Changes in the Morphofunctional State of the Thyroid Gland under the Combined Influence of Hypokinesia and Hypothermia

N. S. Khoch, V. V. Lopukhova, and A. D. Gratsianova

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It is shown that under the influence of a 5-day hypokinesia and hypothermia applied as individual and combined factors the rat thyroid gland develops a compromised response, displaying elements of a reaction to both hypokinesia and hypothermia. Some follicles in the central zone of the thyroid were in substantial functional tension, while in other areas of the organ synthesis and secretion were markedly suppressed. A severe deficiency of thyroid hormones in the organism leads to profound metabolic changes, impairments of thermoregulation mechanisms, and even death of some animals toward the end of the study.

Key Words: *thyroid gland; hypokinesia; hypothermia*

Regulation of the rate and direction of metabolic processes is one of the major effects of the thyroid hormones (TH). Together with other hormones, they determine the specific changes enabling the organism to adapt to extreme factors. However, the information regarding the early structural and functional alterations in the thyroid during stress and adaptation is scant and controversial. There are few publications on the thyroids response to a simultaneous influence of factors which, when applied individually, induce qualitatively opposite shifts of the organ's functional activity. Our aim was to study the morphofunctional state of rat thyroid subjected to the individual and combined influence of hypokinesia and hypothermia.

Department of Human Anatomy, Physiology, and Hygiene, State Pedagogical Institute, Tomsk; Laboratory of Radiation Physiology, Institute of Biology and Biophysics, Tomsk State University. (Presented by M. A. Medvedev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 200-230 g. The animals were divided into four groups. Group I rats were placed in individual "pencil-boxes" for modeling hypokinesia, group II rats were maintained in roomy cages in a thermostatically controlled room at 2-4°C, group III rats were kept in "pencil-boxes" at a low temperature, and group IV animals served as the control and were held in a standard vivarium. The rats were decapitated 1, 3, and 5 days after the beginning of the experiment. Plasma thyroxin (T_4) and triiodothyronine (T_3) contents were determined by the standard radioimmune methods (kits produced by the Institute of Biochemistry, Belorussian Academy of Sciences, Minsk). For morphological studies the thyroid was fixed with Schaffer fluid, dehydrated in ethanols, and embedded in paraffin. Serial sections (5-6 μ thick) were stained with hematoxylin and eosin. These preparations were used for general morphological descriptions and

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TABLE 1. Changes in the TH Content of Rat Blood under the Influence of Hypokinesia and Hypothermia

Experimental conditions	T_4, nmol/liter	T_3, nmol/liler	T_3/T_4, %
Control	42.83±2.19	1.86±0.08	1.00
Hypokinesia			
1 day	31.27±0.29*	1.63±0.07	1.20
3 days	22.95±0.14*	0.93±0.15*	1.00
5 days	15.72±0.19*	0.42±0.04*	0.62
Hypothermia			
1 day	28.91±1.43*	1.55±0.07*	1.25
3 days	31.18±1.74*	1.16±0.07*	1.00
5 days	16.59±0.48*	1.13±0.06*	1.55
Hypokinesia + hypothermia			
1 day	30.86±2.01*	1.65±0.03	1.20
3 days	12.44±0.87*	0.70±0.05*	1.30
5 days	15.87±0.75*	0.90±0.04*	1.30

Note. Here and in Table 2: an asterisk indicates $p<0.05$.

morphometric measurements of the main thyroid structures: inner and outer diameters of the follicles, height of the thyrocytes, and diameter of the nuclei, as well as for calculation of the index of colloid accumulation as described elsewhere [1]. These parameters are known to strongly correlate with thyroid synthesis and secretion and are very precise indications of the functional state of the thyroid in rats [14]. Body temperature is an objective parameter characterizing the intensity of metabolic reactions, and therefore we measured the rectal temperature with a TPEM-1 electric thermometer throughout the experiment (5 days).

RESULTS

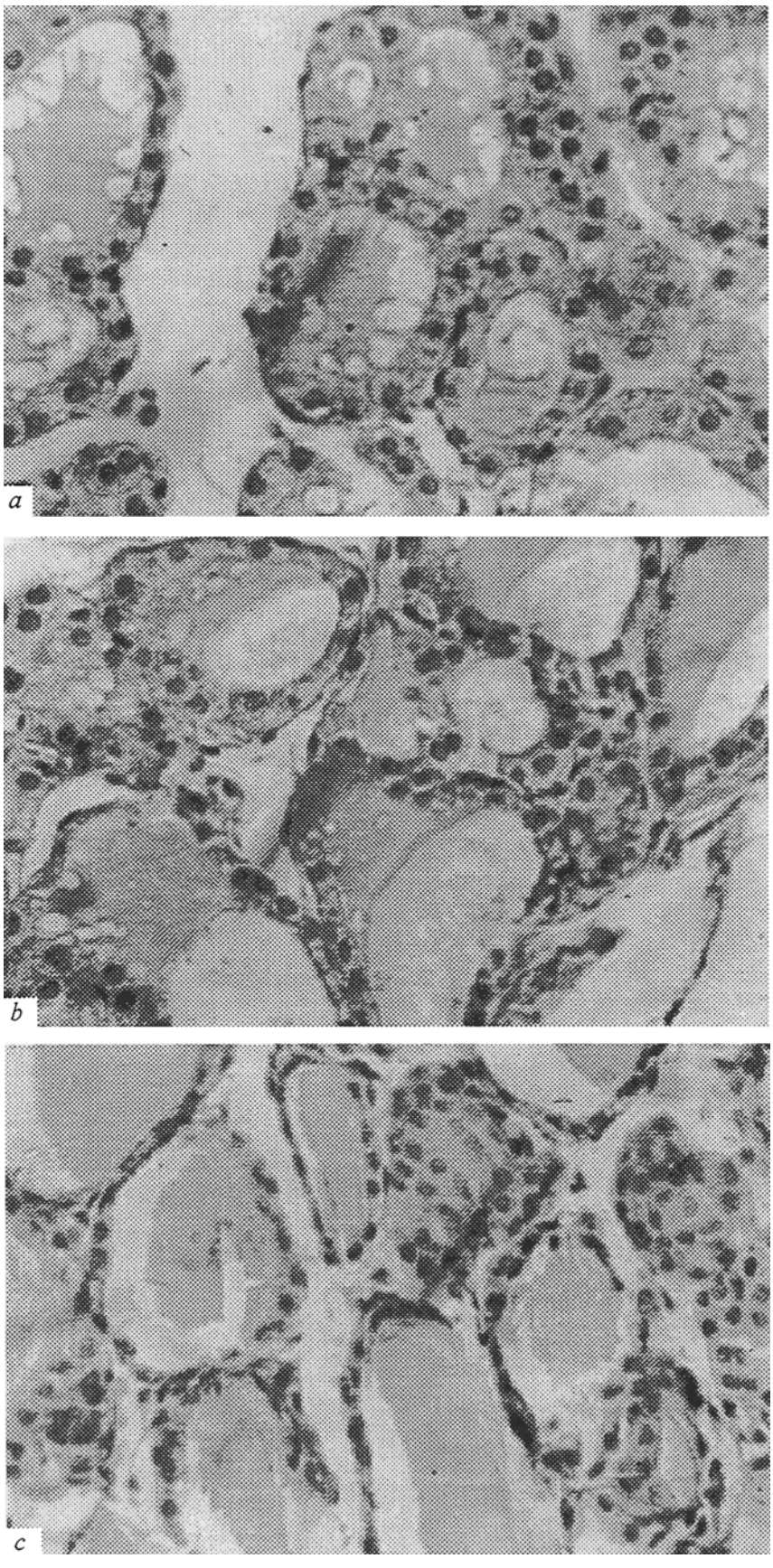
After one day of hypokinesia we recorded a statistically significant decrease in the plasma contents of T_3 and T_4, which progressed during the entire observation period (Table 1). Thyroid insufficiency was aggravated by the shift of the hormonal spectrum (the shift was most pronounced on day 5) toward a prevalence of the less active T_4 (Table 1). However, it should be noted that after one day of hyperkinesia against the background of lowered total thyroid activity the T_3 content was significantly higher than in the control. Most likely this was due to the initial rapidly declining stress-related release of stored hormones [11].

Morphological study confirmed rapid adaptation of the thyroid to diminished energy expenditures of the organism: signs of suppressed functional activity of the thyroid were seen after just one day of hypokinesia: the cells became cuboidal and the follicles became larger. The intrafollicular space was filled with not very dense colloid, and small resorption vacuoles were seen at the periphery of some follicles (a negligible release of hormones into the bloodstream, Fig. 1, *a, b*).

Prolongation of hypokinesia caused a considerable increase in the size of numerous follicles, while the resorption vacuoles in the colloid disappeared, and the index of colloid accumulation rose almost 7-fold compared with the control. The cells

TABLE 2. Morphometry of Rat Thyroid under the Influence of Hypokinesia and Hypothermia

Experimental conditions	Outer diameter, μ	Inner diameter, μ	Thyrocyte height, μ	Nucleus diameter, μ	Colloid accumulation
Control	58.6±2.7	31.6±1.1	13.0±1.6	4.15±0.25	1.20
Hypokinesia					
1 day	67.5±2.8	49.5±1.9*	9.0±1.2*	3.35±0.71	2.75
5 days	91.6±2.8*	81.2±2.4*	5.2±1.5*	2.80±0.54*	7.81
Hypothermia					
1 day	52.3±2.1	25.4±1.6*	13.5±1.3	4.20±0.25	0.94
5 days	39.7±3.1*	10.1±1.4*	15.2±1.1	6.35±0.83*	0.33
Hypokinesia + hypothermia					
1 day (in the center)	53.1±1.4	20.5±1.3*	13.4±1.5	4.21±0.64	0.76
(at the periphery)	59.6±2.1	39.1±2.6*	10.8±1.7	3.73±0.57	1.81
2 days (in the center)	42.3±1.8*	14.9±2.1*	13.8±1.8	4.30±0.75	0.53
(at the periphery	68.4±4.1*	41.5±3.1*	8.4±1.9	3.30±0.63	2.47



of the follicular epithelium became almost flat, and the nuclei became much smaller, dark, and compact (Table 2, Fig. 1, *c*). All these changes are indicative of a marked suppression of thyroid synthetic activity in response to hypokinesia. As a result, in these animals TH deficiency induced a decrease in heat production, which was manifested in a lowered rectal temperature. By the end of the observation period (5th-6th day) this was 1°C lower than in the control group (36.5±0.3°C in the experiment *vs.* 37.6±0.3°C in the control $p<0.05$).

In group II animals, which were maintained at a low temperature, the plasma TH content also decreased as hypothermia was prolonged (Table 1). The dynamics of T_4 was virtually the same as that in group I, whereas the concentration of T_3 declined much more slowly; therefore, at the end of the observation period the relative content of this hormone was 1.55-fold higher than in the control. It is known that the activity of T_3 is 5- to 6-fold higher and its turnover rate is 2- to 3-fold higher than those of T_4 [2]. Therefore, the shift of the hormonal spectrum toward T_3 potentiated the effector thyroid influences on the target cells and stimulated clearance of hormones from the bloodstream.

Morphological observations performed in group II showed that after 1 day of hypothermia the release of stored hormones in the bloodstream increase considerably. As a result, the diameter of numerous follicles decreased, while the number of vacuoles in weakly stained colloid markedly increased (Fig. 2, *a*). By the

Fig. 1. Thyroid gland of intact rats (*a*) and of rats subjected to 1-day (*b*) or 5-day (*c*) hypokinesia. This preparation and the preparations shown in the other figures were stained with hematoxylin and eosin, ×200.

end of the observation period, the mean size of the follicles had further decreased, and many follicles contained no colloid (Fig. 2, *b*). Shrunken follicles with slight epithelial desquamation were seen. The nuclei of the thyrocytes were enlarged and often shifted to the apical pole, which indicates the predominance of basal release [4]. This morphological picture testifies to functional tension of the thyroid and activation of both synthetic and secretory processes in it, as is confirmed by published data [12].

Thus, in this group of rats the initial deficiency of TH in the bloodstream, which became more severe with time, was due primarily to activation of the peripheral metabolism in hypothermia. One of the consequences of activation is the intensification of the main metabolism, which provides almost full compensation for the increased heat losses and enables the rectal temperature to be maintained at a level not statistically different from the control: 37.0±0.4°C (experiment) *vs.* 37.5±0.2°C (control).

A rapid decrease in the blood content of TH was observed in the experiments with the combined influence of these factors (Table 1).

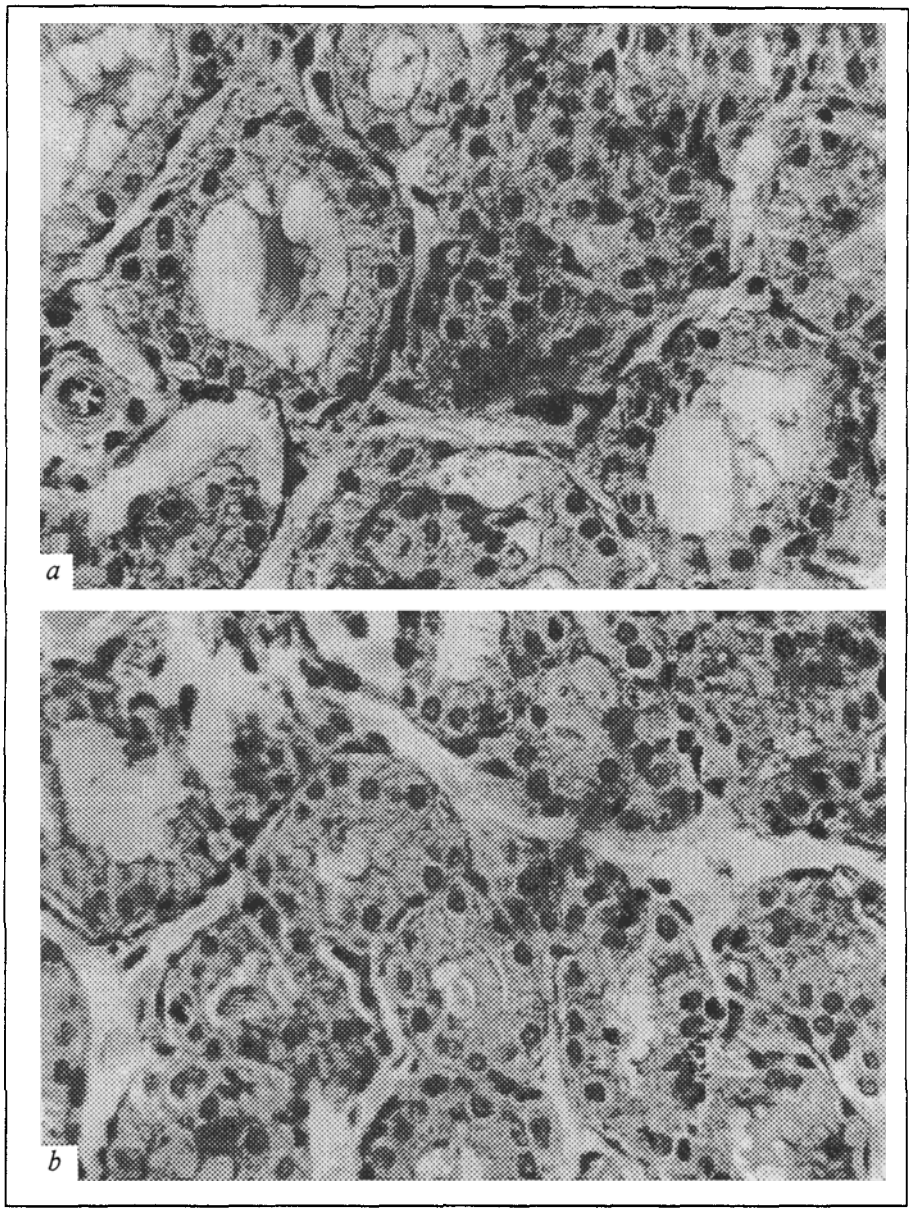


Fig. 2. Thyroid gland after exposure of rats to hypothermia for 1 (*a*) and 5 (*b*) days.

Morphological data obtained on day 1 and, especially, on day 2 of the study showed that hypothermia in combination with hypokinesia did not activate the entire thyroid but only its central zone, where active secretion of TH into the bloodstream occurred. This is confirmed by the very low index of accumulation of colloid, which was weakly stained and contained numerous vacuoles. The mean size of follicles in this area also decreased considerably, and many follicles displayed signs of epithelial desquamation. There were considerable amounts of interfollicular epithelium that had most likely appeared as a result of follicle destruction without the formation of new follicular structures (Fig. 3, *a*). Abundant outgrowth of connective tissue was noted in the interfollicular space. Approximately one-third of the follicles remained active in the central area of the thyroid (the other follicles were destroyed). These observations point to a considerable functional overstrain on this area of the gland.

Meanwhile, hormonal secretion from the peripheral follicles was markedly inhibited, and therefore these follicles were filled with dense masses of intensely stained colloid. Many follicles contained no resorption vacuoles. Epithelial cells became cuboidal and even flat, and the nuclei became smaller and more compact (Fig. 3, *b*). These are indications of inhibited synthesis and secretion in the thyroid. The total region with reduced functional activity was greater than the active region. The secretion of TH in the bloodstream was therefore insufficient, and the blood content of TH continued to drop (Table 1); even

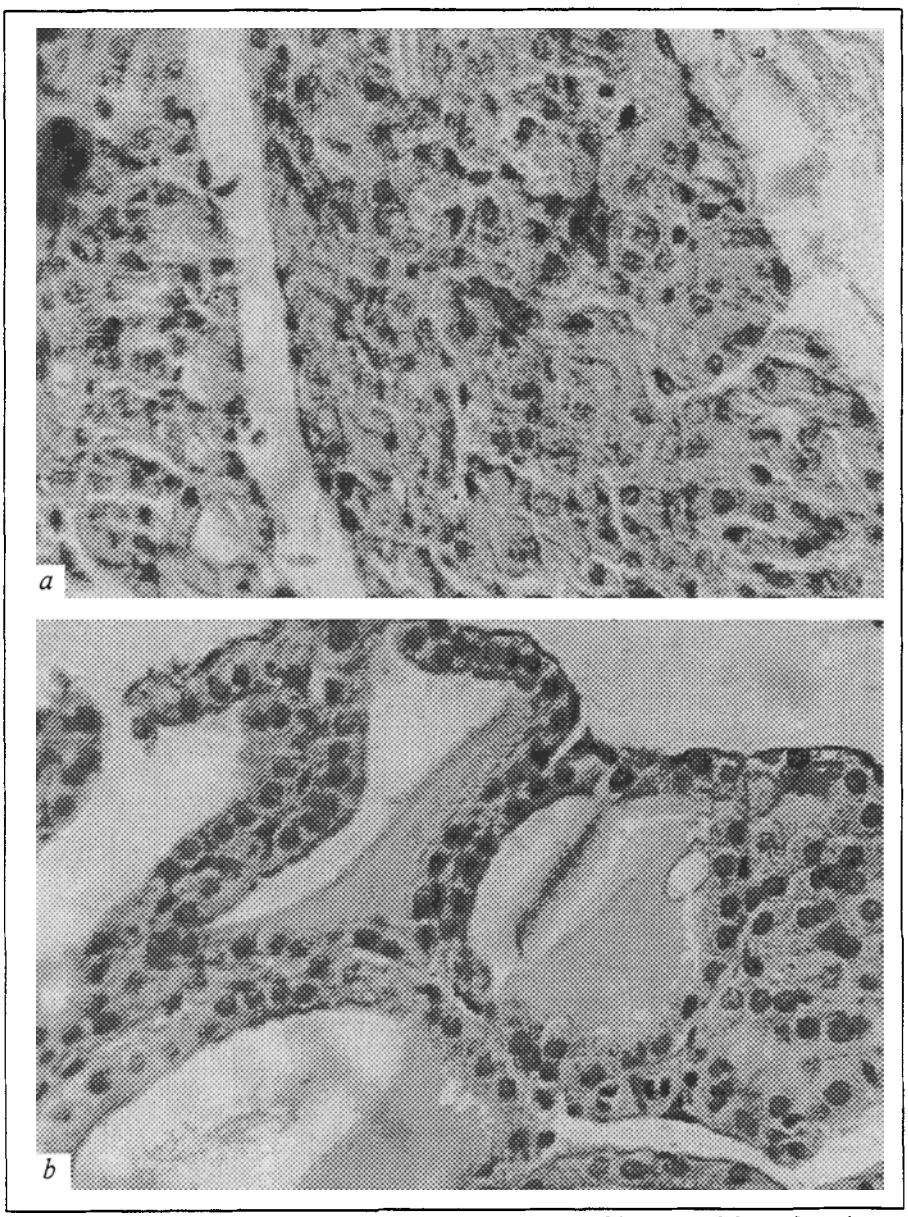


Fig. 3. Thyroid gland after combined influence of hypokinesia and hypothermia on day 2 of exposure: central (*a*) and peripheral (*b*) areas of the gland.

the increase in the relative content of T_3 could not boost the rate of metabolism to the level required for stabilization of the body temperature. The initially lowered body temperature continued to fall and by the 5th day it was 1.5°C lower than in the control: 36.0±0.1°C *vs.* 37.6±0.2°C ($p<0.05$). The slight increase in the blood content of TH was probably due to profound metabolic disorders, a reduced rate of TH clearance being one of their manifestations. Some rats in this group died after being subjected to hypothermia and hypokinesia for more than 5 days.

Analysis of our results showed that hypokinesia (a sharp decrease in the contractile activity of the skeletal muscles, leading to a reduction in proprioception) induces a number of morphofunctional alterations in the organism [5]. The thyroid gland is an organ that quickly responds to hypokinesia by reducing its synthetic and secretory activities. This may be associated with a weaker regulatory stimulation from the higher centers of endocrine regulation that are strongly influenced by proprioception from working muscles [7,8]. The lack of thyroid effector stimulation of the target cells leads to a decline of all metabolic reactions, which are also governed by the functional state of the skeletal muscles [3]. As a result, heat production falls off to such an extent that the organism cannot maintain its core temperature even under a relatively comfortable ambient temperature (23-25°C).

The high activity of the thyroid at low temperatures is geared primarily toward stimulating heat production, since the gland plays a key role in shaping the mechanisms of adaptation to hypothermia. These mechanisms modulate heat productivity of the skeletal muscles, i.e., they increase heat production during muscle contraction [8,13], and stimulate all mechanisms of noncontractile heat generation [11,13]. This is why even prolonged exposure of rats to cold under the chosen experimental conditions did not induce any statistically significant drop in body temperature.

The combined influence of hypokinesia and hypothermia - factors which when applied individually cause opposite changes in the morphofunctional status of the thyroid - induced a compromised response of the thyroid, i.e., elements of the response to both factors were observed. For example, some follicles in the central zone of the gland were in a state of considerable tension, while synthetic and secretory processes were greatly inhibited at the periphery. The fact that numerous follicles in the central zone of the thyroid displayed signs of epithelial desquamation, some follicles were destroyed, the amount of interfollicular epithelium increased without the forma-

tion of new follicles, and there was a proliferation of connective-tissue layers, suggests that only a very small part of the gland was functioning actively. This led to a severe deficiency of TH in the organism, which resulted in profound metabolic changes, impairments of regulatory mechanisms, and even death of some animals subjected to prolonged hypokinesia and hypothermia. The prevalence of the elements of the response to hypokinesia points to a stronger influence of this factor that causes deep-seated multifaceted adverse changes in the organism and thereby lowers its resistance [5,6]. Therefore, the influence of any other factor along with hypokinesia will wreak havoc, particularly in the organs at which both factors are aimed [15].

In this respect, when applied in tandem, hypokinesia and hypothermia (factors that induce qualitatively opposite alterations in thyroid function) cause marked pathological shifts in the structure, synthesis, deposition, and secretion processes occurring in the gland.

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