RETARDED BIOSYNTHESIS OF THYROGLOBULIN SUBUNITS AND THEIR POLYMERIZATION IN VITRO AFTER SUPPRESSION OF TSH SECRETION IN RATS BY CHRONIC ADMINISTRATION OF EXCESS THYROXINE IN THE FORM OF IODINATED CASEIN

By

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ABSTRACT

The effects of short- and long-term (from 3 days to 28 weeks) administration of excess thyroxine in the form of the iodinated casein “Protamone” (0.2 %) to rats on the content of soluble thyroid iodoproteins and on biosynthesis and polymerization of thyroid proteins in vitro were investigated. The concentration of soluble iodoproteins significantly increased (40–80 %) up to 2 weeks of treatment, and after that remained at the same level. 27S iodoprotein markedly increased during thyroxine treatment. The content of DNA in the gland and the thyroid weight were a little lower in the treated rats than in the control. The incorporation rate of [14C]leucine into soluble and microsome-bound proteins in vitro was markedly reduced in the treated animals (30–80 % of control). Thyroxine pre-treatment of rats induced retarded synthesis of thyroglobulin and its subunits in vitro. The inhibition of the synthesis of...
Thyroid stimulating hormone (TSH) stimulates a number of metabolic processes in the thyroid gland (Dumont 1971). The resorption of colloid from the follicular lumen (endocytosis) and subsequently the release of thyroid hormone, which results from intracellular proteolysis of thyroglobulin (TG) by lysosomes are under TSH control. In addition, TSH stimulates all stages of thyroid iodide metabolism and synthesis of TG (Pavlović-Hournac et al. 1971) and its iodination (Wilson et al. 1968). Under normal physiological conditions in the thyroid gland, two important processes take place simultaneously: first, endocytosis of colloid as a process for its transport from lumen follicle into cells and second, exocytosis or secretion of TG from the thyrocytes into the follicular space. There is a harmonious equilibrium between these two processes.

Administration of thyroid hormones causes suppression of TSH secretion (Fukuda et al. 1975) and consequently nearly complete inhibition of endocytosis and release of thyroid hormones, while exocytosis of TG in the thyrocytes is little affected (Björkman et al. 1974). Our previous morphological and functional investigations showed that chronic application of thyroxine to rats in the form of iodinated casein led to almost complete inhibition of thyroid activity (Jovanović et al. 1970–1973).

It is well documented that iodination of TG is the most important factor in the formation of mature, as well as iodinated TG (Seed & Goldberg 1965; Nunez et al. 1966; Inoue & Taurog 1968) and particularly in the polymerization of TG into 27S iodoprotein (Sinadinović et al. 1971, 1973; Frati et al. 1974). Since the last process occurs in the follicular lumen, as well as TG iodination, reduced endocytosis probably influences the formation of 27S iodoprotein.

The aim of the present studies was to obtain more information about the influence of long-term suppression of endogenous TSH secretion on the synthesis and polymerization of thyroid iodoproteins under conditions in which both endocytosis and iodination were inhibited at the same time. Therefore, we performed an experiment in which the influence of TSH on the processes
of iodination and endocytosis of colloid were excluded by chronic administration of thyroxine in the form of the iodinated casein “Protamone” in a dose which is markedly above the physiological level.

**MATERIAL AND METHODS**

*Animal and treatment.* – Male Wistar rats were used in all the experiments. The initial weight of the animals was about 150 to 200 g. The animals fed a diet, prepared in our laboratory, containing approximately 50 μg iodine/kg. The rats were given NaI in their drinking water, so that each animal received about 6 μg iodine per day. Iodinated casein “Protamone” (Agri-Tech. Kansas City, Missouri) as a source of thyroactive compounds, mainly as thyroxine (Mischler & Reineke 1970) was given to rats in a concentration of 0.2% in the diet for various time periods from 3 days to 28 weeks. At various time intervals the following were determined: radioiodine uptake by the thyroid, thyroid weight and DNA content in the gland, content and composition of soluble thyroid proteins and the dynamics of incorporation of a labelled amino acid into soluble and microsome-bound proteins in *vitro*.

Five to ten rats were injected ip with 5 to 30 μCi of Na¹³¹I and their thyroids were used for determination of the 24 h uptake of iodine by the thyroid.

*Thyroid tissue and incubation conditions.* – The thyroid glands were removed immediately after sacrificing the rats, cleared of connective-fatty tissue and weighed. Fresh thyroid tissue was incubated in 2 ml of basic Eagle medium (Eagle 1959) prepared without leucine in the presence of 1.25 or 2.50 μCi of leucine, SA 331 mCi/mM in a Gallenkamp incubator at 37°C in an oxygen-CO₂ atmosphere (95:5 %/%) with gentle shaking.

*Preparation of thyroid extract and protein analysis.* – At the end of the incubation period the thyroid tissue was homogenized in 0.1 m KCl in a glass homogenizer with a teflon pestle. Soluble and microsome-bound (solubilized) proteins were prepared and analyzed as previously described (Sinadinović et al. 1971, 1975). Briefly, the thyroid tissue after homogenization was centrifuged in a Sorvall RC-2B centrifuge at 800 × g for 10 min to remove nuclei and cellular debris; the supernatant was centrifuged at 15 000 × g for 10 min; the post-mitochondrial supernatants were centrifuged at 105 000 × g for 1 h in a Spinco L50 Beckman centrifuge.

The concentration of soluble and solubilized proteins after dialysis in the sample were determined according to Lowry et al. (1951).

Sucrose density gradient centrifugation was performed in the SW 50.1 rotor of a Spinco L50 centrifuge as described in detail previously (Kraincanić et al. 1970; Sinadinović et al. 1971). One ml PBS, pH 7.4 was added to each fraction from gradient (12 drops) and after measuring the absorbance at 280 nm, the proteins were precipitated with cold 10 % trichloroacetic acid (TCA). As protein carrier bovine serum albumin (50 μg/100 μl) was added to each fraction before precipitation. The protein precipitates were filtered through Sartorius filters and the protein collected on the filter was washed twice with 5 to 6 ml of 5 % TCA to remove the non-protein activity. After drying at 105°C the filters were dipped in 10 ml of scintillation solution (0.5% PPO and
0.03% POPOP in toluene). The radioactivity of protein samples was counted in a Nuclear Chicago Liquid Scintillation Counter. The incorporation of $^{14}$C]leucine into protein was expressed as dpm in total incubated tissue, or per 100 mg wet tissue, or per unit soluble protein.

DNA was determined according to the method of Burton (1956).

RESULTS

A. Thyroidal radioiodine uptake

The radioiodine uptake was much lower in Protamone treated animals. As is evident in Fig. 1, the radioiodine uptake was drastically reduced from 9.0–13.4% of the injected dose in control rats to 0.01–0.06% in groups treated for 2 to 26 weeks.

![Graph showing thyroidal radioiodine uptake](image)

**Fig. 1.**
Effect of Protamone on 24 h thyroid $^{131}$I uptake mean ± sd.
B. Thyroid weight and DNA content in the gland

Thyroid weight per 100 g body weight in Protamone-treated and untreated animals is shown in Fig. 2. In treated rats (3 days, 1 and 2 weeks) the thyroid weight was somewhat lower than in controls (6–18%). Similar results were observed after chronic Protamone treatment (10 and 26 weeks).

DNA content per mg wet thyroid tissue of the gland was a little lower in animals treated with Protamone (Fig. 2). In comparison with control rats DNA concentration in animals treated with Protamone for various periods decreased by approximately 10%.

C. The content of soluble thyroid proteins

a) Total protein. – The content of soluble protein per unit tissue weight in the thyroid of Protamone-treated rats was significantly higher. This increase
Influence of Protamone on the content of soluble thyroid proteins (mg % = mg/100 mg wet tissue). Duration of treatment was 1, 2, 26 and 28 weeks. All values are mean ± sd. Each sample of the soluble proteins was obtained from the pooled thyroid glands from at least 5 animals.

in concentration of soluble protein amounted to about 40 to 80 % in comparison with that in the controls. It should be pointed out that the maximal increase was achieved after 7 days of Protamone administration but with extension of treatment no further increase of soluble protein concentration in the thyroid gland was noted.

b) Individual iodoproteins. - The amount of soluble iodoproteins of untreated rats and those treated with Protamone is presented in Table 1 and Fig. 4 for some of the experiments. In the Protamone-treated groups the content of all iodoproteins in the gland was higher, i.e. quantities of TG, 27S and 12S iodoprotein were about two or more times higher than in the control rats. Although the total content of iodoprotein in the thyroid gland after short- and long-term treatment (2, 26 and 28 weeks) remained at the same level, the concentration of TG after long-term treatment was lower than after short-term Protamone administration (Fig. 4 and Table 1). Fraction 3–8S if expressed as per cent of total soluble protein, decreased significantly in the treated animals, but in absolute amounts per 100 mg wet tissue, the level was not much different from the controls (Table 1).
The total and relative content of soluble protein fractions from the rat thyroid after Protamone-treatment for various time periods.

<table>
<thead>
<tr>
<th>Treatment duration</th>
<th>Group</th>
<th>Soluble thyroid proteins</th>
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<tr>
<td></td>
<td></td>
<td>mg/100 mg wet tissue</td>
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<tr>
<td></td>
<td></td>
<td>Total 1) (mean ± sd)</td>
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<tr>
<td>3 days</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>-</td>
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<tr>
<td>2 weeks</td>
<td>C</td>
<td>7.10±0.07 (2)*</td>
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<td></td>
<td>P</td>
<td>12.70±1.80 (2)</td>
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<tr>
<td>26 weeks</td>
<td>C</td>
<td>8.80±1.36 (5)</td>
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<td></td>
<td>P</td>
<td>12.70±1.05 (6)</td>
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<tr>
<td>28 weeks</td>
<td>C</td>
<td>8.10±1.55 (2)</td>
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<td></td>
<td>P</td>
<td>12.50±1.23 (3)</td>
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C: Control groups. P: Protamone-treated groups. 1) Pooled thyroid glands from at least 5 rats were used in each protein determination. 2) Number of determinations (gradient analyses) from 2–3 experiments. Individual proteins (mg/100 mg) were calculated from average values total and per cent of proteins.
Fig. 4.
Influence of Protamone treatment on the composition of individual thyroid soluble iodoproteins (see Table 1).

D. Effect of Protamone pre-treatment on synthesis of thyroid proteins in vitro

a) Incorporation of $[^{14}C]$leucine into total soluble and microsome-bound proteins. – In Table 2 are presented the results of $[^{14}C]$leucine incorporation into total soluble and solubilized proteins of rats treated with Protamone for short and long intervals (from 3 days to 26 weeks). It is evident that the incorporation rate of label into soluble proteins was considerably lower in treated animals (31 to 80 % of control). The degree of inhibition was not too dependent on the duration of Protamone treatment. The incorporation of $[^{14}C]$leucine into total microsome-bound (solubilized) protein of thyroid treated animals was also markedly inhibited (Table 2). The incorporation of labelled amino acid was reduced by nearly 50 % after 3 days of treatment, and a similar inhibition was observed after 2 and 26 weeks of treatment.

b) Incorporation of $[^{14}C]$leucine per 100 mg wet thyroid tissue or 10 mg soluble protein. – In Figs. 5 and 6 are presented the results for incorporation of label into soluble proteins calculated per unit weight of thyroid gland and
<table>
<thead>
<tr>
<th>Treatment duration</th>
<th>Group</th>
<th>Incubation time (min)</th>
<th>[<strong>14</strong>C]leucine incorporated into soluble protein</th>
<th>[<strong>14</strong>C]leucine incorporated into microsome-bound protein</th>
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<tr>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>180</td>
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<td></td>
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<td>DPM</td>
<td>% of control</td>
<td>DPM</td>
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<tr>
<td>3 days</td>
<td>C</td>
<td>22.473</td>
<td>30.0</td>
<td>28.125</td>
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<td></td>
<td>P</td>
<td>9.660</td>
<td>43.0</td>
<td>119.158</td>
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<tr>
<td></td>
<td>C</td>
<td>48.883 ± 1.912</td>
<td>43.2</td>
<td>152.782 ± 19.719</td>
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<tr>
<td>3 days</td>
<td>P</td>
<td>32.578 ± 2.247</td>
<td>74.2</td>
<td>120.445 ± 18.946</td>
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<td></td>
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<td>(3)</td>
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<td>1 week</td>
<td>C</td>
<td>39.715 ± 3.431</td>
<td>73.1</td>
<td>164.040 ± 7.600</td>
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<td>P</td>
<td>29.047 ± 824</td>
<td>73.1</td>
<td>75.690 ± 2.840</td>
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<td>(3)</td>
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<td></td>
<td></td>
<td>P &gt; 0.01</td>
<td>P &gt; 0.01</td>
<td>P &gt; 0.01</td>
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<tr>
<td>2 weeks</td>
<td>C</td>
<td>58.491 ± 3.005</td>
<td>47.5</td>
<td>182.493 ± 6.251</td>
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<tr>
<td></td>
<td>P</td>
<td>27.805 ± 4.785</td>
<td>47.5</td>
<td>100.030 ± 8.378</td>
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<td></td>
<td></td>
<td>(3)</td>
<td>(3)</td>
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<td></td>
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<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
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<tr>
<td>26 weeks</td>
<td>C</td>
<td>9.487</td>
<td>52.2</td>
<td>166.369</td>
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<tr>
<td></td>
<td>P</td>
<td>4.955</td>
<td>52.2</td>
<td>50.991</td>
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M ± sem from 3 experiments. In each experiment thyroid glands from 3 or 5 animals were incubated together.
Fig. 5.

Time-course of incorporation of $[^{14}\text{C}]$leucine into soluble thyroid proteins (DPM/100 mg wet thyroid tissue). Values represent mean ± SEM of the results obtained from three parallel incubations at each time period. Each sample represents pooled thyroid glands from 3 rats.

per unit weight of soluble protein. The rats were pre-treated with Protamone for 3, 7 and 14 days (Fig. 5) and 2, 26 and 28 weeks (Fig. 6). In each experiment Protamone pre-treatment inhibited $[^{14}\text{C}]$leucine incorporation into soluble protein. It should be pointed out that the degree of inhibition was not correlated with the duration of Protamone administration. Incorporation of labelled amino acid into microsome-bound protein per unit weight of gland was also markedly reduced in the treated animals.

c) Sucrose density gradient analysis of newly synthesized soluble proteins. – In both groups, after 60 min incubation of thyroid tissue a marked incorporation of $[^{14}\text{C}]$leucine into TG was observed. The amount of newly formed TG was lower in animals previously treated with Protamone in vivo in comparison with the corresponding controls. In addition, it can be seen, that in the treated rats a relatively high percentage of radioactivity was found in 12S protein
Fig. 6.
Time-course of incorporation of $[^{14}\text{C}]$leucine into soluble thyroid proteins (dpm/10 mg of soluble proteins). Each incubation presents results of a single experiment, but all incubations were performed at least three times. Each sample represents pooled thyroid glands from 3 or 5 animals.

(Fig. 7, B), while in the control rats no radioactivity was observed in this gradient region (Fig. 7, A). Similar dynamics of $[^{14}\text{C}]$leucine incorporation were observed after shorter term Protamone treatment (1 and 2 weeks).

**DISCUSSION**

In the thyroid gland of rats chronically treated with an iodinated casein containing thyroactive compounds an increase in all soluble proteins, except for
Sucrose density gradient ultracentrifugation patterns of soluble thyroid proteins of rats untreated (A) and treated with Protamone for 28 weeks (B) after 3 h incubation of thyroid tissue in vitro in the presence of [14C]leucine. Similar patterns of [14C]leucine labelled soluble proteins were obtained after 1 and 2 weeks of Protamone pre-treatment.

The direction of sedimentation was from right to left

the 3–8S protein fraction was observed. Our earlier histological and electron microscopic examinations showed that after Protamone treatment of rats for various time intervals the follicular lumen was filled with dense colloid, the epithelium became low-prismatic in shape and the amount of endoplasmatic reticulum was markedly reduced (Jovanović et al. 1970–1973). Recently, Smeds et al. (1977) observed that the relative volume of the follicles during thyroxine treatment (from 2 to 21 days) did not change, but the relative volume of the follicle lumen increased progressively with thyroxine administration.

In Protamone-treated animals an increase was observed in total soluble iodo-proteins in vivo (Fig. 3) and a decrease in the rate of synthesis of TG and sub-units in vitro (Fig. 7). The increase of thyroid iodoproteins per unit thyroid
weight, particularly TG and 27S iodoprotein is a result of two processes: a) reduced endocytosis and proteolysis of iodoproteins pre-existing in the follicle lumen and b) continued synthesis and secretion of thyroid proteins.

The amount of 12S iodoprotein in treated animals was higher than in the controls, particularly at some later time intervals of the treatment. At the same time, the content of TG in treated animals was lower after long-term treatment than after short-term. Since radioiodine uptake by the gland was drastically reduced (several hundred times), the finding of 12S protein fraction (absorbance and radioactivity) in treated animals (Fig. 7, B) might be connected with inhibition of TG iodination. This iodoprotein might have been formed by the dissociation of poorly iodinated TG molecules or its presence may be the result of retarded aggregation of 12S subunits or both. The presence of radioactivity in 12S protein suggests that previous suppression of TSH secretion with excess thyroxine decrease the rate of TG synthesis, not only by inhibition of synthesis of TG subunits, but possibly also by their retarded aggregation into TG. The appearance and accumulation of 12S protein in the thyroid gland of hypophysectomized rats (Pavlović-Hournac et al. 1967) and rats treated with antithyroid substances (Sinadinović et al. 1969) has been observed previously.

A significantly higher value for 27S iodoprotein in the thyroid of treated animals is probably the result of long-term interaction between well iodinated TG molecules in the follicles which were filled with the relatively immobilized colloid. This finding supports the view that, besides adequate TG iodination, there are other factors such as increased storage of TG in lumen follicles and immobilization of colloid for a long period, which influence the conversion of TG into its dimer, i.e. 27S iodoprotein (Sinadinović et al. 1973).

Since the DNA content in the thyroid glands of treated rats does not change significantly, the decrease in the incorporation of [14C]leucine into soluble and microsome-bound proteins is not related to any changes in the number of epithelial cells, but is probably the result of a reduction of the endoplasmatic reticulum and the intensity of metabolic processes and therefore of changed capacity of the thyrocytes for protein synthesis. It seems that the main effects of TSH are directed to endocytosis and intracellular proteolysis of colloid, while the extent of its influence on TG synthesis is considerably smaller (Pavlović-Hournac & Delbauffe 1976).

The duration of Protamone treatment after several days is not correlated with the above mentioned changes in the gland (the content of soluble iodoproteins, the incorporation rate of label into proteins, radioiodine uptake) and with oxygen consumption, body weight and other parameters as observed previously (Jovanović et al. 1970–1973) suggesting that the thyroid–pituitary axis becomes adapted to the chronic administration of excess thyroxine.
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