Cultured Thyroid Cells Respond to Exogenous Thyroxine[1] (35717)

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Do thyroid cells respond to thyroxine, a hormone that these cells elaborate? Introduction of this hormone into growth medium does influence a cell line derived from the dissociation of the steer thyroid gland. As has been reported (1–3), these cells have been serially propagated for as long as 8 months, during which a number of specialized features remain demonstrable (*e.g.*, in responding to thyrotropin (TSH), synthesizing iodoproteins, preserving morphological and ultrastructural features, *etc.*).

Materials and Methods. After explanting of slaughterhouse specimens of steer thyroid and digestion with collagenase (1 mg/ml Hanks' BSS) for about 2 hr, the thyroid cells were cultured as monolayers in Eagle's MEM supplemented with 10% fetal bovine serum and antibiotics in 37° incubators gassed with a mixture of 5% CO_2 and 95% air. The cells were subcultured ("passed") about every 14 days, utilizing trypsin (0.05%) and gentle agitation for release and dispersion.

Determinations of plating efficiency (PE) were made by seeding approximately 150 dispersed cells (fourth pass) in the exponential phase of growth into 60-mm plastic petri dishes containing medium supplemented with thyroxine (1.78×10^{-8}–1.78×10^{-5} *M*). The number of colonies visible after growth for 2 weeks and staining with methylene blue were expressed as a fraction of the initial number of cells.

To study the incorporation of labeled leucine, radioautographs were prepared (4) by overgrowing about 1×10^5 cultured thyroid cells (eighth pass) onto microscope slides placed in glass petri dishes. After growing for several days, the cells were tagged for 1 hr with ^{3}H-Leucine (sp act, 2 Ci/mmole) diluted to 1 μCi/ml of medium. After repeated rinsing of the cells with Hanks' BBS, thyroxine was added to the medium bathing the cells for various intervals (10 min–24 hr) prior to fixing and dipping the slides into melted (43°) Kodak NTB-2 emulsion. Subsequent to photographic processing and staining of the slides with Harris hematoxylin, the number of developed grains over 200 cells were counted.

Results. The effect exerted by L-thyroxine on cellular proliferation and aggregation of these cultured thyroid cells as expressed by PE is indicated in Table I. PE determinations made with five replicate dishes demonstrate that T_4 does augment colony formation relative to the controls, by as much as 39% in the presence of 1.78×10^{-7} *M* (Student's *t* test, $p < .001$).

Corroborating evidence that the thyroid cell line responds to T_4 was obtained from radioautographic studies of the incorporation of ^{3}H-leucine as an index of polypeptide syn-

TABLE I. Effect of L-Thyroxine (T_4) on Plating Efficiency (PE) of the Thyroid Cell Line (Fourth Pass).

T_4 concentration (*M*)	PE[a] (%)
No T_4 (control)	17.2 ± 1.0 (SD)
1.78×10^{-8}	20.2 ± 1.4
1.78×10^{-7}	23.9 ± 1.0
1.78×10^{-6}	23.4 ± 1.3
1.78×10^{-5}	21.4 ± 0.8

[a] PE entries are the means and standard deviations of five replicate dishes, scored for visible colonies 2 weeks after seeding of single cells and staining with methylene blue.

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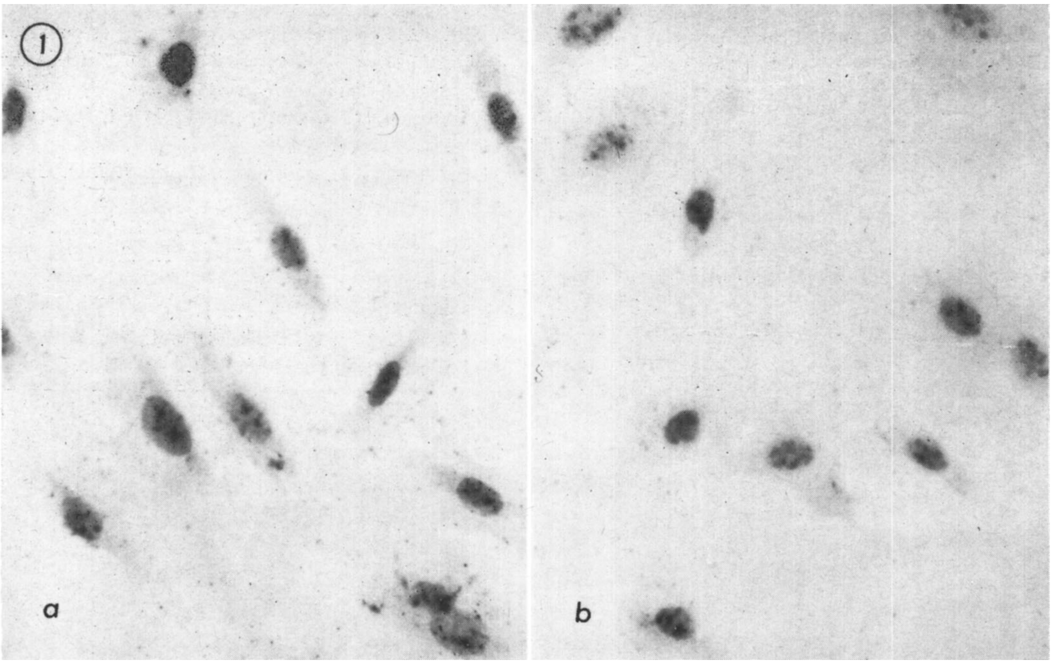

FIG. 1. Radioautographs of the cultured thyroid cells (eighth pass) labeled with ^{3}H-leucine (1 μCi/ml). Exposure: 6 days (Harris hematoxylin; × 390). (a) Stimulated with thyroxine ($1.78 \times 10^{-6} M$) for 1 hr. Counting of developed grains over 200 cells indicates an enhanced ^{3}H uptake of 19.6% per unit area relative to the control population. (b) Control cells.

thesis. Evidently, treatment with T_4 enhances the synthesis of proteins; thus, exposure to 1.78×10^{-6} M for 1 hr elevates the mean grain count per unit area by 19.6%, relative to that for the control cells (Fig. 1). Incidentally, 27.8% is the corresponding figure for such cells exposed to TSH (1 mu/ml) for 1 hr (3).

Discussion. These findings imply that thyroxine affects the growth and metabolism of the cultured thyroid cells much as it does the end-organ *in situ*. Thus, titers that increase the PE of the thyroid cell line are similar to the T_4 levels circulating in the serum as well as those reported active in several other cell lines (4–7). Furthermore, PE of the cultured thyroid cells appears to vary biphasically with T_4 dosage, a relationship characterizing many of the peripheral effects induced by thyroxine and triiodothyronine (8). The T_4 concentration that enhances ^{3}H-leucine localization in the cultured thyroid cells is likewise similar to those which have been demonstrated both *in vivo* (9) and *in vitro* (10) to stimulate extrathyroidal protein biosynthesis. Both TSH (11, 12) and 3′,5′-cyclic adenosine monophosphate (13) promote growth and protein synthesis in the thyroid cell, raising the question as to whether the same or different mechanisms mediate the T_4 interactions being reported.

Since the thyroid cell line preserves some degree of differentiation and persists in responding to TSH, a search seems indicated for the corresponding effects *in situ* of endogenous thyroid hormones on the thyroid follicular cell. If confirmed *in vivo*, such intracellular effects of T_4, and presumably also of T_3, would have to be taken into account in the operation of the feed-back mechanism associated with the thyroid–pituitary axis.

Summary. Thyroxine (1.78×10^{-8}–1.78×10^{-5} M) enhances the colony-formation of serially propagated steer throid cells. Radioautographs indicate that thyroxine likewise stimulates the incorporation of ^{3}H-leucine by these cultured cells. The analogous effects *in situ* of thyroxine on the thyroid follicular cell should be sought.

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