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Chronic Effect of TSH on Human Thyroid Tissue in Organ Culture (41538)

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Abstract. The chronic effect of TSH on thyroidal cAMP concentrations and release of thyroid hormones was investigated using human thyroid tissue in organ culture. Normal human thyroid slices were placed in HAM's F-10 synthetic culture medium in Falcon organ tissue culture dishes, and incubated at 37° in a humidified atmosphere of 5% CO_2 in air. Medium was changed everyday and daily T_3 or T_4 release was determined using concentration of T_3 or T_4 in the medium. After incubation, slices were transferred to the medium containing 10 m*M* theophylline and incubated without TSH for an additional 30 min to determine thyroidal cAMP concentrations. Thyroidal cAMP concentrations in slices incubated with 10 mU/ml of TSH increased significantly at 2, 6, and 24 hr and even on the 6th day of incubation. Daily T_3 release was significantly increased above control from the 3rd day and daily T_4 release from the 4th day to the 11th day of incubation with 10 mU/ml of TSH. Histologically, almost all follicles were structurally maintained even on the 11th day of incubation. These results suggest that both thyroidal cAMP concentrations and release of thyroid hormones are stimulated chronically by TSH. This organ culture system is useful for investigating chronic effects of various materials on human thyroid tissue.

Organ culture is an ideal method to observe chronic effects of various materials on human thyroids *in vitro* since the structure of the follicles is essential for thyroid glandular function. However, only a few studies have been reported on the function of human thyroid tissue in organ culture (1–5) and none of them studied the adenylate cyclase–cyclic AMP (AC–cAMP) system in tissues and release of hormones from tissues.

The present experiments were undertaken, therefore, to observe chronic effects of TSH on thyroidal cAMP concentrations and release of hormones using human thyroid tissue in organ culture.

Materials and Methods. *Organ culture.* Normal human thyroid tissue was obtained from tissues surrounding solitary nonfunctioning adenomas in untreated euthyroid patients. Immediately after thyroid tissues were excised, they were transported to the laboratory in HAM's F-10 synthetic culture medium (Flow Laboratories, Mclean, Va.) containing 10% fetal bovine serum (Grand Island Biological Co., Grand Island, N.Y.), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 4°. Slices (1.0–1.5 mm thick) were made with a Stadie–Riggs microtome. These slices (15–20 mg) were rinsed with F-10 medium and transferred on the surface of Millipore filters in 750 μl of F-10 medium with various concentrations of bovine TSH (thytropar, Armour Pharmaceutical Co., Phoenix, Ariz.) in Falcon organ tissue culture 3037 dishes and incubated at 37° in a humidified atmosphere of 5% CO_2 in air (Fig. 1).

Medium was changed daily since the amount of medium was insufficient for the size of slices to obtain good culture conditions. The change of medium was performed by transferring each slice together with the Millipore filter to another organ culture dish containing fresh culture medium.

All of the above procedures were performed under sterile conditions.

Release of T_3 and T_4 from thyroid slices. T_3 and T_4 concentrations in medium were measured by radioimmunoassay (RIA) using

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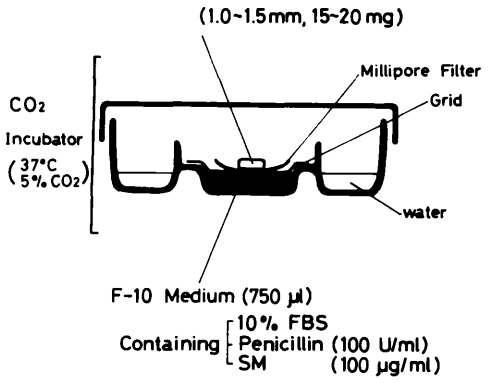

FIG. 1. Method for organ culture of normal human thyroid tissue.

commercial kits (T_3 RIA Kit and T_4 RIA Kit, Dainabot RI Laboratory, Chiba-Pref., Japan). Release of T_3 or T_4 from thyroid slices were calculated from concentrations of T_3 or T_4 in the medium and tissue weight, and expressed as picograms or nanograms per milligram weight per unit of time, respectively.

Measurement of dialyzable T_3 and T_4 concentrations. Dialysis of incubation medium was carried out as previously described (6). Six-tenths milliliter of medium was diluted with 0.6 ml of phosphate buffer containing 100 units/ml of penicillin and 100 μg/ml of streptomycin, pH 7.4, ionic strength 0.15. One-milliliter aliquots of diluted medium were then pipetted into dialysis sacs (Visking Nojax cellulose, 27/32) and were dialyzed against 5 ml of above-mentioned phosphate buffer, overnight at 37°. The concentrations of T_3 or T_4 in dialysates were measured by conventional RIA and expressed as nanograms per deciliter per milligram weight of tissue.

Thyroid cAMP concentrations. After incubation with or without TSH, slices were transferred to Falcon organ tissue culture dishes with 750 μl of F-10 medium containing 10 m*M* theophylline and incubated without TSH in a CO_2 incubator for an additional 30 min. Slices were then quickly frozen in dry ice-acetone. The frozen tissue was immediately weighed and homogenized in 1 ml ice-cold 50% acetic acid. The homogenates were centrifuged at 10,000*g* for 15 min at 4°, and the supernatants were evaporated at 50° and dissolved with 0.05 *M* sodium acetate buffer, pH 6.2. The cAMP concentrations in the buffer were measured by RIA using cyclic AMP KIT-125 (obtained from Mitsui Toatsu, Ltd., Tokyo, Japan) and expressed as picomoles per milligram weight of tissue.

Histological examination. In some experiments, tissues were fixed in isotonic buffered formalin (10%, v/v). Thin sections were cut, stained with hematoxylin and eosin, and examined under a light microscope.

Results. Daily T_3 and T_4 release into incubation medium with or without 10 mU/ml of TSH was measured until the 11th day of incubation (Fig. 2). Daily T_3 release increased

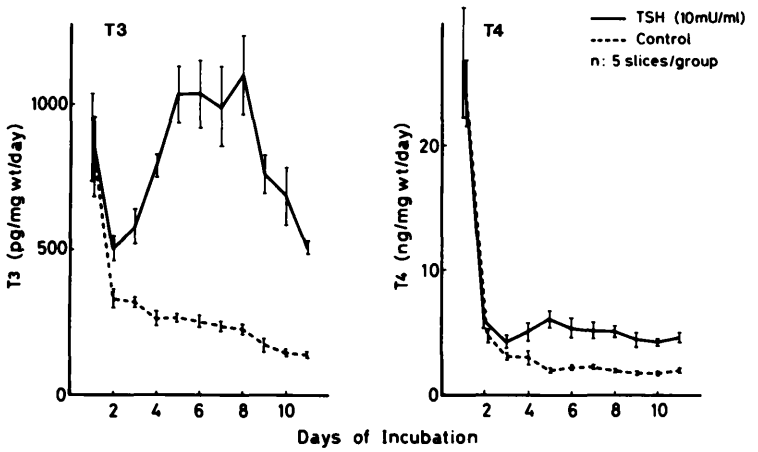

FIG. 2. Chronic effect of 10 mU/ml of TSH on T_3 and T_4 release from cultured human thyroid slices. The incubation medium was changed everyday and values represent daily T_3 and T_4 release. Bars indicate ±SE.

gradually until the fifth day of incubation with TSH, and significant differences between values in TSH-stimulated slices and control slices were observed from the third day until the 11th day of incubation. The release of T_4 paralleled that of T_3 and daily T_4 release in TSH-stimulated slices was significantly increased from the 4th day of incubation.

Cyclic AMP concentrations in slices incubated with 10 mU/ml of TSH increased significantly within 2 hr and decreased thereafter until 24 hr. However, no further significant decrease was observed after 24 hr throughout 6 days of incubation. Even after 6 days of incubation, thyroidal cAMP concentrations were significantly higher in TSH-treated slices than in controls (Fig. 3).

Dialyzable T_3 and T_4 concentrations in incubation medium were measured on the sixth day of incubation with TSH. Dialyzable T_3 and T_4 concentrations increased significantly from 1.37 ± 0.14 (control slices) to 2.81 ± 0.18 (TSH-stimulated slices) ($P < 0.005$) and from 8.5 ± 1.8 to 16.7 ± 1.5 ng/dl/mg wt ($P < 0.05$), respectively.

When thyroid slices were incubated with various doses of TSH (0.01–10.0 mU/ml), T_3 release on the fifth or sixth day of incubation increased in a dose-response manner and the increase of T_3 release was significant at TSH levels as low as 25 μU/ml (Fig. 4, Table I).

Figure 5 shows the histology of the slices

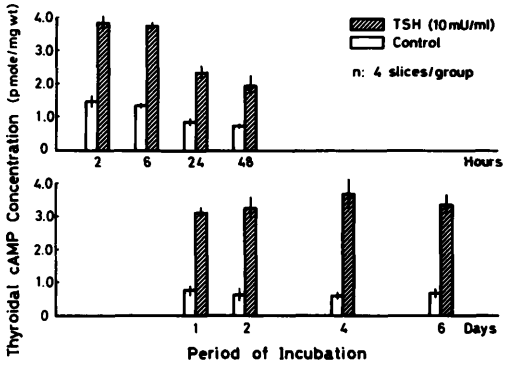

FIG. 3. Cyclic AMP concentrations in slices incubated with or without TSH for 2, 6, 24, and 48 hr (upper panel), and 1, 2, 4, and 6 days (lower panel). Thyroidal cAMP concentrations were measured after slices were transferred to Falcon organ tissue culture dishes with F-10 medium containing 10 mM theophylline and incubated without TSH in a CO_2 incubator for an additional 30 min.

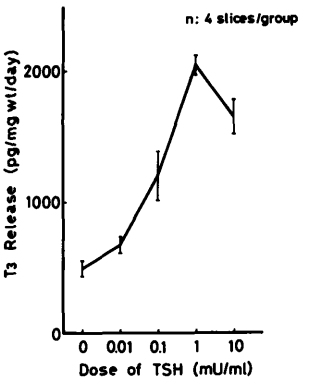

FIG. 4. Dose-response curve between doses of TSH and amounts of T_3 release on the sixth day of incubation from human thyroid slices. Bars indicate ±SE.

after 11 days of incubation with or without 10 mU/ml of TSH. The follicular epithelium appeared healthy after 11 days of incubation with or without TSH. In slices incubated with TSH, significant reduction of colloid storage and peripheral vacuollation of colloid were observed. The height of the epithelial cells was greater in TSH-stimulated slices.

Discussion. In previous publications (1–5), some thyroid functions were measured in human thyroid tissue in organ culture, i.e., iodine uptake (1, 3), its distribution among thyroidal iodoaminoacid (3), and release of labeled hormone from thyroid tissue (3). Other reports describe the feasibility of applying tissue culture to postmortem tissues (2), results from organ culture of human tumors (4), and various pathological human thyroids (5). In the present experiments, release of hormones increased significantly until the 11th day of incubation with TSH. Furthermore, cyclic AMP concentrations were significantly higher in slices incubated with TSH than in controls even on the sixth day of incubation.

It has been reported that thyroid cells in tissue culture grew for similar periods of time as in the present study (7–10). In the absence of TSH, thyroid cells grew as a monolayer and have characteristics typical of epithelial cells. In the presence of TSH, thyroid cells differentiate to form two-dimensional follicles. The lumina of these follicles contain dense material reacting with thyroglobulin antisera and the cells can concentrate iodide and synthesize iodinated thyroglobulin (7, 8). Further-

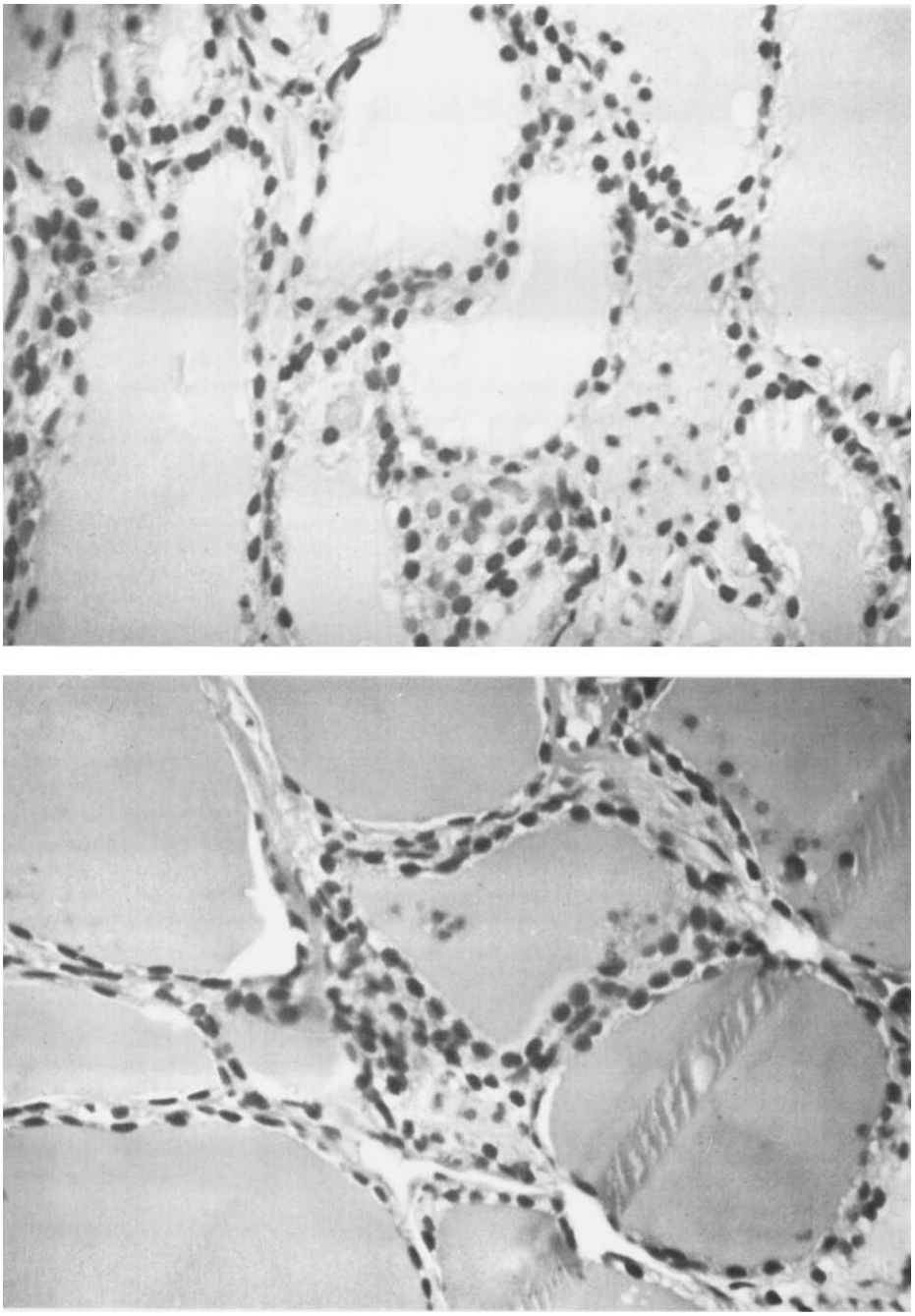

FIG. 5. Histology of the slices after 11 days of incubation with (right) or without (left) 10 mU/ml of TSH. Original magnification ×400.

TABLE I. EFFECTS OF LOW DOSES OF TSH ON T_3 RELEASE ON THE SIXTH DAY OF INCUBATION FROM HUMAN THYROID SLICES

	Dosages (μU/ml incubation medium)			
	Control	12.5	25.0	50.0
T_3 release (pg/mg wt/day)	232.13 ± 19.43[a]	300.45 ± 34.58	375.45 ± 48.90*	642.98 ± 75.56*

[a] Values shown represent the mean ± SE of the results obtained from four slices each group.
* Significantly higher than control ($p < 0.05$).

more, cAMP concentrations in cultured thyroid cells increased in response to TSH (10–12). However, a maximum increase of cAMP concentrations was observed after 10 to 30 min of stimulation (11, 12), and then cAMP concentrations declined rapidly attaining basal levels at the fourth day of culture (10). While the release of hormone from cultured thyroid cells was also increased significantly by TSH stimulation, it was maximal at 24 to 48 hr, and progressively diminished (13, 14). Thus, thyroid cells in tissue culture tend to lose their responsiveness to TSH after a period of a few days.

TSH-induced refractoriness has been reported not only in cultured thyroid cells (14), but also in studies of tissue immersed in buffer (16–18). In those reports, the effects of TSH on adenylate cyclase–cAMP system, glucose oxidation, phospholipid synthesis, organification of iodide, and colloid droplet formation were observed in thyroid slices previously incubated with TSH for several hours. On the other hand, there have been several reports of patients with TSH-producing tumors whose thyroids were continuously secreting excessive thyroid hormones indicating relative nonrefractoriness (19, 20). In the present study, using normal human thyroid slices, both thyroid cAMP concentrations and release of thyroid hormones were stimulated chronically by TSH.

The organ culture is a useful system to investigate chronic effects of various materials on human thyroid tissue under conditions independent from neural and humoral control.

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