Structural and Functional Alterations in Thyroid Gland as Elements of Chronic Endotoxicosis

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Chronic endotoxicosis leads to pronounced structural changes in the thyroid gland and hypothyrosis characterized by reduced weight and activity of the thyroid parenchyma.

Key Words: *chronic endotoxicosis; thyroid hormones; thyroid gland; hypothyroidism*

High prevalence of the states aggravated by the development of endogenous intoxication stimulated studies of the basic mechanisms and pathogenesis of polyorganic insufficiency. The pathology of chronic intoxication in so-called "barrier" target organs (liver, lungs, kidneys, and intestine) is explained rather comprehensively [3,4,8]. At the same time, changes in endocrine organs under conditions of chronic endogenous intoxication (CEI) are scarce and contradictory [2,9]. It is known that tissue structure of the thyroid gland (TG) is changed under the effect of various damaging factors.

Our aim was to study structural changes in TG and to examine the profile of thyroid hormones at various terms of CEI.

MATERIALS AND METHODS

The experiments were carried out on male and female Wistar rats (n=35) weighing 201±11 g. The animals were maintained under vivarium conditions on a standard ration with natural day-night cycle and water *ad libitum*. CEI was simulated in rats with predominant hepatic damage produced by multiple injection of low doses of *S. typhimurium* (Sigma) lipopolysaccharides (LPS) in a dose of 0.2 mg/kg combined with 0.5 ml/kg CCl_4 [6]. Structural alterations were assessed on postinjection days 30, 60, and 90 in 3 experimental groups (n=10 in each group). Intact rats comprised the control group (n=5). After the experiments, the rats were sacrificed with 100 mg/kg nembutal.

CEI was verified by plasma content of medium-molecular-weight substances and their oligopeptide fractions, MDA concentration, activity of hepatic and renal acylase. In addition, CEI was assessed by characteristic pathomorphological alterations in the liver (changes in volume fraction of hepatocytes, structure of the connective tissue, and size of Kupffer cell nuclei) and kidneys (volume fraction of interstitial tissue, urinary space, and tubular index) [6].

Plasma concentrations of thyreostimulating hormone (TSH), total and free thyroxin, triiodothyronine, and thyroglobulin were determined in blood serum by ELISA using Stat Fax 2100/2600 (AWARENESS Technology) and Vector-Best kits.

The organs were routinely fixed in neutral 10% formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin according to van Gieson.

Morphometry of TG was carried out in accordance with established principles of systemic quantitative analysis [1,7]. The following parameters were analyzed: the relative volume of the follicular and interfollicular epithelium, colloid, stroma, and vessels; the mean external diameter of the follicles, the thickness of follicular epithelium, and the diameter of thyrocyte nucleus (not less than 30 measurements were performed in each series). The degree of damage was assessed by the number of intact cells and by the count of hypersecretory, ex-

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tremely depleted, dystrophic, and autolytic endocrinocytes.

The data were analyzed statistically using mean values, standard deviation, and representativeness error [5].

RESULTS

On postinjection day 30, the concentrations of medium-molecular-weight substances and their oligopeptide fractions surpassed the corresponding control values by 2.69 and 2.32 ($p<0.05$) times, respectively (Table 1). Then, the content of CEI markers increased, and on postinjection day 90, the corresponding values surpassed the control by 3.18 and 2.53 times (Table 2).

On postinjection day 90, morphometry revealed a significant decrease in the volume fraction of hepatocytes to 75.8±9.9% *vs.* 56.5±3.9% in the control ($p<0.05$). In addition, the fraction of the connective tissue increased by 5.18 times ($p<0.05$). Similar changes were revealed in the kidneys: on postinjection day 90, the fraction of interstitium increased by 275%, while the urinary space decreased by 77% compared to the control ($p<0.05$).

The study of hormonal profile during CEI attested to the development of hypothyroid state (Fig. 1): plasma level of thyroid hormones significantly decreased ($p<0.05$). On postinjection day 90, triiodothyronine concentration decreased more markedly than that of thyroxin (to 78.2±7.4% and 81.4±9.9% from the control), primarily due to hormones not bound to plasma proteins. In contrast, plasma content of TSH increased insignificantly to postinjection day 30 and surpassed the control by only 1.02 times, which probably reflects a compensatory trend. However, further development of CEI promoted exhaustion of TSH: this parameter decreased to 68.6% of the control on postinjection day 90. The observed changes could be functional and transitory responses to the action of the toxic agent and disappeared after the end of exposure.

Morphological study on postinjection day 30 showed normoplastic structure of TG: follicles of various size and shape (predominantly round shape of medium size) with marked content of light homogenous colloid in all follicles and with occasional marginal vacuolation of the colloid were seen (Fig. 2, a).

Thyrocytes had predominantly cubic shape, but in some sites we observed low-prismatic cells

TABLE 1. Biochemical Indices of CEI in Rats (*M*±*m*)

Parameter	Control group	CEI, days		
		30	60	90
Medium-molecular-weight substances, arb. units	0.16±0.02	0.43±0.05*	0.49±0.05*	0.51±0.06*
Oligopeptides, mg/liter	115.5±13.8	268.5±29.8*	289.4±30.5*	291.0±36.4*
MDA, mmol/g lipids	5.33±0.43	11.40±0.94*	10.53±1.25*	11.08±1.35*
Hepatic acylase, μcat/g	27.91±1.93	9.06±0.87*	7.58±0.73*	5.97±0.68*
Renal acylase, μcat/g	35.61±2.90	18.21±1.78*	13.61±1.45*	9.61±1.12*

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Morphometric Indices of CEI Degree in Rats (*M*±*m*)

Index	Control group	CEI, days		
		30	60	90
Liver morphometry				
volume fraction of hepatocytes, %	75.8±9.9	79.3±10.4	62.9±4.7*	56.5±3.9*
volume fraction of connective tissue, %	1.6±0.1	3.9±0.6	6.2±0.4*	8.4±1.2*
mean size of nuclei in Kupffer cells, μ	23.98±3.64	21.56±1.96*	20.88±3.16*	18.29±2.94*
Kidney morphometry				
volume fraction of interstitial tissue, %	7.2±0.4	9.2±0.9	14.5±1.3*	19.7±4.4*
urinary space, arb. units	1.4±0.1	1.2±0.1	1.2±0.1	1.1±0.6*
tubular index, arb. units	0.8±0.1	1.6±0.2	0.6±0.1*	0.5±0.1*

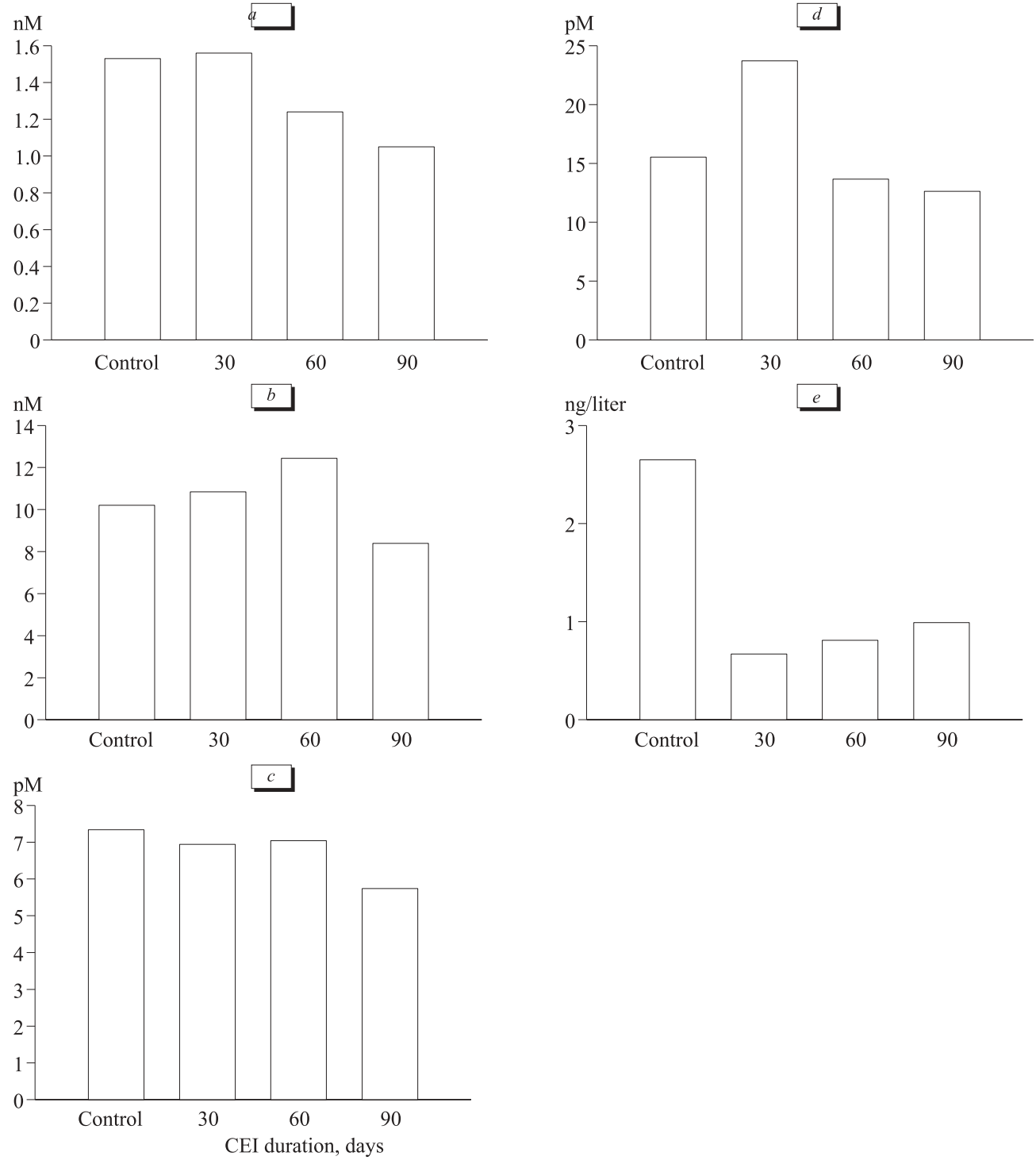

Fig. 1. Serum profile of TG hormones in rats during CEI. *a*) TSH; *b*) total triiodothyronine; *c*) free triiodothyronine; *d*) free thyroxin; *e*) thyreoglobulin.

with disturbed polar differentiation. The cells had centrally located nucleus and light cytoplasm. Pronounced diffused hemorrhagic signs were observed in the intra- and extrafollicular spaces (Fig. 2, *b*). The appearance of diapedetic hemorrhages into the intra- or extrafollicular space can be explained by increased vascular permeability due to direct damaging action of endogenous toxic substances.

On postinjection day 60, the histological pattern of TG was characterized by pronounced polymorphism. Numerous hypertrophic follicles with flattened follicular epithelium were observed. Com-

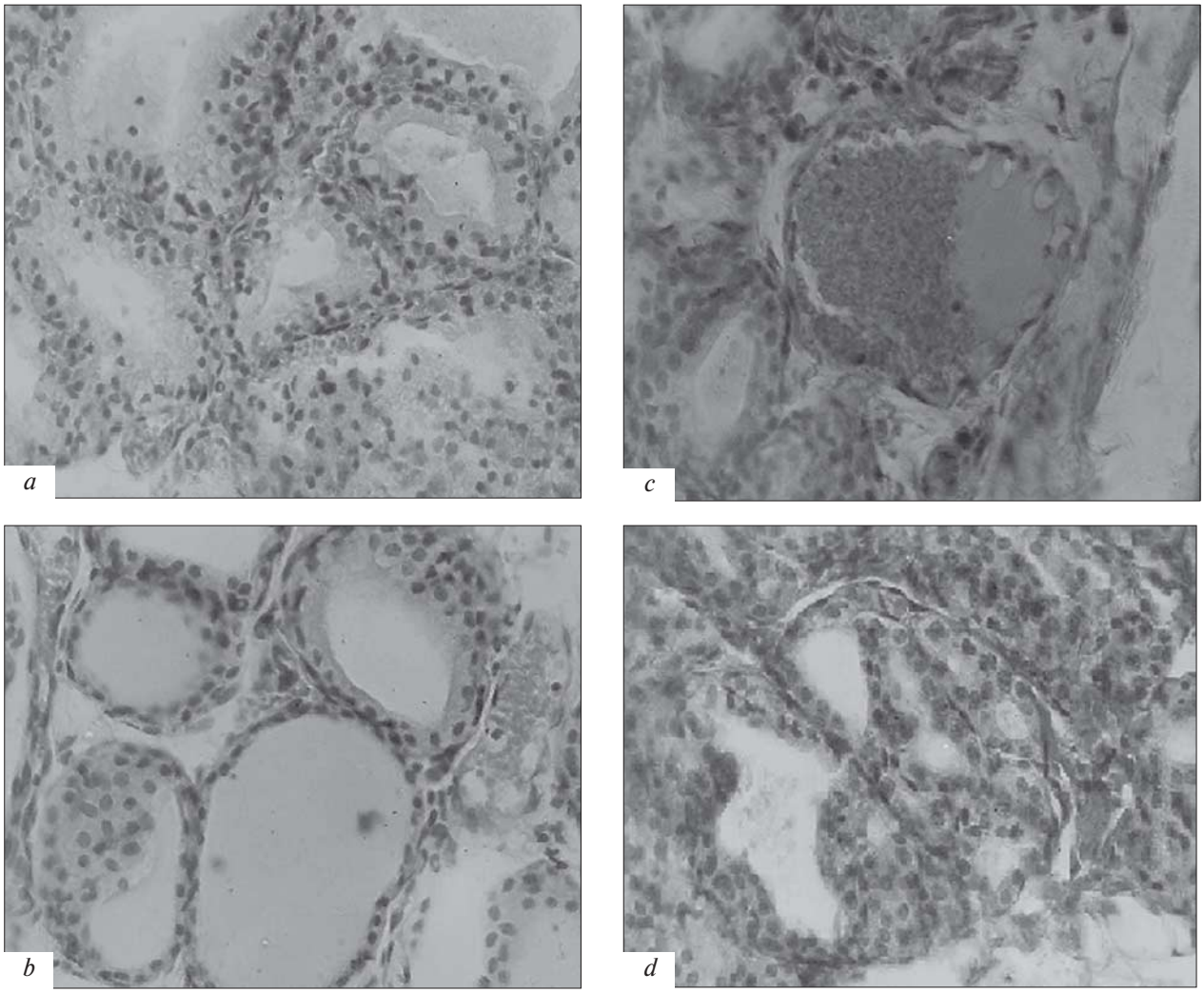

Fig. 2. TG during chronic endotoxicosis. *a*) postinjection day 30: normoplastic structure with cubic thyrocytes; *b*) day 30: intrafollicular accumulation of hemorrhagic exudate; *c*) day 60; *e*) day 90: low content of the colloid in follicles and decrease in follicular size. The shape of thyrocytes is cylindrical. *a-c*): hematoxylin and eosin staining, ×120; *e*) van Gieson staining, ×120.

pression and destruction of follicles were accompanied by degeneration and desquamation of the epithelium into follicles filled with eosinophilic colloid. Moderate intrafollicular proliferation of thyrocytes was accompanied by the formation of Sanderson cushions (Fig. 2, *c*).

On postinjection day 90, follicles formed clusters (thyreons) with common circulatory bed at the level of intrafollicular arteries. There were many small round follicles, which formed lengthy fields. The structure of the thyroid gland became parenchymatous with low content of colorless and locally vacuolated colloid in shrunk follicles. Daughter follicles appeared. Thyrocytes were flattened, their nuclei were rounded, and their cytoplasm was locally vacuolated. We observed focal irregular sclerosis of the stroma with thickening of the capsule and connective tissue septa, which terminated blindly in the parenchyma. The connective tissue proliferated tightly twined round the working follicles and even partially replacing them (Fig. 2, *d*). In most cases, we found hemorrhages of various degrees of severity and incidence ranging from perivascular detection of individual erythrocytes to hemorrhage into a follicle or stroma as well as signs of focal hemosiderosis.

In intact animals, TG had follicular-colloid structure (Table 3), which attested to moderate functional activity typical of euthyreotic state.

On postinjection day 90, the size of TG follicles decreased, and the follicular epithelium flattened. Synchronous decrease of the diameter of nuclei in thyroid epithelium and the amount of colloid was documented (Table 3).

During the progress of pathologic process in TG, the compensatory and adaptive mechanisms were initiated, which manifested morphologically

TABLE 3. Morphometric Indices of TG during CEI in Rats (*M*±*m*)

Index	Control group	CEI, days		
		30	60	90
Mean external diameter of follicles, μ	125.4±11.7	118.9±27.6	115.1±27.8*	91.3±5.2*+
Thickness of follicular epithelium, μ	31.0±9.1	25.1±5.7*	29.8±18.3*	16.5±2.5*+
Diameter of nuclei in thyroid epithelium, μ	10.0±1.4	8.4±1.8	7.2±2.4*	7.1±0.6*
Relative colloid volume, %	18.7±0.4	17.9±0.9	19.9±1.2	10.5±0.9*+
Relative volume of follicular epithelium, %	74.9±3.1	72.4±9.1	50.2±7.4*+	60.9±2.3*
Relative volume of interfollicular epithelium, %	3.9±0.1	4.5±0.1	4.3±0.1	5.2±0.9*
Relative volume of the stroma, %	1.1±0.1	2.4±0.1	16.0±1.1*+	18.5±1.9*+
Relative volume of vascular bed, %	1.3±0.1	2.9±0.1	9.5±0.9*+	14.9±1.2*+

Note. $p<0.05$ compared to *control and +previous term of CEI.

by the development of hyperplastic processes involving the interfollicular epithelium. On postinjection day 90, the relative volume of the follicular epithelium decreased at the expense of interfollicular epithelium. The described changes developed in parallel with pronounced increase of the volume of stromal-vascular component ($p<0.01$).

Therefore, CEI is accompanied by thyroid dysfunction (hypothyroidism), which morphologically manifested by structural changes in TG characterized by a decrease in the volume of functionally intact parenchyma and by moderation of its activity. This state is associated with the development of uncontrolled thyrotoxic crises resulted from the formation of autonomic foci of hormonopoiesis. Thyroid hormones enter the systemic circulation and produce secondary damaging effect to the target organs, thereby participating in CEI maintenance and inducing polyorganic insufficiency.

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