Cadmium (Cd) was administered for 12 months to female rats in drinking water at the concentration of 5 and 50 mg/l, and its effect on the structure and functions of the thyroid follicular cells was assessed. The function of the follicular cells was evaluated by measuring the levels of serum thyroid hormones such as T3 and T4, and TSH. The structure of these cells was assessed under a light microscope. Cd, in spite of its low accumulation in the thyroid, damaged dose-dependently the structure and function of the thyroid follicular cells. At the exposure to 5 mg Cd/l, changes in their structure, but not in the function, were observed. However, the intoxication with 50 mg Cd/l led to both structural and functional damage. Our findings seem to suggest that people exposed to Cd can be at a risk of thyroid damage.

Key words: rats, cadmium, thyroid gland, structure, function.

Cadmium (Cd) is well known to damage various organs and tissues, especially the kidneys, lungs, testes and bones, in humans and experimental animals (2, 19). The available literature provides some data that Cd can also affect the thyroid (7, 10, 18). Yet the influence of Cd on the thyroid is poorly understood.

The thyroid is an endocrine gland whose follicular cells and parafollicular cells (C cells), synthesize and secrete important regulatory hormones, including triiodothyronine (T3), tetraiodothyronine (T4) and calcitonin (CT). Iodothyronines (T3, T4), synthesized in follicular cells, are necessary for normal growth and development (20). Parafollicular cells, via CT secretion, play an important role in the regulation of calcium and phosphate metabolism (15, 16).

We have already reported that Cd affects the thyroid parafollicular cells (13, 14). The present study was aimed to evaluate the effect of chronic Cd exposure on the function and structure of the thyroid follicular cells.
Material and Methods

Experimental design. Twenty-one inbred young female Wistar rats (initial body weight ~100 g), divided into three groups of 7 animals each, were given drinking water containing 0 (control group), 5 and 50 mg Cd/l (as CdCl₂) for 12 months. The animals were housed in environmentally controlled conditions and had free access to drinking water (redistilled water or water solutions of CdCl₂), 24-h consumption of which was monitored during the whole experiment, and rat chow (LSM dry diet; Motycz, Poland). At the end of Cd administration, after overnight fasting, all the rats were anesthetized with vetbutal (30 mg/kg b.wt., i.p.). Blood samples, taken from the heart, as well as both thyroid lobes (together with parathyroids) were collected for analysis.

The study was carried out in accordance with national and international laws and Guidelines for the Use of Animals in Biomedical Research and was approved by the Local Ethic Committee for Animal Experiments in Bialystok.

Estimation of Cd concentration. Cd concentration in the whole blood and the thyroid was determined by flameless atomic absorption spectrometry (Atomic Absorption Spectrophotometer Z-5000, HITACHI, Japan) as reported previously (1, 8).

Estimation of T₃, T₄ and TSH (thyroid-stimulating hormone). T₃, T₄ and TSH serum levels were determined radioimmunologically, using commercially available kits (T₃-PROP MJ-109/F, T₄-RIA-PROP MJ-110/F, and TSH-IRMA MI-112 Orion Diagnostica, respectively). A mini-gamma spectrophotometer (LKB, Finland) was employed. All assays were performed in duplicate.

Thyroid preparation for morphological examination. The thyroid lobes were fixed in Bouin’s fluid at room temperature for 24 h, embedded in paraffin, sectioned at 5 μm and routinely stained with haematoxylin and eosin (H&E). Histological structure of follicular cells of the thyroid was evaluated under a light microscope (NIKON ECLIPSE E 400, USA).

Statistical analysis. Data are mean ± SEM. The results were analysed statistically by one-way analysis of variance (ANOVA). Pearson correlation was performed for the relationship between some of the parameters. The level of significance was P < 0.05.

Results

Cd intake. An average Cd intake, calculated on the basis of fluid consumption, was 0.130 ± 0.002 mg/24 h (ranged from 0.28 to 0.82 mg/kg b.wt./24 h) for 5 mg Cd/l and 1.162 ± 0.017 mg/24 h (ranged from 2.25 to 6.5 mg/kg b.wt./24 h) for 50 mg Cd/l.

Thyroid weight. Cd had no effect on absolute and relative thyroid weights (data not shown).

Blood and thyroid Cd concentration. The exposure to Cd resulted in an increase in the concentration of this metal in the blood and its accumulation in the thyroid, depending on the level of exposure (Fig. 1). Cd concentration in the blood positively correlated with its accumulation in the thyroid (r = 0.942, P < 0.001).
T3, T4 and TSH levels in serum. The exposure to both Cd concentrations had no influence on the serum T3 concentration (Table 1). At 50 mg Cd/l, a decrease (by 20.5%, P < 0.01) in T4 level and an increase (by 27.7%, P < 0.005) in the T3/T4 ratio, compared to the control, were noted (Table 1). Serum concentration of TSH in the 50 mg Cd/l group tended to increase, but the difference was not statistically significant (Table 1). Serum T4 level and T3/T4 ratio correlated negatively (r = 0.598, P < 0.005) and positively (r = 0.791, P < 0.001), respectively, with blood Cd concentration. There was also a correlation between Cd concentration in the thyroid and the serum level of T4 (r = 0.576, P < 0.01) and T3/T4 ratio (r = 0.768, P < 0.001). There was no correlation between serum T3 and TSH, and Cd in the blood and thyroid.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>T3</th>
<th>T4</th>
<th>T3/T4 ratio</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td>x10³</td>
<td>(IU/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>0.614 ± 0.037</td>
<td>31.09 ± 1.87</td>
<td>19.79 ± 0.50</td>
<td>0.053 ± 0.006</td>
</tr>
<tr>
<td>5 mg Cd/l</td>
<td>0.614 ± 0.022</td>
<td>28.29 ± 1.42</td>
<td>21.57 ± 0.70</td>
<td>0.051 ± 0.005</td>
</tr>
<tr>
<td>50 mg Cd/l</td>
<td>0.626 ± 0.030</td>
<td>24.71 ± 0.33</td>
<td>25.28 ± 1.05</td>
<td>0.069 ± 0.014</td>
</tr>
</tbody>
</table>

Microscopic examination of the thyroid follicular cells. The morphological picture of the thyroid follicular cells in the rats exposed to Cd differed from the proper structure observed in the control animals (Fig. 2, Table 2). Changes of a similar kind, but of various intensity, were observed at both levels of exposure. At 5 mg Cd/l these changes were slight, whereas at 50 mg Cd/l they were seriously advanced (Table 2).
Table 2

Results of light microscopy examination of the structure of thyroid follicular cells in rats given drinking water containing Cd for 12 months

<table>
<thead>
<tr>
<th>Change</th>
<th>$5 \text{ mg Cd/l}$</th>
<th>$50 \text{ mg Cd/l}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Some follicles of the thyroid were lined with high-toned epithelium and had light cytoplasm</td>
<td>$0$</td>
<td>$7$</td>
</tr>
<tr>
<td>Presence of desquamated epithelial cells inside the follicles</td>
<td>$3$</td>
<td>$0$</td>
</tr>
<tr>
<td>Mononuclear cell infiltration of follicle connective tissue</td>
<td>$7$</td>
<td>$0$</td>
</tr>
</tbody>
</table>

The change was evident: + in a few follicles, ++ in many follicles, +++ in almost all follicles; the figures show the number of animals of the group in which the change was observed.

Fig. 2. Sections of the thyroid of rats. A. Control group. Follicles lined with cubic epithelium lie on the periphery of the gland. B. A group exposed to $50 \text{ mg Cd/l}$ for 12 months. The presence of desquamated epithelial cells inside the follicles, mononuclear cell infiltration of follicles in connective tissue and follicles lined with high-toned epithelium and light cytoplasm can be observed. H&E, x80.

**Discussion**

The present study was conducted to assess the influence of chronic intoxication with Cd on the structure and function of the thyroid follicular cells. The function of follicular cells was evaluated by measuring the levels of serum thyroid hormones such as T3 and T4, and pituitary TSH. The structure of these cells was...
assessed under a light microscope. The applied experimental model can reflect situation of exposure to Cd which may take place in human life (3, 5, 8, 19).

Follicular cells of the thyroid are designed for hormone synthesis and secretion. T3 and T4 are the predominant circulating thyroid hormones synthesized and secreted by follicular cells in vertebrates. T3 is considered a biologically active thyroid hormone and most of the circulating T3 is generated by extra-thyroidal deiodination of T4, taking place mainly in the liver (6, 11). However, T4 is synthesized only in the follicular cells of the thyroid (6). Serum levels of thyroid hormones, including T3, T4 and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals. Changes in the serum concentration of these hormones can reflect disturbances in their glandular synthesis and/or secretion as well as disorders in their extra-thyroidal peripheral metabolism. Thyroid hormones are metabolized in peripheral tissues (by deiodination, conjugation, deamination and decarboxylation) and alterations in their metabolism may significantly influence the function of thyroid hormone metabolites at the cellular level (4, 9, 11).

The thyroidal Cd accumulation in the Cd-exposed rats was very low, compared to its accumulation in other organs of these animals, especially in the liver and kidney (data not shown). Similarly, in the thyroid samples from adults not exposed occupationally, the concentrations of Cd were considerably lower than in the kidney, being a main and age-independent place of its storage (17). In the available literature there is a lack of any other data referring to Cd concentrations in the thyroid gland.

In spite of low Cd retention in the thyroid, less or more serious damage to the thyroid follicular cells was observed in the female rats chronically exposed to this heavy metal. Cd influenced dose-dependently the structure and function of these cells. At the lower exposure, changes in the structure, but not in the function, of the thyroid follicular cells occurred, whereas at the higher Cd concentration both structural and functional damage to these cells was observed.

It has been suggested that Cd interferes with the thyroid function at the glandular level as well as at the peripheral level by inhibiting the conversion of T4 to T3 (4, 6, 7, 11). Since the thyroid gland is the only organ involved in T4 synthesis, the decrease in the serum level of this hormone in the Cd-exposed rats, together with unchanged concentration of T3, may suggest that Cd influences the production and/or secretion of T4 by follicular cells. Yoshizuka et al. (18) have speculated that the metal accumulated in the mitochondria of thyroid follicular epithelial cells can inhibit the synthesis and release of thyroid hormones influencing the oxidative phosphorylation of these organelles. The probability of Cd interference in the synthesis and/or secretion of T4 by the thyroid follicular cells is supported by the results of morphological examinations. These revealed a damaging action of Cd, at both levels of exposure, on the structure of follicular cells. A tendency towards an increase in the serum TSH concentration observed at the higher level of exposure to Cd is a likely response to decreased serum T4 level. The lack of significant response of TSH to decreased serum T4 level may suggest Cd interference with pituitary regulation of thyroid hormones production and secretion (12). As the concentration of T4 was reduced and that of T3 remained unchanged, the T3/T4 ratio was elevated.

Most of the circulating T3 originates from the extra-thyroidal tissues. The peripheral deiodination of T4 to T3, taking place mainly in the liver, is dependent on 5'-monodeiodinase (5'-D) activity (4, 11). At this stage of studies it is difficult to explain why T3 serum concentration in the Cd-treated female rats was unchanged. At least two possible mechanisms should be taken into account. The first is likely to be
connected with a lack of Cd influence on T3 thyroidal production and secretion as well as on its extra-thyroidal production from T4. The second mechanism may be associated with the enhanced extra-thyroidal transformation of T4 to T3 to compensate decreased glandular synthesis and/or secretion of T3. As the morphological damage to the parafollicular cells, decreased serum T4 level and a tendency to increase that of TSH were noted, the mechanism is also probable. On the other hand, an inhibiting effect has been reported of Cd on 5'-D activity, being a seleno-enzyme containing a seleno-cysteine residue as its active site (4, 11). Cd can inhibit 5'-D activity through binding to sulphydryl groups of this enzyme.

In the available literature there are some data on the effect of Cd on thyroid hormone concentrations in serum. But they are very often inconsistent. Various directions of changes in the serum T3, T4, and TSH as well as their lack have been reported depending on an experimental model used (6, 7, 12, 18). Nishijo et al. (10) showed a decrease in serum free T4 in women and an increase in T3 level of both sexes from a Cd-polluted area as compared to those from a non-polluted region.

In summary, the observations made in this study, together with our previous findings (13, 14), indicate that Cd damages the structure and function of both follicular and parafollicular cells of the thyroid. Severity of the disturbances increases with the level of exposure. Our results unambiguously show that Cd affects the thyroid follicular cells at the glandular level but they do not allow recognition of Cd effect on the thyroid function at the peripheral level. We have obtained important data on Cd interference in the thyroid gland, yet this problem requires further studies since mechanisms of this action are still poorly recognized.

References