# Gonadotropic Activities of Thyrotropic Tumors; Demonstration by Immunohistochemical Staining[1] (37203)

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Thyrotropic pituitary tumors (TtT) can be readily induced in mice by sustained deficiency of thyroid hormones (TH), brought about by surgical or radiothyroidectomy or by agents which block TH synthesis (1). These tumors are transplantable only in TH-deficient mice. Unexpectedly, female athyroid mice bearing grafted TtT exhibited marked ovarian hyperplasia with follicular maturation, hemorrhagic follicles and formation of large corpora lutea (1). After several years of successive transplantations in radiothyroidectomized mice, these tumors usually gave rise to autonomous variants (mutants). These variants grew at first better in athyroid than in euthyroid hosts, but upon subsequent passages, some grew better in euthyroid than in athyroid hosts. One such variant, named "reversely responsive," possessed high gonadotropic and minimal thyrotropic potency (2).

These biologic findings have been amply confirmed, but until recently, the relationship between thyrotropic (TtH) and gonadotropic (GtH) activities remained puzzling. The discovery of a structural relationship between these 2 hormones (3–5) indicates the existence of a subunit common to TtH and GtH and another that is hormone-specific. (The term GtH includes LH = luteinizing hormone, FtH = FSH = folliculotropic hormone, and hCG = human chorionic gonadotropin.) The same gonadotrope is known to secrete LH and FtH; hCG is known to have predominantly LH activity.

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Earlier conventional, rather insensitive bioassays failed to disclose the presence of gonadotropic activity of TtT (1). This paper reports on immunohistochemical staining (IHCS) of normal and neoplastic thyrotropes and normal and hyperplastic gonadotropes, utilizing antisera to GtH and TtH and their subunits, named G$\alpha$ and G$\beta$ and T$\alpha$ and T$\beta$, respectively. The findings are in agreement with the biochemical studies (3–5) and explain the biologic GtH activity of TtT (1). The radioimmunoassays (RIA) thus far done have shown elevated serum GtH levels in mice bearing TtT.

*Materials and Methods. Animals and tumor strains.* Young adult $LAF_1$ mice of both sexes were obtained from the Jackson Laboratory. They were injected with $\sim$60 $\mu$Ci of $^{131}I$ after being kept on low iodine diet for about 2 wk. The dependent tumor strains TtT 100 and 76 have never given rise to autonomous variants since their isolation 5 and 2 yr ago, respectively. The autonomous mutants arose from an older TtT 87 strain which has steadily given rise to autonomous variants that grow well in both athyroid and euthyroid mice. For other details on isolation and characterization of TtT, see (1). Since gonadotropic tumors are not available for comparative studies, pituitaries of normal and ovariectomized mice and rats were used as controls. Rats of the W/Fu strain were obtained from ARS/Sprague–Dawley.

*Immunohistochemical staining. Radioimmunoassays.* IHCS was performed by the technics of Nakane and Pierce (6) or Mason *et al.* (7). The results of the 2 technics are

essentially identical; the latter is somewhat more sensitive. Anti-rat LH and anti-rat FtH were obtained from NIAMD; anti-hCG from Drs. G. T. Ross and P. Nakane. Anti-mouse TtH was prepared with purified mouse TtH obtained from Drs. R. W. Bates and P. G. Condliffe. The anti-$\alpha$ and $\beta$ hCG (anti-G$\alpha$, anti-G$\beta$) were kindly given to us by Dr. G. T. Ross, the anti-$\alpha$ and $\beta$ bovine TtH (anti-T$\alpha$, anti-T$\beta$) by Dr. J. G. Pierce. All preparations were also stained with antisera to the other types of pituitary hormones (described and illustrated in a WHO monograph on the Pathology of Tumours in Laboratory Animals, in press). The reactions with anti-adrenotropic hormone and anti-mammotropic hormone were negative. Some salient conclusions were confirmed by sequential staining of 3 $\mu$m sections and by differential staining of 2 hormones in the same section (unpublished data). The introduction of faint counter-staining with diluted 5% Harris' hematoxylin for 3 min after IHCS proved valuable in visualization of the various cells not seen by IHCS.

Preliminary serum LH levels were determined by double antibody RIA using cross-reacting rat LH.

*Results and Comments. Immunohistochemical staining.* The findings on the interrelationship between the 2 glycoprotein hormones and their subunits are surveyed in Table I and illustrated in Figs. 5–9. The appearance of the 2 normal glycoprotein and the 2 "acidophilic" polypeptide hormones are illustrated in Figs. 1–4.

The anti-TtH stained well normal thyrotropes of both mice and rats. Their staining intensity diminished after thyroidectomy but could be restored by administration of L-thyroxine. (This, the well-known "rebound phenomenon," was demonstrated by IHCS). Anti-TtH stained slightly also normal gonadotropes, but much less intensely than normal thyrotropes (Fig. 5). Anti-TtH also stained well the dependent TtT cells (Fig. 6). Anti-hCG and anti-LH stained well the gonadotropes (Fig. 8), but not the thyrotropes. However, anti-LH *did* stain TtT cells (Fig. 7); anti-GtH usually stained fewer TtT cells and stained them less intensely than anti-TtH, as illustrated in Fig. 7. These observations are sufficient to explain the hitherto puzzling observation that TtT-bearing mice have marked biologic gonadotropic activity.

Subsequently, the same cells were also stained with antisera to the $\alpha$ and $\beta$ subunits of hCG and of TtH. Anti-T$\beta$ (1/300) stained normal thyrotropes, but not gonadotropes; anti-G$\beta$ stained well gonadotropes (1/10), but not thyrotropes (1/5). Figure 9 shows that the anti-G$\beta$ serum stained the hypertrophied ovariectomy-gonadotropes about as well as did anti-rat LH (Fig. 8). The corresponding anti-$\alpha$ sera gave negative or weak reactions when relatively concentrated antisera were used. It is possible that the $\alpha$ subunits are weak antigens. Our tests show that their antisera gave weak or no reactions with the complete hormones. It is noteworthy that when the anti-$\alpha$ sera were positive, they stained the thyrotropes and gonadotropes about equally well.

The response of mouse and rat pituitary cells to the anti-LH and anti-hCG used was nearly identical. The anti-FtH stained gonadotropes at varying intensities, on the whole, less intensely than did the anti-LH serum.

*LH content of sera.* A comprehensive study of changes in GtH concentrations following radiothyroidectomy, in sera of mice bearing various types of TtT, and following gonadectomy will be fully reported later. The observations thus far have shown that all 3 dependent TtT (3 mice) have moderately elevated serum LH compared to 6 radiothyroidectomized and 3 normal mice.

*Discussion.* The discovery of structural and immunologic relationships between thyrotropes and gonadotropes by Pierce *et al.* (3), Canfield *et al.* (4) and Vaitukaitis *et al.* (5) is in harmony with our earlier biologic observations of the close relationship between these 2 glycoprotein hormones. The review of their excellent work, supplemented with IHCS by Baker, Pierce and Cornell (8) (which overlaps with our studies) is beyond the scope of this report. It was surprising to find that anti-TtH stained not only normal thyrotropes, but also normal gonadotropes (Fig. 5), albeit much more weakly.

Immunologic cross-reactions can often be explained on the basis of impurities of anti-

TABLE I. Immunohistochemical Staining of TtH and GtH with Their Respective Antisera to the Full Hormones and Their Subunits.

| Cells | Antisera to | | | | | |
|---|---|---|---|---|---|---|
| | TtH | $T\alpha$ | $T\beta$ | GtH | $G\alpha$ | $G\beta$ |
| Mouse pituitary | | | | | | |
| Normal thyrotrope | 1/30 +++[a]<br>1/300 +++ | 1/30 +<br>1/300 — | 1/300 +++ | 1/10 — | 1/5 — | 1/5 — |
| Normal gonadotrope | 1/30 +<br>1/300 — | 1/30 +<br>1/300 — | 1/30 — | 1/25 +++ | 1/5 — | 1/10 ++ |
| Pituitary tumor | | | | | | |
| TtT 100 (dependent) | 1/50 +++ | 1/30 +<br>1/300 — | 1/300 +++ | 1/25 ++ | 1/5 — | 1/10 + |
| Rat pituitary | | | | | | |
| Normal thyrotrope | 1/50 +++<br>1/500 +++ | 1/30 +<br>1/300 — | 1/300 +++ | 1/10 — | 1/5 + | 1/5 — |
| Normal gonadotrope | 1/50 +<br>1/500 — | 1/30 +<br>1/300 — | 1/30 — | 1/100 +++<br>1/500 +++ | 1/5 + | 1/10 ++ |
| Ovariectomized rat | | | | | | |
| Normal thyrotrope | 1/50 +++ | 1/30 +<br>1/300 — | 1/300 +++ | 1/25 — | 1/5 +<br>1/25 — | 1/10 — |
| Hypertrophied gonadotrope | 1/50 + | 1/30 +<br>1/300 — | 1/30 — | 1/25 ++<br>1/50 ++ | 1/5 +<br>1/25 — | 1/10 ++<br>1/25 + |

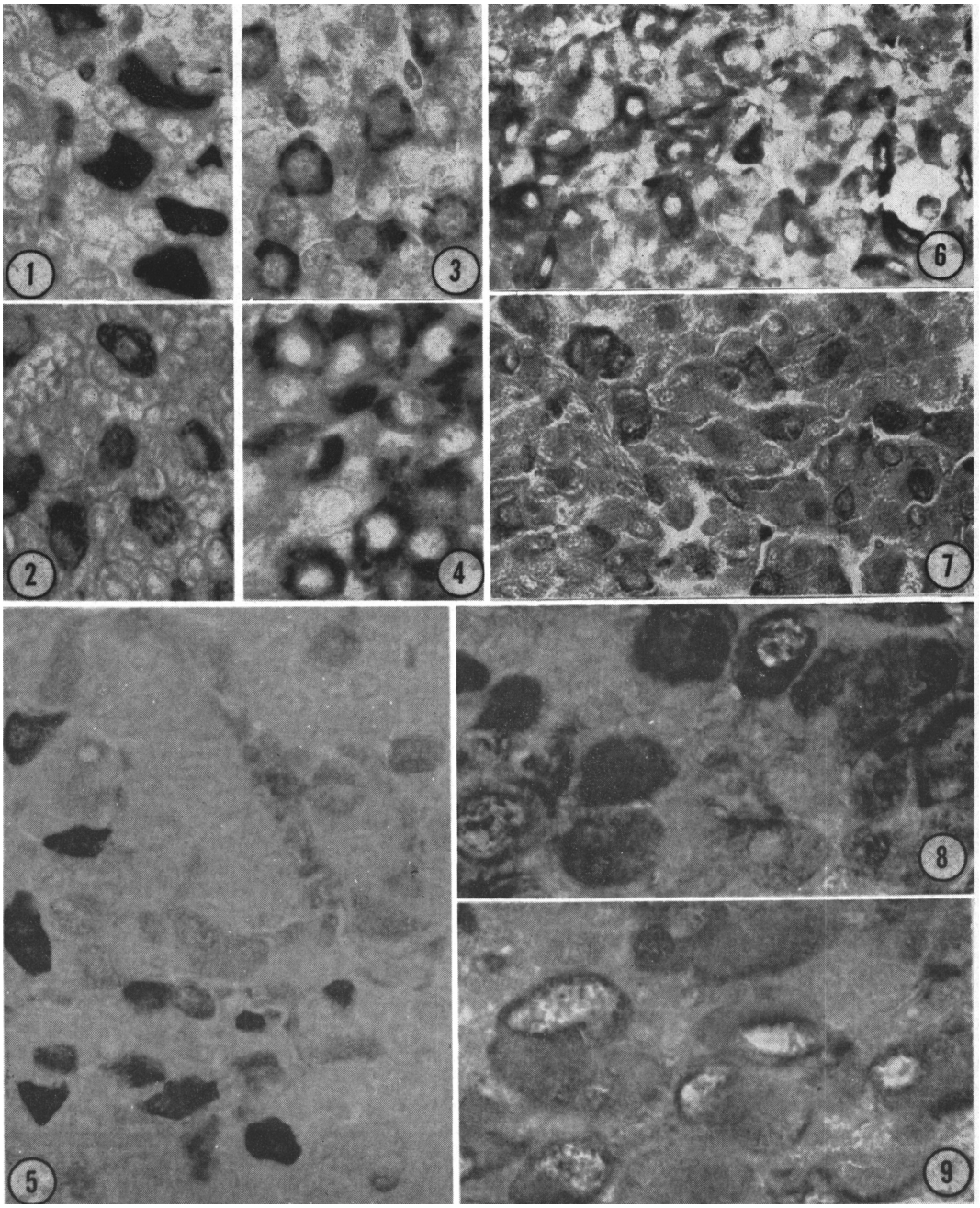
[a] Dilution of antisera and intensity of the staining reaction (—, negative; +, weak; ++, moderate; +++, strong).

gens and antisera used. This is not the case as concerns the multipotentiality of TtT, because it was first indicated by biologic observations. The TtT cells originate in normal thyrotropes and for many years of subpassages grow only in athyroid hosts. It is most unlikely that these dependent TtT carry with them normal gonadotropes during many years of successive passages during which these tumors fail to grow in normal mice.

With neoplastic transformation, the original TtH potency markedly declines, while the GtH potency persists. The same holds for somatotropic activity (9). The formation of autonomous TtT seems to be due to irreversible morphologic and cytogenetic alterations. It is reminiscent of the acquisition of hormonal activity by nonhormone-secreting organs which is usually explained by derepression of the genetic code. This derepression differs only quantitatively from that of TtT, the former being more drastic.

Note that in most of the reported studies, antisera and antigens were not from the same species and often hCG was used as a substitute source for pituitary GtH. Nevertheless, these studies are highly informative because of the high degree of hormone-specific group reactions between the hormones of different species or different organs (placenta vs pituitary). Further, the intensity of the reactions has often not been adequately quantitated. The specificity and the group reactions (antigenic determinants) of the various hormones of various species, utilizing up-to-date immunologic technics, are yet to be mapped. Cloning of tumors, establishing the hormonal features of the progeny of single cells, and isolation of hormones from tumors with multiglandular disturbances are also desirable, for the dogma that the hormones secreted by tumor cells are the same as those of normal cells may be incorrect.

*Conclusions.* The observations made are explained (a) by the existence of antigenic and biologic group-reactive common subunits between these 2 glycoprotein hormones and (b) by change in the genetic code associated with derepression in the course of neoplastic




FIGS. 1–4. Normal pituitary cells: (1) thyrotropes ×800; (2) gonadotropes ×600; (3) somatotropes ×800; (4) mammotropes ×800; FIG. 5. Normal pituitary stained with anti-TtH. Dark-staining cells are thyrotropes; light-staining, gonadotropes. ×600. FIG. 6. TtT 100 stained with anti-TtH ×500. FIG. 7. Same as Fig. 6 stained with anti-LH ×400. FIG. 8. Pituitary of ovariectomized rat stained with anti-LH ×400. FIG. 9. Same as Fig. 8 stained with anti-Gβ ×400.

transformation.

*Summary.* Mice with thyrotropic pituitary tumors (TtT) invariably exhibit biologic gonadotropic activity. By immunohistochemical staining (IHCS), anti-thyrotropic hormone (TtH) in high dilutions was found to visualize strongly normal thyrotropes; the same sera also stained normal gonadotropes, albeit rather slightly and only when applied in higher concentrations. Anti-gonadotropic hormone (GtH) stained the gonadotropes intensely, but not the normal thyrotropes. However, it did stain TtT cells. Antisera to the corresponding $\beta$ subunits stained normal and tumor cells almost as specifically as the antisera to the corresponding complete hormones. The anti-$\alpha$ sera were very weak and gave negative or weak, nonspecific reactions. Preliminary radioimmunoassays (RIA) indicated elevated GtH levels in sera of TtT-bearing mice.

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