyme and H_2O_2. However, the peroxidase enzyme does not appear to be required for further oxidation of $OSCN^-$ to the toxic metabolites. One of the end products of this SCN oxidation process is sulfate, and sulfate is detected in the thyroid as the result of SCN metabolism (31, 34). Together, these findings suggest that toxic SCN metabolites may well occur in the thyroid (Fig. 6), especially in the I-deficient stimulated thyroid gland, where SCN trapping and metabolism are increased as is H_2O_2 synthesis. Moreover, the production of toxic metabolites would be concentrated at the apical membrane level or in the lumen, where I and SCN are concentrated and H_2O_2 generated. This could be related to the apical lesions as blebs.

SE deficiency in this experiment had two major effects. It dramatically increased the SCN-induced necrosis and promoted thyroid fibrosis. Indeed, necrotic cells were present in twice the number in SE-deficient thyroids compared with SE-supplemented glands regardless of whether necrosis was induced by SCN or by I overload. The precise mechanism by which SE deficiency increases tissue sensitivity to necrosis is not known. The initial hypotheses speculated on the importance of the cytosolic GPX enzyme, the first selenoprotein identified in mammals (19), in protecting cells against free radical damage (13). Since then, the role of this GPX enzyme has to be balanced considering the increasing number of identified selenoproteins involved directly or indirectly in

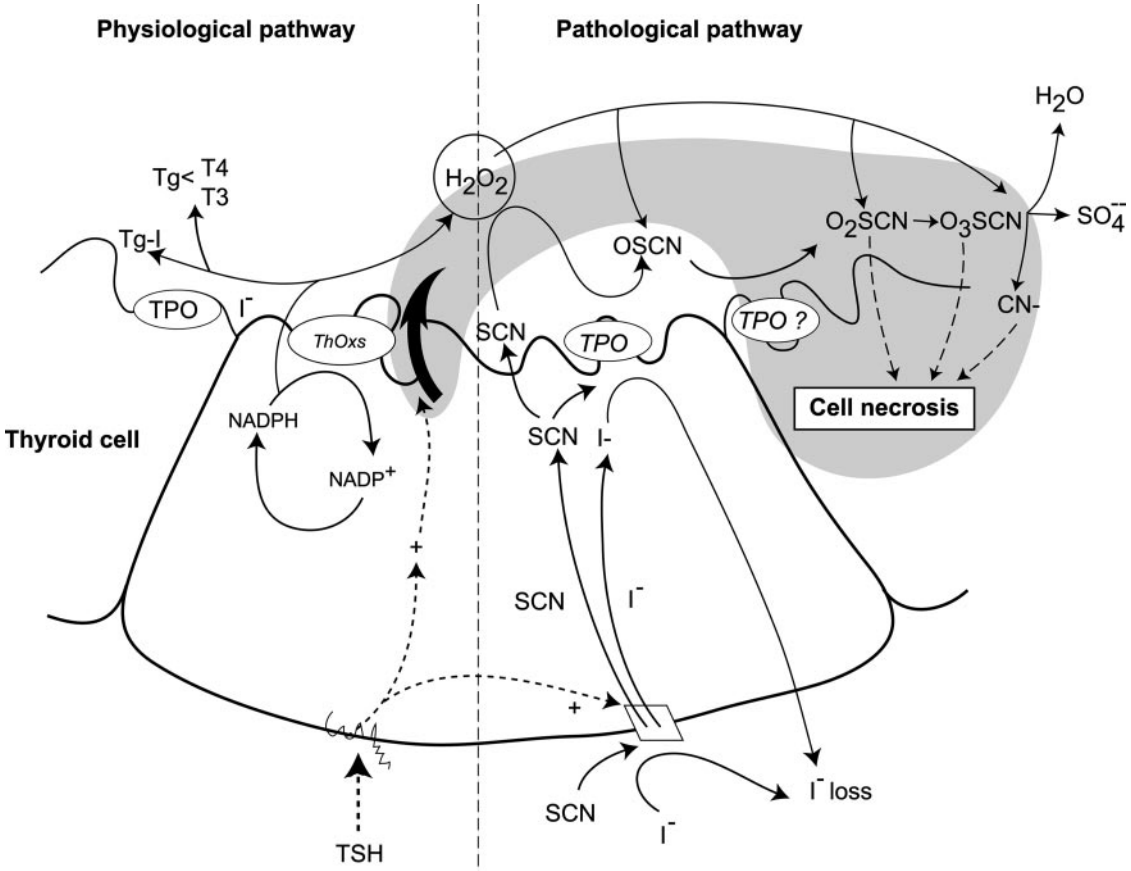

FIG. 6. Goitrogenic and toxic effects of SCN at the thyroid level. In *gray frame*, Hypothesis of the production of toxic metabolites from SCN and H_2O_2, leading to cell necrosis. O_2SCN, cyanosulfurous acid; ThOxs, thyroid oxidases; O_3SCN, cyanosulfuric acid.

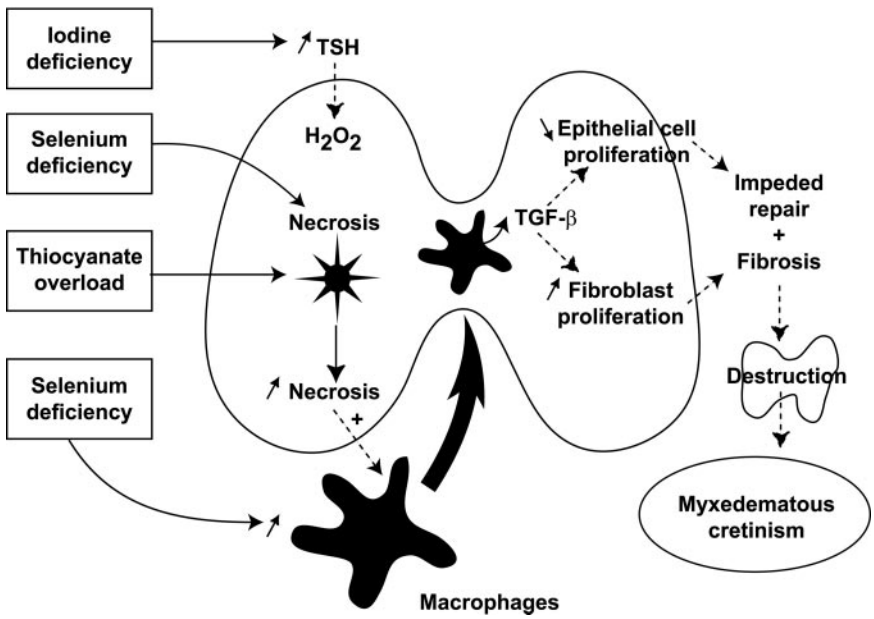

FIG. 7. Hypothesis for the mechanism by which an interaction between I and SE deficiencies and SCN overload induces thyroid necrosis and promotes thyroid fibrosis and, as a consequence, myxedematous cretinism if these conditions are met early in life.

cell defense in a increasingly complex system. Indeed, in some experiments the restoration of SE protection against free radical damage in the liver correlated to SE protein P levels and not to GPX levels (27). The thyroid also could be peculiarly sensitive to cytosolic GPX deficiency. Indeed, in the thyroid, cytosolic GPX is alleged to play a particularly important role by inhibiting protein iodination in the cytosol (44).

An evolution to fibrosis of the SE-deficient thyroids has been described in experiments in which SCN was not in-

volved (26, 27). Therefore, the evolution to fibrosis is not specific to toxic SCN effects, but, rather, is a characteristic of SE deficiency itself. Indeed, the cascade of events leading to fibrosis, which has been described in previous SE experiments (27), has also been observed in the present SCN experiment. In short, in SE deficiency these events are 1) an increased inflammatory reaction in response to necrosis, and 2) the presence of TGFβ-producing macrophages. In I-treated animals, the evolution of the thyroid to fibrosis was prevented by the administration of antibodies against TGFβ (27). Thus, the combination of the three nutritional factors, I and SE deficiencies plus SCN overload, leads to an interaction of these factors that induces inflammation and fibrosis.

The toxic effects of I followed acute administration, whereas those of SCN were prolonged over 15 d. Nevertheless, both treatments had similar consequences.

As represented in Fig. 7, the following model is proposed for the interaction between the combined I and SE deficiencies and the SCN overload. Together, these interactions lead to thyroid destruction. 1) Iodine deficiency causes stimulation of the gland, which is an essential condition for thyroid destruction to occur in the present experimental model. This stimulation leads to increased H_2O_2 synthesis by the thyroid gland. 2) Cell necrosis itself is triggered by SCN metabolism in the highly stimulated thyroid gland. In addition, because of the goitrogenic effects of SCN *per se*, its administration helps to sustain thyroid overstimulation, and this would contribute to render the necrotic effect chronic. 3) Selenium deficiency would initially increase the sensitivity of the thyroid gland to necrosis. Up until then, despite the amount of necrosis, thyroid repair would probably be possible, as the potential of the thyroid to proliferate is not impaired.

However, 4) a second effect of SE deficiency would be to increase the inflammatory reaction that follows necrosis, a process in which macrophages secrete an excess of TGFβ. An excess of TGFβ would definitely jeopardize thyroid repair and enhance fibrosis. This could lead to thyroid destruction and irreversible hypothyroidism. As the nutritional combination herein described has been documented in central Africa, the acute interaction described in our experimental model could explain the chronic process leading to thyroid atrophy and elevated prevalence of myxedematous cretinism in this goiter endemia. As smoking greatly increases serum SCN levels, this combination could also occur sporadically in areas where cassava is not a nutrient.

Acknowledgments

We thank Mrs. M. Stalmans-Falys and Mrs. N. Botterman for their technical help, Mr. Ph. Thiry for help in adapting the technique of SCN measurement, and Mrs. C. H. Heering for the final revision of the manuscript.

Received July 16, 2003. Accepted September 11, 2003.

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This work was supported by Grants 3.4530.93, 3.4506.967 1.5.141.91F, and 1.5.154.93F from the Fonds de la Recherche Scientifique Médicale, the Ministère de la Politique Scientifique, and the Capital Humain Program of the European Economic Community.

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