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Thyroid Inhibition of Rats Bearing Transplantable, Hormone-Producing Pituitary Tumors* (32958)

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Previous reports from this laboratory (1) have described the significantly reduced uptake of radioactive iodine, and the histology of an understimulated thyroid gland, present in rats implanted with hormone-secreting pituitary tumors. Thyroid inhibition also has been described in rats implanted with a malignant nonendocrine tumor (2,3) and in humans with a variety of cancers (4).

The tumor strain employed here was the MtTW5 which secretes prolactin and growth hormone but not TSH or ACTH. The hormonal properties of this tumor have been previously described (5,6).

It has been suggested that the thyroid inhibition of rats implanted with tumors (2) and of humans with cancers or chronic debilitating illnesses (7) is operated through nonspecific mechanisms such as a decrease in the thyroxine binding proteins, diminution which is just a participant in the generalized decrease of protein synthesis that goes on in those diseases. These possibilities were explored.

Since thyroid suppression in the rats implanted with the pituitary tumors are not the result of the gland becoming unresponsive to TSH (1), our attention was directed to the pituitary content of TSH and to some of the aspects of intrathyroid iodine metabolism that are affected by TSH.

Materials and Methods. 1) *Correlation of tumor size and thyroid inhibition of iodine uptake.* Thirty-two adult Wistar-Furth female rats were placed on regular Purina rat chow and tap water *ad libitum*. They were implanted s.c. in the right subscapular area with the MtTW5 tumor and were distributed in four cages containing eight rats each. On days 5, 8, 12, 15, 20, 29, 36, 49 postimplantation, one rat from each cage was randomly selected, injected with 2 μC of carrier-free sodium ^{131}I per 100 gm of body weight, and 18 hours later anesthetized with ether, and bled by cardiac puncture. Their thyroids and anterior pituitary glands were removed and weighed. One ml of serum and the thyroids suspended in 1 ml of saline were counted in a well scintillation counter.

2) *Levels of free thyroxine* (T_4) in plasma were determined according to the equilibrium dialysis technique described by Ingbar *et al.* (8). The serum extracted from one single rat was insufficient to carry out these studies on an individual basis, hence the serum from 2–4 rats was pooled.

Control and tumor animals were matched by age and sex, but not by weight, because by virtue of the very same tumoral effects, the tumor-bearing rats were heavier.

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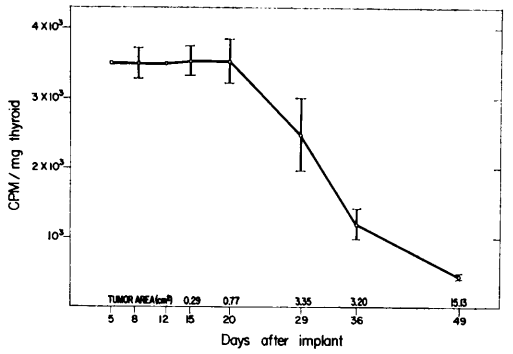

FIG. 1. Correlation of tumor area and days postimplant with inhibition of thyroid ^{131}I incorporation.

3) *The TSH content of the pituitary glands* was determined by the MacKenzie bioassay technique with minor modifications (9). The pituitaries used had been previously removed, pooled and kept frozen until the day of the assay, when they were homogenized in cold saline, diluted, and injected into the test mice. Care was taken that equal amounts of pituitary weight from each group were injected into the assay animals (40 μg of dry wt. /mouse).

4) *Effects of varying doses of stable iodine on thyroid function.* Rats with pituitary tumor MtTW5 and a nontumor group were matched by age and sex. These groups were subdivided into four subgroups and injected i.p. with 2 μC of carrier-free sodium ^{131}I/100 gm of body weight. The first subgroup received carrier-free isotope. The second subgroup was given, in addition, 0.002 mg of potassium iodide (KI)/100 gm of body weight. The third subgroup received 0.08 mg of KI/100 gm of body weight, and the fourth subgroup received 0.4 mg of KI/100 gm of body weight.

Four hours after injection, the rats were killed with ether, blood was taken by cardiac puncture, the thyroid was rapidly removed, blotted, weighed and homogenized in 2 ml of 10% trichloroacetic acid (TCA). The precipitate was removed by centrifuging at low speed. One ml of serum was also precipitated with 2 ml of 10% TCA and separated into soluble and insoluble fractions. Pieces of mammary gland were removed and weighed. The radioactivity in these tissues was determined by using a well scintillation counter. At the time of the experiments, the MtTW5 tumor had been implanted for 26–52 days.

Results. The data in Fig. 1 present the correlation between tumor size and inhibition of thyroid radioactive iodine uptake. As shown, 20 days after inoculation the pituitary tumor MtTW5 was measurable. During this period thyroid function measured by the uptake of radioactive ^{131}I was constant. However, it is seen that a reduction in ^{131}I uptake occurred by day 29 after the implant when the average tumor area was 3.35 cm^2. A further decrease in radioiodine uptake occurred by days 36 and 49 when tumor areas were, respectively, 3.2 cm^2 and 15.13 cm^2.

The pituitary weight per 100 gm of body weight was significantly reduced by day 49 ($p<0.01$ when compared with the weight of the pituitary by day 12 postimplant) (see Table I).

The TSH activity in the rat pituitary glands is presented in Fig. 2. The pituitaries

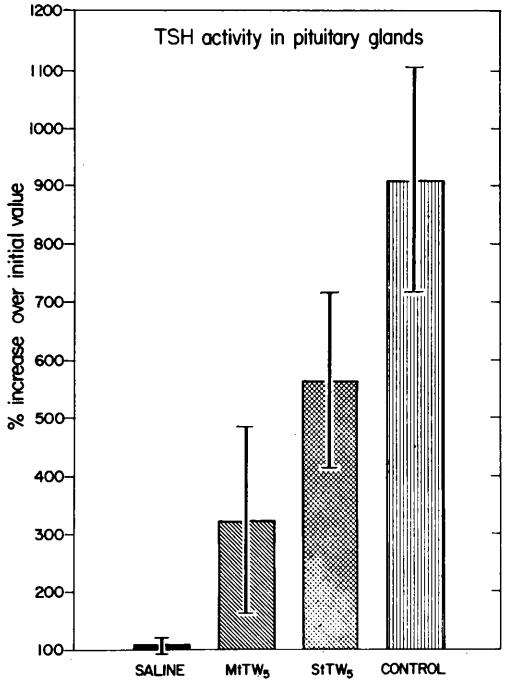

FIG. 2. Results are expressed as the percentage increase of the radioactivity of the 2-hour sample over the baseline sample minus the percentage increase of the group injected with saline. Student t test: control vs MtTW5 $p<0.05$; StW5 vs control and MtTW5: NS.

of MtTW5-implanted rats contained, per unit of weight, significantly less TSH than the pituitaries of the control rats. The TSH content of the pituitaries of another group of rats implanted with a tumor that only secretes growth hormone, the StW5 (10), is also shown, but they did not differ significantly from controls or MtTW5-implanted rats.

The data in Table II show that there were not significant differences in the percentage of free T_4 (PFT_4). On calculating the absolute amounts of circulating free T_4 (AFT_4), it is seen that they were significantly less in the MtTW5-implanted animals than in the controls. The values of the other tumorous animals studied (StW5) were intermediate between those two.

The effects of varying doses of stable iodine on thyroid function were studied next and the data are presented in Table III. Previously it was demonstrated that when no carrier ^{127}I was given, the percentage uptake of the thyroid gland and the T/S ratio in propylthiouracil (PTU)-blocked rats were significantly greater in the control animals than in the tumor-bearing ones (1). In the present study using unblocked thyroid glands it is seen that when 2 μC of carrier-free or 2 μC/0.002 mg of stable iodine/100 gm of body weight were injected, nontumor animals incorporated significantly greater amounts of isotope into their thyroid glands, either organified or not ($TPB^{131}I + T^{131}I$), as compared to the tumor-bearing rats. At higher doses of stable iodine, this difference in incorporation between the tumor-bearing and control rats disappears and may be even reversed. When the control animals are loaded with ^{127}I, in increasing concentrations, they manifest a progressive decrease in the intrathyroid to serum ratio ($TPB^{131}I + T^{131}I/S^{131}I$). The tumor-bearing rats also show a progressive diminution, but the decrease is not as sharp as in the controls so that at the higher dose levels of ^{127}I, the ratios are higher in the tumor-bearing animals than in the controls.

The ratio $TPB^{131}I/T^{131}I$, which we have chosen to express as the degree of iodine organification, was not significantly different between the controls and the animals implanted with MtTW5 tumor, when no ^{127}I

TABLE I. Correlation of Days Postimplant of Tumor MtTW5 and Body, Thyroid, and Pituitary Weights.[a]

	Days after implant																			
	5		8		12		15		20		29		36		49					
Body wt. (gm)	118	±5	119	±4	117	±9	136	±7	137	±4	156	±1	165	±3	191	±11				
Thyroid wt. (mg)	5.87 ± 0.54		6.36 ± 0.78		6.10 ± 0.40		6.76 ± 0.21		7.09 ± 0.42		8.31 ± 0.17		7.00 ± 0.31		7.91 ± 0.23					
Pituitary wt. (mg/100 gm of body wt.)	—		—		3.62 ± 0.1126		4.57 ± 0.2406		4.39 ± 0.7142		—		3.27 ± 0.2739		2.45 ± 0.04					

[a] Values presented are mean ± SEM.

TABLE II. Percentage and Absolute Free Thyroxine in Plasma.[a]

	PFT_4	AFT_4 (mμg/100 ml)	Probability (vs control)
Controls (4)[b]	0.1827 ± 0.011	10.40 ± 0.61	—
MtTW5 (4)	0.1552 ± 0.004	7.03 ± 0.19	<0.005
StW5 (3)	0.1643 ± 0.016	9.85	<0.9

[a] Values presented are mean ± SEM.

[b] Numbers in parentheses indicate number of pooled samples of serum tested.

was injected. Both tumor and nontumor implanted animals show diminished iodine organification with increasing amounts of stable iodine, but the slope of this decrease in the tumor animals is not as steep resulting in less organification in control animals when injected with large amounts of carrier iodine.

Mammary gland uptake of ^{131}I was studied and found not to be affected by injecting varying amounts of carrier iodine. The mammary glands of tumorous animals (prolactin-producing) trapped iodine in much greater amounts than did those of the control animals. Yet, this iodine trapped by these mammary glands did not seem to be at the expense of the iodine trapped by the thyroid since the serum concentrations of ^{131}I were higher in the MtTW5-bearing rats.

Discussion. The correlation of tumor size with the thyroid inhibition indicates that MtTW5 tumors are rather slow to produce thyroid inhibition since they need to attain a large size before showing the inhibitory effects and since the other hormonal effects of this tumor were present long before the thyroid inhibition occurred. In any event the animals bearing the larger tumors did not appear sick or lethargic and ate well. The significant reduction in pituitary weight has been reported by MacLeod (11) to occur in animals implanted with GH and/or LTH secreting tumors. A decrease in size and

TABLE III. Effects of Varying Doses of Stable Iodine on ^{131}I Incorporation into Thyroid.[a]

Group	Controls			
^{127}I injected (mg)	0.00	0.002	0.08	0.4
$TPB^{131}I + T^{131}I/S^{131}I$	115.71 ± 43	15.32 ± 3.53	0.95 ± 0.1	0.176 ± 0.016
$TPB^{131}I/T^{131}I$	93.81 ± 12	14.58 ± 3.11	4.32 ± 1.04	0.622 ± 0.265
$TPB^{131}I$/10 mg	82,974 ±6862	49,280 ±3310	5778 ± 541	504 ± 84
$T^{131}I$/10 mg	1091 ± 146	3850 ± 510	1684 ± 224	993 ±216
$S^{131}I$/ml	1344 ± 326	4259 ± 876	7809 ± 824	7034 ±656
No. of rats	9–15	5	5	5
Group	MtTW5			
^{127}I injected (mg)	0.00	0.002	0.08	0.4
$TPB^{131}I + T^{131}I/S^{131}I$	23.66 ± 6[b]	3.14 ± 0.84[b]	1.43 ± 0.11[b]	0.236 ± 0.01[c]
$TPB^{131}I/T^{131}I$	116.53 ± 12[d]	20.47 ± 2.20[d]	20.55 ± 2.64[c]	6.244 ± 0.93[c]
$TPB^{131}I$/10 mg	44,134 ±6387	21,280 ±2130	7689 ± 753	1668 ± 87
$T^{131}I$/10 mg	410 ± 51	1030 ± 30	498 ± 96	425 ± 86
$S^{131}I$/ml	1615 ± 148	7349 ±1002	5440 ± 204	8000 ±359
No. of rats	10–15	2	4	5

[a] Animals fed regular purina rat chow and tap water *ad libitum*. Values presented are mean cpm ± SEM. Animals were sacrificed 4 hours following the injection of $^{131}I/^{127}I$. TPBI and TI = thyroid protein bound and nonbound iodine, respectively. SI = serum nonbound iodine. Tumors were implanted 26–52 days earlier. Test of statistical significance between nontumor and tumor groups injected with identical amounts of stable iodide is defined as [b] $p < .05$, [c] $p < .01$, [d] NS = nonsignificant.

in number of cells of the "thyrotroph" may also be involved.

The decrease of the TSH content of the pituitary glands would suggest that the thyroid hypofunction in the tumor-bearing rats is mediated through a reduction of the circulating TSH. It is quite possible that the pituitary content of a hormone does not necessarily correlate with the circulating level of such hormone, as would happen if the turnover of synthesis and release were too rapid. However, as it has been shown previously, their thyroid function can be restored to normal when injected with TSH (1), therefore lending presumable evidence that their circulating TSH is insufficient to maintain normal thyroid function.

Galton and Ingbar (2) found that rats implanted with the malignant, nonendocrine carcinosarcoma Walker 256 had decreased thyroid function as measured by ^{131}I uptake, PBI and release of hormone from the thyroid. The calculated AFT_4 found in the animals implanted with the carcinosarcoma was about two times greater than that of the nontumorous rats. They suggested that these higher levels of AFT_4 would in turn feed back on the pituitary and decrease TSH synthesis and release, thus resulting in diminished radioactive iodine uptake and release as $^{131}I—T_4$. The implication is that the thyroid hypoactivity of the tumor rats was similar to that of "sick" patients in whom a decrease in thyroxine binding prealbumin (TBPA) leads to a relative and absolute increase of free T_4 (7).

Whereas disease states including neoplastic disease may decrease thyroid function by feedback at the hypothalamo–hypophysial level secondary to elevated serum free thyroxine levels, the data presented here show that pituitary tumors which are not debilitating cause a decrease in pituitary content of TSH through another mechanism.

The behavior of the thyroids in normal intact and tumor implanted rats following the administration of increasing concentrations of iodine, i.e., the decrease of intrathyroid to serum ratio, and the decrease in the degree of organification, was quite consistent with the induction of an acute Wolff–Chaikoff effect (12). Dilution of ^{131}I by ^{127}I may have contributed to the apparent decrease of intrathyroid to serum ratios, but it fails to explain the differences observed in the control animals compared to the tumor bearing rats. Previously it was shown that the serum iodine concentration was not altered by pituitary tumors (1).

The significantly higher amount of organification in tumor animals at the moderate and high concentrations of ^{127}I is somewhat reminiscent of the behavior of the rats that have "escaped" from the inhibitory effects of iodine. The decreased T/S ratio in both PTU-blocked and not blocked glands of tumor bearing animals when no ^{127}I was added is consistent with this hypothesis (13). Even though the tumor implanted animals eat and drink more than the controls, this intake of foodstuff was not enough to increase the plasma concentrations of iodine, which have been reported as minimal to provoke the induction of and the escape from the Wolff–Chaikoff effect (14).

The data of Braverman and Ingbar (13) show that the percentage of organification in intact rats that had escaped from the inhibitory effects of iodine is close to that of hypophysectomized rats. Also, as Raben demonstrated (14), high levels of TSH make the animals more sensitive to the inhibition by iodine. The converse is true: the less TSH and/or the less avidity of the thyroids for iodine, the less likely it is for the inhibition of organification to occur in the presence of high concentrations of ^{127}I.

Recently, Gona described (15) the goitrogenic effects of prolactin on the thyroids of frogs. When given prolactin, some stages of their metamorphosis were impaired, and this impairment could be prevented or neutralized by administering thyroxine. Prolactin caused the thyroids to trap less ^{131}I and this reduction was not changed by TSH. The histology of the thyroids of the frogs given prolactin suggested that they already were subjected to marked stimulation by their endogenous TSH. Prolactin also has been reported to specifically inhibit the coupling reaction of the tyrosines (16) but this has not been confirmed by others.

Even though the rats implanted with

MtTW5 tumors are chronically exposed to large amounts of prolactin, their thyroid inhibition seems of a different nature than the one induced by prolactin in the frogs because the histology of the gland and the response to TSH are different.

In humans with acromegaly, an increased clearance of iodine, a decreased ^{131}I uptake by the gland and slightly decreased thyroid hormone formation has been reported (17). The possibility of increased renal clearance of iodine decreasing the share of iodide trapped by the thyroid in the MtTW5-implanted rats has been excluded, as the thyroid inhibition persists in nephrectomized tumor-bearing rats compared to nephrectomized controls (1).

Summary. Implantation of rats with growth hormone- and prolactin-secreting pituitary tumors cause weight reduction of the host's pituitary gland. This change was accompanied by a decrease in the pituitary content of TSH and a fall in ^{131}I uptake by the thyroid gland in rats with prolactin- and growth hormone-secreting pituitary tumor MtTW5. These results correlated well with the finding that the proportion of organified iodine and the intrathyroid to serum iodine ratio, in the presence of high concentrations of stable iodine, were higher in the tumor bearing rats. The amount of circulating free thyroxine was measured and found to be slightly lower in tumor-bearing rats. It is suggested that the pituitary tumor hormones have a direct suppressive effect on the host's pituitary gland production of TSH and thus indirectly decrease thyroid function.

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Hepatic Deposition of ^{131}I Labeled Amylase in Dogs: Comparison of Enzymatic and Isotope Measurements* (32959)

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When ^{131}I labeled amylase (α-1, 4-glucan 4-glycanohydrolase) preparations are injected into dogs, the enzyme is first rapidly bound to blood cells and then slowly released to the plasma. As a result, it takes about 3 hours after injection for the plasma amylase-^{131}I to reach maximum values. After these are attained, the plasma concentration of the labeled enzyme declines (1). It has been found

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