Growth Hormonal Activity of Thyrotropic Pituitary Tumors[1] (37057)

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It was a casual observation many years ago (1) that autonomous thyrotropic tumors (TtT) have marked somatotropic activity. This was not apparent in dependent TtT, which grow only in athyroid mice and have much more highly elevated serum thyrotropic hormone (TtH) levels than the autonomous tumors. A simplistic explanation seemed satisfactory but proved to be wrong: it was thought that the above observations could be explained by assuming that the somatotropic stimulation was due to sustained elevation of thyroid hormone levels manifested by marked hyperplasia of the thyroid gland in mice bearing autonomous TtT. This explanation was first contradicted by immunohistochemical staining (IHCS) of TtT that suggested the presence of large quantities of somatotropic hormone (StH = growth hormone, GH) in dependent tumor cells. Therefore, radioimmunoassays (RIA) were performed for StH content in sera of mice bearing various types of transplanted TtT and the weight changes in various organs were more closely followed.

Materials and Methods. The sera were obtained from: (a) normal mice bearing autonomous TtT; (b) radiothyroidectomized (rte) mice bearing fully thyroid hormone deficiency-dependent TtT; (c) nontumor-bearing mice, 130–364 days following rte; (d) normal mice; and (e) normal mice bearing a mammosomatotropic tumor (strain MtT .38).

Animals and tumor strains. Young adult LAF_1 mice of both sexes were obtained from the Jackson Laboratory. They were injected with ~60 μCi of ^{131}I after being kept on low-iodine diet for ~2 wk. The dependent tumor strains TtT 100 and 76 have never given rise to antonomous variants since their isolation 5 and 2 yr ago, respectively; strain 97, our oldest, rarely did so, but is still dependent. The autonomous mutants arose from an older TtT 87 strain which steadily gives rise to autonomous variants. The latter grew well in both athyroid and euthyroid mice. For other details of isolation of TtT and their characteristics, see the reports reviewed earlier by Furth and Clifton (1).

Radioimmunoassays. RIA for StH were done by a double antibody procedure for rat StH (2). Before assaying the present samples we tested the cross-reactivity of rat and mouse StH with a preparation of StH extracted from mouse pituitaries by Dr. C. Hellerstrom, Uppsala, Sweden. A parallel displacement of ^{125}I-rat StH was produced by both rat StH standards and mouse StH in a calculation range between 0.5 and 500 ng/ml. For the present study, all determinations were done in duplicate. Due to limitation of the quantity of sera obtainable from single mice, not all wanted assays for various pituitary hormones could be carried out on sera of the same animal; however, enough assays were done on the sera of various types of animals to obtain informative data.

Immunohistochemical staining. This was performed by the technic of Nakane and Pierce (3) with the following anti-hormones: anti-mouse TtH prepared by us using purified mouse TtH obtained from Dr. R. W. Bates and Dr. P. G. Condliffe; and anti-rat StH prepared by us with rat StH obtained from Dr. A. E. Wilhelmi. Staining with antisera

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TABLE I. StH Content of Sera from TtT-Bearing Mice and Controls.[a]

Animal	Test	StH values (ng/ml)	
		Individual	Mean
With autonomous TtT 87 in normal hosts	1	♀ 320; ♀ 118	219
	2	♀ >200	(200)
With dependent TtT 100 and 76 in rte hosts	1	♂ 29; ♂ 11; ♀ 32	24
	2	♀ 69; ♀ 32	50.5
Rte	1	♂ 1.5; ♀ 1.5; ♀ 2.0; ♀ 4.5	2.4
	2	♀ 15	(15)
Normal	1	♂ 1.0; ♀ 2.0	1.5
	2	♂ 4.8; ♀ 14.0	9.4
With MStT (MtT.38)	1	♀ 280; ♀ 170	225
	2	♀ 132	(132)

[a] Each value was obtained for a different mouse.

to other pituitary hormones is described in a WHO monograph on "Pathology of Tumours in Laboratory Animals" (in press).

Results with comments. RIA for StH are shown in Table I. The changes observed in the TtT-bearing mice are of such a magnitude and consistency that we can be almost certain that they are not an artifact. The 2 high values in a normal and a rte control female mouse are in the range found in rats during estrus or proestrus. The concentration of StH in sera of mice bearing dependent TtT was about 10–20 times greater, and that of mice bearing autonomous TtT 100 times greater, than in normal mice. No difference was observed between normal and non-TtT-bearing athyroid mice. However, the spread of individual values between normal and non-TtT-bearing athyroid mice was too great for detection of small differences between these 2 groups of animals. It is noteworthy that the serum StH concentrations in a mammosomatotropic tumor-bearing mouse (strain MtT .38) were within the high range of those of autonomous TtT-bearing mice.

All dependent TtT-bearing mice were radiothyroidectomized shortly preceding tumor grafts. The dependent tumors were palpable after 4–6 mo latency period. The first few transplant generations of the autonomous tumors grew somewhat faster in athyroid than in euthyroid hosts and were palpable in about 3–4 mo. In later subgraft generations, they grew faster in euthyroid than in athyroid mice. Whereas the dependent tumors are rather uniform, the autonomous tumors are heterogeneous, having only one feature in common: ability to grow in euthyroid hosts.

RIA for TtH, done by Dr. J. Hershman, gave the following values (mU/ml): normal, 0.2–0.6; rte, 1.8–5.9; dependent TtT, 579–8400; and autonomous TtT, 10–250.

The results of IHCS parallel and amplify the RIA data on the increase of StH levels in mice bearing dependent and autonomous TtT. IHCS was done in a much larger number of mice bearing the various types of tumors than were the RIA. As illustrated in Fig. 1a, anti-TtH sera stained the dependent tumors, grown in rte hosts, very intensely; autonomous tumors were stained either slightly (Fig. 2a) or not at all. However, the very slight to moderate stimulation of the thyroid and the RIA indicate that some TtH activity was invariably present in autonomous tumors. RIA seem to be more sensitive indicators of TtH activity than IHCS. When the TtH levels were moderately elevated in autonomous TtT-bearing mice, the sections of the thyroid showed disseminated adenomatoid nodules (1).

When stained with anti-StH, both autonomous and dependent TtT were positive (Figs. 1b and 2b). The number of cells intensely stained was usually fewer in the autonomous

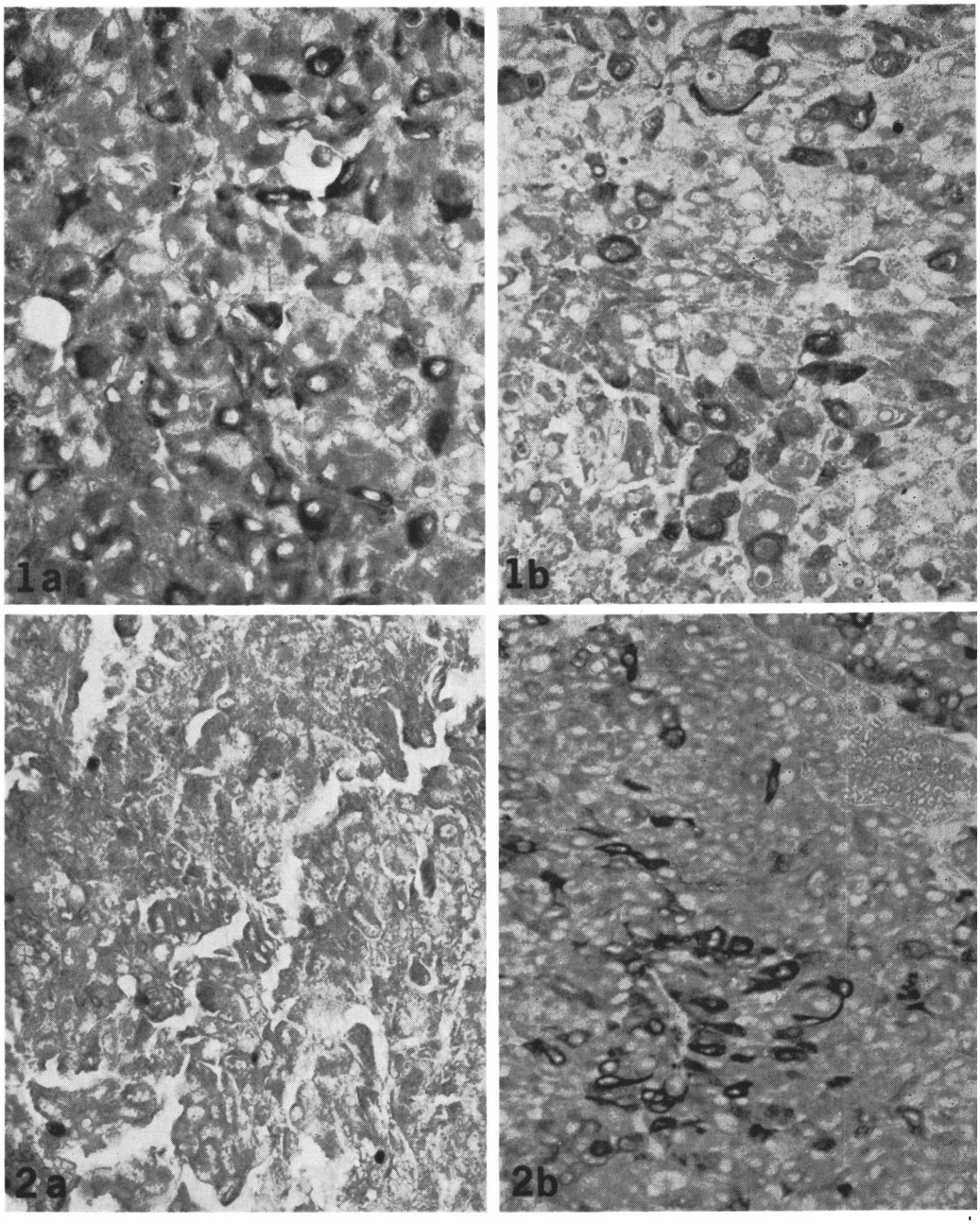

FIG. 1a. Dependent transplanted TtT (100) stained with anti-TtH; (b) same tumor as (a), stained with anti-StH.

FIG. 2a. Autonomous TtT (87) stained with anti-TtH; (b) same tumor as (a) stained with anti-StH.

than in the dependent tumors. The intensity of staining with StH varied with both types of tumors. A moderate number of cells were usually intensely stained; the majority were mildly stained or unstained. This is an apparent contradiction to the findings with RIA that indicated higher serum StH levels in autonomous than in dependent tumors. This

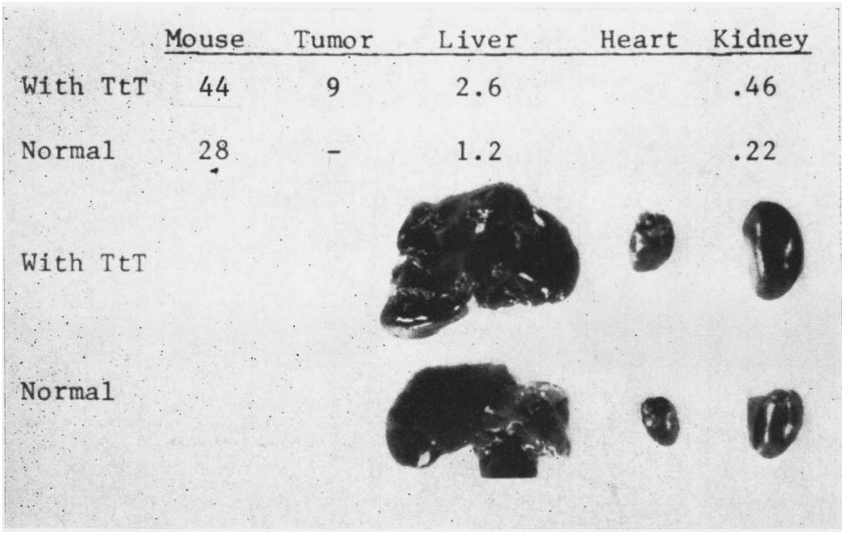

FIG. 3. StH activity with TtT (87) and controls (wt in g).

suggests that the acquisition of the ability of thyrotropes to secrete StH occurs already during the formation of a dependent TtT cell. It should be recalled that IHCS indicates storage, whereas RIA indicates release of hormone produced. It is noteworthy in this connection that in hypothyroid rats, after injection of sodium pentobarbital a decreased response of StH release was demonstrable (4). It is possible that autonomous tumor cells release the hormone faster than the dependent tumor cells and that the latter do so faster than the normal cells. This phenomenon requires clarification by quantitation of hormone production, storage and release under various experimental conditions.

The increase in *biologic StH activity* was more conspicuous in animals bearing autonomous tumors than in those bearing dependent tumors. This was suggested by increases in the body weight (including tumor weight) and in weights of organs known to be stimulated by StH. Figure 3 is an illustration of increased StH activity in a normal host bearing an autonomous TtT vs a well-matched control. The body weights of tumor-bearing animals and of their organs were estimated in a large number of animals sacrificed simultaneously with matched controls, but body and organ weights were actually determined in only a small number of animals. While the data seem sufficient to conclude

TABLE II.

No.	Tumor type and host	Wt (g)				
		Body[a]	Tumor	Liver	Kidney	Spleen
1a	Auton.(strain 87) in normal ♂	38	8.5	2.26	0.313	0.381
1b	Matched normal ♂	33	—	1.76	0.269	0.094
2a	Auton.(strain 87) in normal ♀	44	9	2.61	0.455	—
2b	Matched normal ♀	28	—	1.24	0.215	—
3a	Depend.(strain 97) in rte ♂	36	3	2.22	0.320	0.070
3b	Matched rte ♂	31	—	1.56	0.250	0.080
4a	Depend.(strain 76) in rte ♀	40	6.5	1.86	0.287	0.254
4b	Matched rte ♀	28	—	1.48	0.221	0.126
5a	Auton. MStT(strain 38) in normal ♀	34	0.9	2.75	0.328	0.296
5b	Matched normal ♀	31.5	—	1.90	0.271	0.189

[a] Body weights include tumor weights.

that StH activity is manifest in both autonomous and dependent TtT-bearing animals, precise quantitation remains to be done in a larger number of animals for the following reasons: (a) wasting, with marked loss of body weight, is usually encountered with chronic pneumonia and with very large tumors and (b) parallel data on various types of nonhormone-related tumors were not available. In general, somatotropic activity was better expressed by increase of weights of organs as the liver and kidney than by increase of body weight minus tumor weight, as illustrated by the data in Table II.

On multihormonal changes induced by thyroidectomy. The observations made thus far indicate that thyroidectomy-induced TtT are associated with a multiglandular disease manifested by gonadotropic, somatotropic and some mammotropic activities. It is most unlikely that TtT grown for years in successive passages in athyroid hosts would carry with them normal pituitary cells of various types.

The gonadotropic activity noted earlier (1, 5) is best explained (a) on the basis of a subunit common to gonadotropic hormones (GtH) and TtH and/or (b) by derepression of the genetic code associated with neoplastic transformation of normal thyrotropes. The former explanation cannot be applied to StH activity, since no structural relationship is known to exist between the glycoprotein TtH and the polypeptide StH. Further, GtH levels in sera of dependent and autonomous tumors were about the same (unpublished data). Whereas StH serum levels increased following autonomous transformation of TtT cells, serum TtH dropped to very low levels.

The high StH levels associated with autonomous tumors are best related to the derepression of the cytogenetic code of normal thyrotropes when these undergo neoplastic transformation. However, other explanations are possible and further studies are needed to elucidate the basic mechanisms observed in the course of transformation of normal, "fixed" types of cells into varied types of autonomous cells in the apparent absence of carcinogens, including viruses.

We suppose that the elevated mammotropic hormone (MtH = prolactin) activity is secondary to gonadotropic hyperplasia, estrogens being known to be the physiologic stimulators of prolactin; this remains to be verified.

Summary. Dependent TtT grown in athyroid mice have elevated serum StH (growth hormone) levels with variable, usually slight, biologic StH activity. When these tumors become autonomous, their StH activity increases while their thyrotropic activity decreases. This is demonstrable by biologic changes in the tumor hosts, by IHCS and by RIA of sera for StH and TtH. These changes are best related to neoplastic transformation of TtT cells that seems to be associated with derepression of the genetic code of normal thyrotropic cells.

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