Prolactin and Thyrotrophin Content of Functional Transplantable Pituitary Tumors. (22806)

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Transplantable functional pituitary tumors described recently were classified on the basis of their hormonal secretion as indicated by changes in tumor-bearing hosts(1). Three main types have been studied extensively: adrenotrophic, thyrotrophic and mammary gland stimulating. The adrenocorticotrophin and melanophore stimulating activity of an adrenotrophic tumor(2) and the thyrotrophin (TSH) activity of some thyrotrophic tumors have been determined. The following is a study of prolactin content and a more complete account of the TSH content of various tumors.

Methods. Prolactin was assayed in pigeons by the cropgland weight increase method (3,4). Thyrotrophin was assayed in baby chicks by following the depletion of I^{131} from the thyroid(5). Each powder was a pool of tumors from several mice. All lyophilized or acetone dried tumor powders were put through a Wiley mill using a 60 mesh screen and suspensions of such powders in 0.1 M $NaHCO_3$ solution were injected using a 22 gauge needle. Heparinized blood was collected from abdominal aorta of mice. After centrifuging, the plasma was kept frozen until injected. Prolactin was extracted from 2 tumor powders using 65% ethanol at pH 9.5-10. The alkaline alcoholic extracts were adjusted to the isoelectric region, pH 5-7, and prolactin precipitated in refrigerator by addition of ethanol to a concentration of 85%. This precipitate was washed with acetone and dried. After removal of tumor, adrenals, gonads, pituitary and obvious abdominal metastases, the bodies of 9 radiothyroidectomized mice, which had had large dependent thyrotrophic tumors (Strain 77D) were frozen. Later they were ground in a Waring blendor with 3 volumes of 1% saline and then centrifuged. The cloudy liquid phase was adjusted to pH 4.7 with N/1 HCl and again centrifuged. The clear supernatant at pH 4.7, which should contain any TSH present, was adjusted to pH 7 and lyophilized for bioassay. Seven grams of powder were obtained.

Material. Tumor Strains: Thyrotrophic tumor strain 77D was induced in a C57Bl male mouse by 275 μc of I^{131}, remained dependent for 4 consecutive passages. In one line autonomy was acquired in the fifth subpassage. Thyrotrophic tumor strain 101D was induced in a C57Bl female mouse by 400 μc I^{131}, remained dependent through 2 subtransfers, but one line developed autonomy in third transfer. The present study was made with dependent tumor lines. The origin and characteristics of the mammotrophic tumor strains in mice(6) and rats(7) have been described. Characteristics of the adrenotrophic strain 2 induced by total body ionizing irradiation have been reported(1).

Results. TSH and Prolactin content of tumor powders: There is a very high potency of the 2 dependent TSH tumors (77D and 101D) (Table I). This is about 10 times that of powders from cattle or sheep pituitaries. The other tumors all gave negative tests or borderline responses at the high dose level used. These borderline tests represent concentrations of TSH that are only 1% that found in bovine pituitaries and 0.1% that found in TSH tumors.

Thyrotrophic and adrenotrophic tumors gave negative tests for prolactin. The stilbestrol dependent mammotrophic rat tumor (FR4) was uniformly positive but had only 10% concentration of prolactin found in bovine pituitaries. Powders of mouse mammotrophic tumor (OG4) gave variable results indicating that they had about 10-40% of prolactin potency of the rat tumor (FR4).

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TABLE I. Prolactin and Thyrotrophin Potency of Dried Powder of Six Transplantable Tumors.

Species	Transplantable pituitary tumor: Strain No.	Type of tumor	TSH potency, USP U/g	Prolactin: Total dose, mg	Prolactin: Potency, IU/g
Mouse	77D	Thyrotrophic, dependent	200	200	<15
	101D	" "	100	"	"
	OG2	Adrenotrophic, autonomous	<.2	"	"
	OG3	Mammotrophic, "	"	"	"
	OG4	" "	.35	10*	10±
	"	Sample A, ♂—acetone dried	<.2	200	<15
	"	" B, ♀— " "	"	"	"
	"	" C, ♂—lyophilized	"	"	"
	"	" D, ♀— "	"	"	20
Rat	FR4	Mammotrophic, dependent	<.1	"	50
Bovine anterior pituitary gland powder			10-20	20	500

* Local cropsac assay.

TABLE II. Prolactin Potency of Crude Extracts of Mammotrophic Tumor Powders.

Tumor powder: Species	No.	Type	Amt extracted, g	Extract: Yield, dry wt, g	Prolactin: Total dose, mg	Prolactin: Potency, IU/g	Prolactin: Tumor powder, IU/g*	TSH: Potency, USP U/g	TSH: Tumor powder, USP U/g*
Mouse	OG4A,B,C	Autonomous	10.5	1.2	200	44	5	<.15	<.02
Rat	FR4	Dependent	1.3	.24	20	300	56	"	<.03

* Potency of original tumor powder calculated from potency of extracts.

Crude extracts were made of pooled mammotrophic mouse tumors (OG4, samples A, B, and C) which had given negative tests for prolactin and of rat tumors (FR4) in order to test the feasibility of concentrating the activity before assaying. Both extracts tested negatively for TSH and positively for prolactin (Table II). The autonomous mouse tumor OG4 contained perhaps as little as 5 I.U./g of prolactin, or about 10% that of the dependent tumor FR4 and 1% that of bovine pituitary powder. The extract of the dependent tumor FR4 had a potency of 300 U/g which indicated a potency of 56 U/g for the original tumor powder. This confirmed the prolactin potency of 50 U/g obtained by direct assay of FR4 powder.

TSH content of tissues of the mouse: Because the blood level of TSH is high and the tumors are fairly vascular, it was thought worthwhile to determine the TSH content of various tissues of the body (Table III). Over 98% of the TSH activity was found to be in the tumor and only about 1% in the blood stream. The small amount found in the body could be due to unidentified metastases and residual blood.

Discussion. From the data in Table III it can be calculated that a 5 g tumor weighs 2,500 times the pituitary of the mouse and contains at least 5,000 times as much TSH. Also, the serum concentration of TSH is over 2,000 that of normal human blood. Only negative tests have been obtained with normal mouse serum.

TABLE III. TSH Potency of Pituitary Gland, Blood Serum, Tumor and Body of Mice with TSH Producing Tumors.

Tissues measured	TSH potency—USP units: Wet wt basis	Dry wt basis, U/g	Total potency, U/mouse
Tumor	40 U/g	200	200 (1)
Blood serum	1-2 U/ml	10	1-2 (2)
Body minus tumor	.1 U/g	.5	2 (3)
Pituitary	20 "	100	.04(4)

(1) Basis of a 5 g tumor. (2) Basis of 1 ml serum. (3) Basis of 20 g body wt. (4) Basis of 2 mg pituitary.

The TSH concentration in human blood serum is about 0.5 U/liter, or about 2 units per man(8). A human pituitary contains only about 5 to 10 units of TSH so that the total amount of TSH in the blood stream is as much as 20 to 40% of that in the pituitary gland. Hence, it is surprising that in mice with TSH tumors the blood content is only 1% that of its source, *i.e.*, the tumor. In other words, the pituitary content/blood content ratio in normal man is 5/1, but the tumor content/blood content ratio in mice is 100/1. The supernormal hormone concentration in the tumor tissue and blood of mice with transplantable dependent thyrotrophic pituitary tumors is most unusual and these materials may, therefore, be unusually valuable in physiological and chemical studies. It may be noted that these pituitary tumors release TSH at a site distant from the hypothalamus.

Meyer and Clifton(9) have recently shown that primary pituitary tumors induced by stilbestrol pellets in rats have the same prolactin concentration as normal rat pituitaries. These results cannot be correlated with our present data because their potency values were only relative and not in terms of International Units.

Summary. The prolactin and thyrotrophin content of various transplantable pituitary tumors was studied. 1. Prolactin was found in stilbestrol induced and stilbestrol dependent rat pituitary tumor and also in an autonomous mammotrophic mouse tumor in concentrations only 10% or less that found in bovine pituitaries. These tumors contained little or no thyrotrophin. 2. Two autonomous adrenotrophic tumors contained no detectable prolactin or thyrotrophin. 3. Two dependent thyrotrophic tumors contained no detectable prolactin while the thyrotrophin concentration was ten times that of bovine pituitaries. A five gram thyrotrophic tumor contains 5,000 times more thyrotrophin than one mouse pituitary. Blood levels of thyrotrophin were 2,000 times normal.

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