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Thyroxine and Triiodothyronine Influence Cultured Thyroid Cells: Alterations of Morphology and Mucosubstances (39314)

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An important question bearing on the regulation of the thyroidal hormones as well as on their peripheral metabolism is whether thyroid cells themselves respond to their own secretions. Utilizing the steer thyroid cell line, it has been shown that exogenous thyroxine does influence the formation of colonies and the synthesis of proteins (1). The cultured cells used have been serially propagated for many months during which at least some aspects of differentiation were preserved, including responses to thyrotropin (TSH) and the long-acting thyroid stimulator (LATS), the synthesis of iodoproteins, and the maintenance of cytological and ultrastructural features. Especially noteworthy among these specialized responses are the highly ordered arrays resembling follicles that the dispersed cultured cells assume following each subculture for the first 3 months; this morphological pattern is, we have shown, independent of TSH (2). Further experimentation with this same cell line reveals that thyroxine (T_4) and triiodothyronine (T_3) affect morphological organization and the production of mucosubstances, the subjects of this communication.

Materials and methods. Maintained as monolayers, the cultured thyroid cells were grown in water-saturated incubators, gassed with 95% air–5% CO_2 at 37°, using as the growth medium Eagle's MEM (3) supplemented with 10% fetal bovine serum and penicillin-streptomycin. They were subcultured ("passed") about every 2 weeks. The mean doubling time for this line is about 48 hr (2). The determinations being reported here were carried out on cells from the third pass, which were allowed to overgrow onto pairs of microscope slides contained in duplicate petri dishes and exposed for 96 hr to T_4 (8.9×10^{-7} *M*), T_3 (8.9×10^{-7} *M*), or as controls, to neither hormone. These preparations were fixed in Hanks' ethanol (1:1), followed by absolute alcohol. PAS-alcian blue staining at pH 2.5 (4) was carried out for demonstrating mucosubstances by exposing hydrated cells for 30 min to 1% alcian blue in 3% acetic acid, and rinsing in tap water for 5 min. The preparations were then oxidized for 10 min in 1% periodic acid and rinsed in tap water for 15 min. Finally, the slides were bathed in Schiff's Reagent for 60 min, 0.5% sodium bisulfite for 3 min, and washed in distilled water.

Results. Evaluation of the preparations indicates that even as late as the third pass, when the thyroid cell line was about 6-weeks old (more than 20 doubling periods having elapsed), the architecture and synthetic activity of these cells are strongly influenced by the exogenous T_4 and T_4. While both control and treated populations assume ordered arrays, many more of the cells exposed to the thyroidal hormones comprise semicircular or ringlike structures, reminiscent of partial or complete "follicles" (Fig. 1a, c). As compared to the influence exerted by T_4 (Fig. 1b) on these cultured thyroid cells, T_3 appears to be the more active in producing more numerous completed follicles possessing larger diameters and thicker septa (Fig. 1d).

As with the formation of follicles by these cultured cells, the synthesis of mucosubstances is also enhanced as a consequence of adding either thyroid hormone (Fig. 1a–d). The borders of the follicles of the populations exposed to T_4 or T_3 are frequently PAS-alcian blue positive and occasionally the "lumens" of T_3-treated cells contain large PAS plaques. As compared to the corresponding controls, the cells treated with either hormone have more alcian blue-staining granules associated with nuclei and pseudopodia. As judged by intensity of stain

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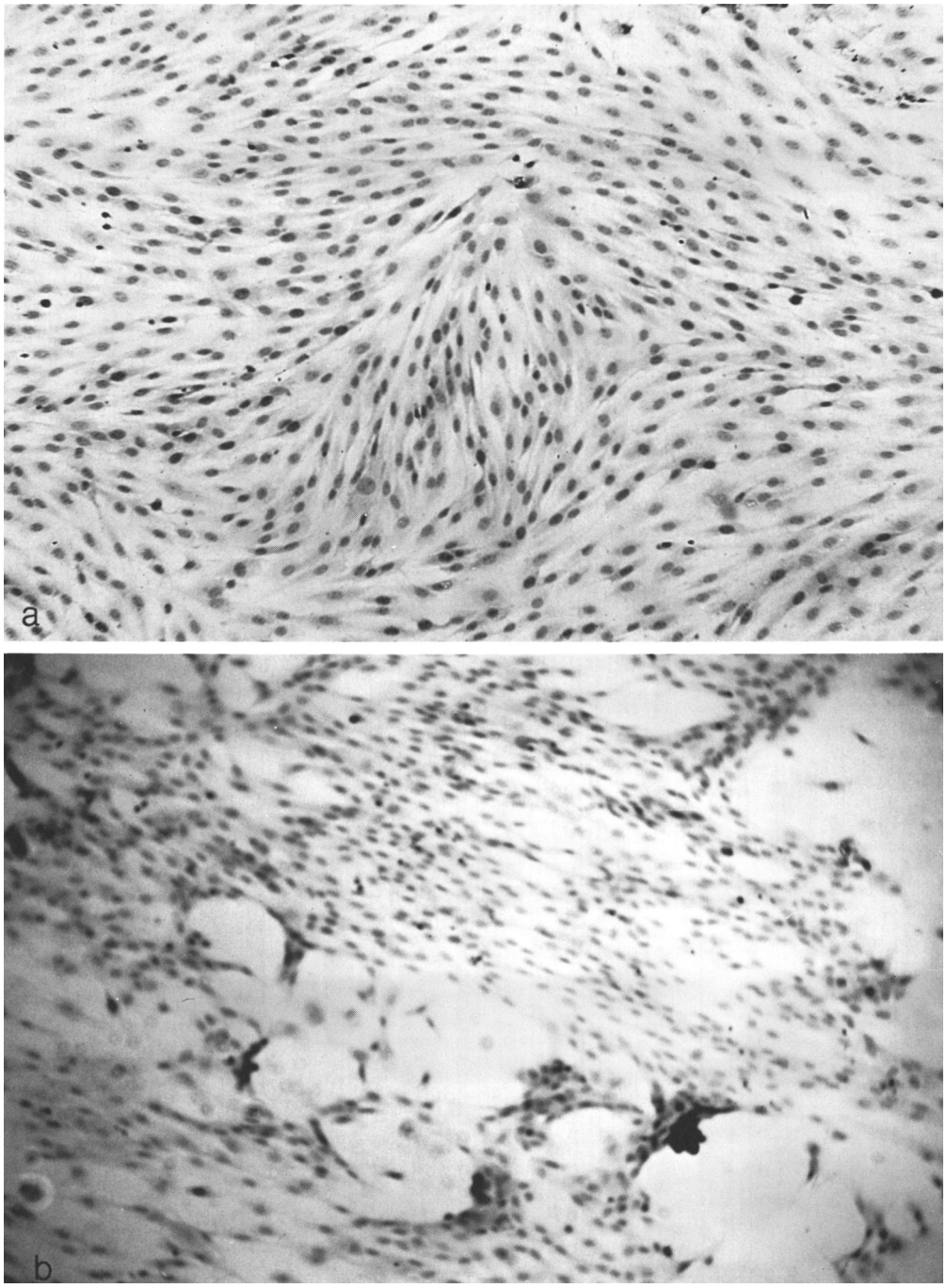
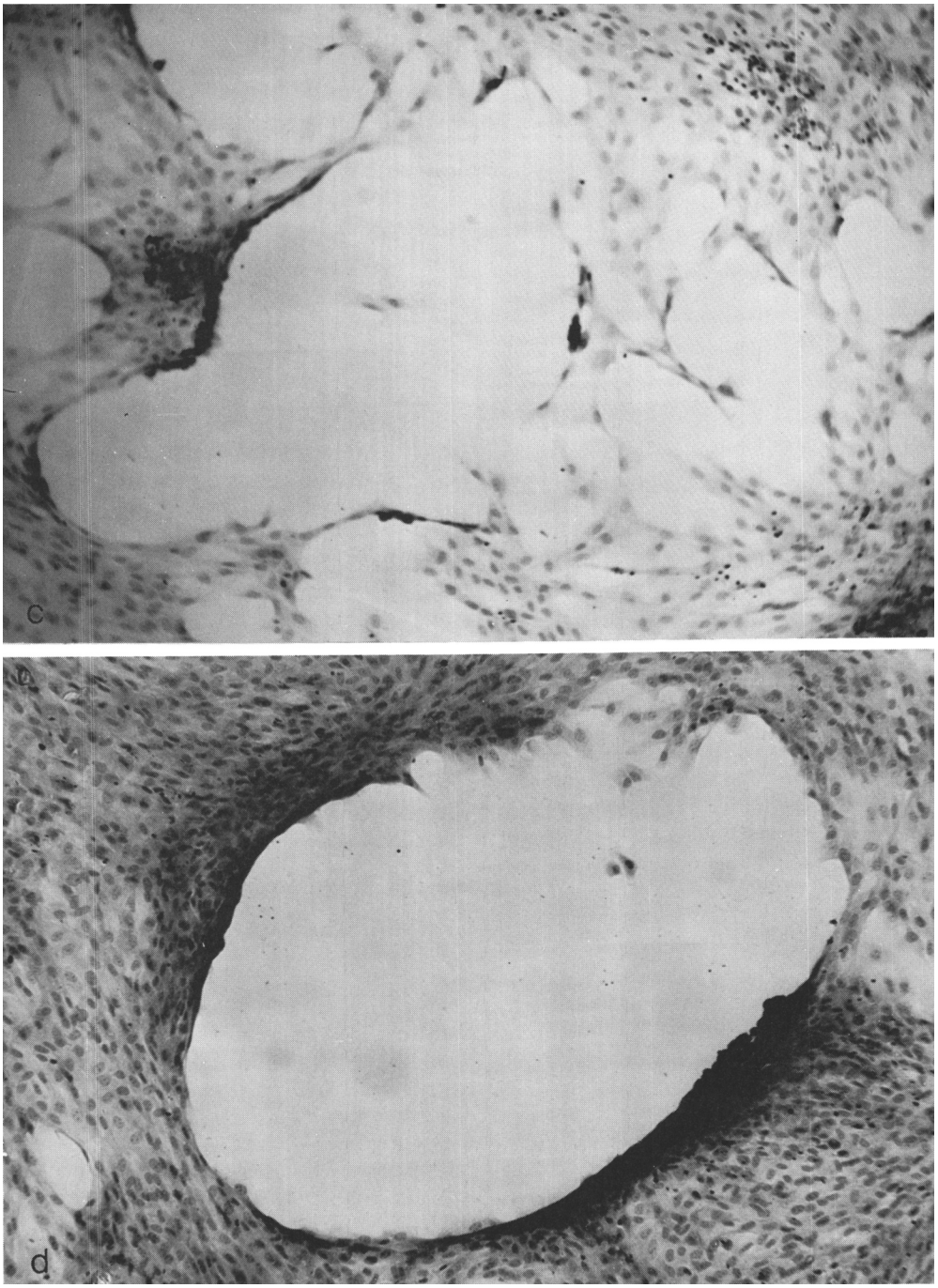


FIG. 1. Effect of exposure for 96 hr to 8.9×10^{-7} *M* T_4 or T_3 on dispersed individual cells of the steer thyroid line (third pass) stained with PAS-alcian blue (pH 2.5) to demonstrate mucosubstances (× 50.8). (a) Control (no hormone) cells: Although the cells organize into structural arrays, "follicular" rings are very rarely encountered; PAS-alcian blue granules are distributed throughout the field. (b) T_4-treated cells: Follicular arrays are numerous, but many are incomplete rings or segments of small diameter; PAS-alcian blue particles are more numerous,



especially over many of the follicular walls and adjoining regions. (c) T_3-treated cells: More complete follicles of larger diameter with thickened "apical" walls appear throughout the field. Alcian blue and PAS staining over the follicular periphery and surroundings are frequently pronounced; occasionally (not illustrated) large PAS plaques localize over some "lumens." (d) T_3-treated cells: Another field (same magnification) illustrating a very large follicle; its dimensions as well as the intensity of staining are much greater than those of T_4-treated cells.

and by the number and size of the dye-reaction particles, T_3 is once again more potent than T_4 (Fig. 1d).

Discussion. Like the formation of colonies and proteins shown previously (1), the cellular organization and the elaboration of mucosubstances are influenced by the thyroidal hormones, implying that T_4 and T_3 can act directly on the thyroid cell. For making the current pilot determinations, a hormonal titer (8.9×10^{-7} *M*) was chosen that had been shown earlier to evoke peripheral-like responses in the T-1 human kidney cell line (5). Obviously what yet needs characterizing in the steer thyroid cell line is the effects elicited by a broad range of T_4 and T_3 concentrations, especially those due to the lower hormonal levels that prevail *in vivo*. Even so, the findings made to date with the cultured thyroid cells point to lines of further investigation for ascertaining whether the thyroidal hormones have a role in intraglandular autoregulation, a subject of interesting conjecture (6–9).

As has been argued elsewhere (2), the serial propagation of cultured cells derived from isolated thyroid cells over many months, during which differentiated morphological and functional features are preserved, suggests that the adult steer cell may retain the potential for glandular morphogenesis and regeneration. In promoting the transformation of dispersed thyroid cells into highly ordered arrays, the thyroidal hormones may be expressing roles that correspond *in vivo* to those responsible for the morphological and embryonic development of the thyroid gland. Support for this notion comes also from the earlier observation already referred to (1), that T_4 and T_3 in enhancing colony formation by these cultured thyroid cells foster adhesion and aggregation which are requisite *in vivo* for follicles to exist.

A morphological transformation occurring over many passes and promoted by T_4 and T_3, the highly organized structures assumed by the isolated thyroid cells resemble follicles. The experimental evidence for this conclusion includes the localization of radioiodide and the release of labeled iodoproteins by these arrays in response to TSH and LATS; at the ultrastructural level it is noteworthy that TSH also stimulates rapidly the formation of electron-dense droplets and peripheral microvilli in these cultured thyroid cells (2), phenomena that closely simulate *in vivo* behavior (10). These morphological roles played by the thyroidal hormones, moreover, must be specific since they are not shared with TSH, which as has been noted, does not influence the development of folliclelike structures in the steer thyroid cell line nor in that derived from the adult porcine gland recently studied by others (11).

Assuming it also occurs *in vivo*, the influence of the thyroidal hormones on the synthesis of mucosubstances demonstrated here may bear at least on two aspects of thyroid gland metabolism. For the ontogenesis to occur that leads to the development of the thyroid gland, thyroid cells must mutually adhere and aggregate as has been remarked, a process known to require mucosubstances (12). Secondly, the effect of T_4 and T_3 on the elaboration of mucosubstances and proteins (1) may signify a relationship to thyroid hormone formation and secretion, possibly connected to autoregulation.

Summary. Further experimentation with the steer thyroid cell line indicates that the formation of "follicles" is enhanced by exposure to 8.9×10^{-7} *M* thyroxine (T_4) or triiodothyronine (T_3) for 96 hr. The production of mucosubstances by the cultured thyroid cells is also increased by treating with T_4 or T_3, effects demonstrable after staining with PAS-alcian blue at pH 2.5. It is suggested that these *in vitro* effects bear on morphological organization and synthetic activity of the thyroid gland as well as on autoregulation and intraglandular homeostasis that may occur *in situ*.

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