Transplantable Thyroid Tumors in the Rat: Development of Normal-appearing Thyroid Follicles in the Differentiated Tumors, and Development of Differentiated Tumors from Iodine-deficient, Thyroxine-involuted Goiters

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SUMMARY

Rats fed an iodine-deficient diet for 18 months developed benign goiters and goiters containing a differentiated tumor. Implants of benign, iodine-deficient goiters grew large papillary-follicular tumors in thyroidectomized, iodine-deficient rats. There were no "takes" of the same implants in the normal rats fed regular diet.

Implants of iodine-deficient goiters containing either a pure papillary or a pure follicular tumor grew large papillary-follicular tumors in thyroidectomized, iodine-deficient rats. The same implants in the normal, regular diet-fed rats grew small differentiated tumors which contained aggregates of normal-appearing thyroid follicles. This observation suggests that the thyroid tumor cell can differentiate to form normal-appearing thyroid follicles.

Rats fed an iodine-deficient diet for 18 months were then given regular diet and daily injections of 5 μg sodium L-thyroxine for 2 months. The goiters were involuted. The implants of these iodine-deficient, thyroxine-involuted goiters in thyroidectomized, iodine-deficient rats grew either large papillary-follicular tumors or small nodules of hyperplastic tissue. The transplants of the benign nodules into thyroidectomized, iodine-deficient rats developed into large papillary-follicular tumors. Involution of iodine-deficient goiter by thyroxine does not prevent the development of transplantable thyroid tumors. Some of these phenomena may be correlated with the pathogenesis of thyroid carcinoma in man.

INTRODUCTION

Transplantable thyroid tumors are produced in the rat and mouse by maintaining prolonged increased secretion of thyrotropin in isologous, inbred donors and recipients of thyroid tissue. (1, 2, 9–11, 17). The donor thyroid glands are severely hyperplastic and sometimes contain differentiated tumors. The first-generation transplantable tumors are usually differentiated, functional, and thyrotropin dependent. The tumors of later generations are often poorly differentiated, nonfunctional, and thyrotropin independent.

Williams and Doniach (15) observed growth of thyroid autotransplants in hypophysectomized rats and in rats with thyrotropin secretion suppressed by administration of large doses of thyroxine. This approach has not been used in the investigation of transplantable thyroid tumors.

Leblond and Isler (8) demonstrated that feeding of iodine caused involution of thyrotropin-dependent β-nodules of iodine-deficient goiters in rats, while autonomous γ-nodules increased in size and number. This phenomenon also has not been examined in the development of transplantable thyroid tumors. In such an experiment thyroxine could be substituted for iodine.

This is a report of experiments designed to examine the development of transplantable thyroid tumors in rats by transplantation of iodine-deficient, thyroxine-involuted goiters fed an iodine-deficient diet.

MATERIALS AND METHODS

Animals

Fischer strain 344 inbred male rats were kept in a large cage at a constant temperature (28°) and 12-hr cycles of exposure to light. They were fed regular Purina chow pellets (2.5 μg iodine/g) and tap water or a Remington iodine-deficient diet (wheat gluten 18%, brewers' yeast 10%, yellow corn 70%, calcium carbonate 1.0%, sodium chloride 1.0%, and iodine 0.02 to 0.05 μg/g) and distilled water.

Donors of Thyroid Tissue. Iodine-deficient Rats. Twelve animals were fed an iodine-deficient diet for 18 months. Six animals were used for study of iodine metabolism. The iodine-deficient goiters of the other 5 rats were transplanted into 131I-thyroidectomized and iodine-deficient rats and normal rats kept on regular diet.

Iodine-deficient, Thyroxine-treated Rats. Twelve animals were fed an iodine-deficient diet and distilled water for 18

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Iodine-deficient Rats. Ten-week-old normal rats were fed an iodine-deficient diet were litter mates of the Tx-ID2 rats. Another 2 weeks later they received transplants of thyroid tissue. Twelve hr before sacrifice iodine-deficient rats and iodine-deficient, thyroxine-treated rats were treated by injection with 10 μCi 131I i.p. The iodine-deficient, thyroxine-involuted thyroid tissue was implanted only into Tx-ID rats.

Recipients of Thyroid Tissue. 131I-Thyroidectomized, Iodine-deficient Rats. Ten-week-old normal rats were fed an iodine-deficient diet and distilled water. Two weeks later, they were treated by injection with 500 μCi 131I i.p. Another 2 weeks later they received transplants of thyroid tissue.

Normal Rats on Regular Diet. Normal rats fed a regular diet were litter mates of the Tx-IDÉ rats.

Sacrifice

All animals were anesthetized with 1.0% Pentothal. Blood for 127I and 131I studies was taken from the heart and the animals were then bled from the aorta. The thyroid glands, pituitary glands, and transplantable thyroid tumors were immediately removed and weighed. The tissue was handled on ice. Tissue samples were stored at -20°C. In Tx-ID rats the extent of thyroidectomy was verified by histological examination of the larynx and trachea for thyroid remnants. In all animals the organs were examined with magnifying glasses (10 X) for metastases.

Histological Examination

Thyroid glands used for transplantation and study of iodine metabolism were histologically examined. Several samples from each thyroid lobe was fixed in 10% formalin. From each sample of tissue several microscopic sections were taken. Several samples from each transplantable tumor were similarly taken. Failure of “takes” was confirmed by histological examination of subcutaneous tissue at the implantation site.

Transplantation

Transplantation of Thyroid Tissue. Donor animals were bled and the thyroid glands were removed under sterile conditions. The glands were weighed before and after sections for histological examination were taken. Thyroid tissue was finely minced in 0.9% sterile NaCl solution and thoroughly mixed; 20 mg (0.1 ml) were implanted with a No. 18 gauge needle under the skin of both lumbar areas.

Iodine Metabolism

Measurement of 131I Uptake, Protein Bound 131I, and 131I-labeled Compounds. Tumor and thyroid tissue were homogenized in cold borate buffer solution, pH 8.5. The radioactivity of muscle, homogenates of the tumor, and thyroid tissue were determined in a well-type scintillation detector. The protein bound 131I was determined after precipitation of the tissue protein with 20% trichloracetic acid. The 12 hr uptake of 131I by the thyroid glands and tumors as well as the protein bound 131I were done and calculated according to the method previously described (9).

Iodine-deficient Goiters and Transplantable Thyroid Tumors Developed from Them

Iodine-deficient Goiters and Pure Papillary or Pure Follicular Tumor-containing Goiters. Rats were healthy and euthyroid. The thyroid glands were enlarged (433.9 ± 31.4 mg), hyperemic, and grossly nodular. In 4 animals the thyroid glands were hyperplastic. Few β- and no γ-nodules were found. In the remaining 2 rats a well-differentiated follicular tumor was present in addition to hyperplastic tissue (Table 1). The mean thyroidal 131I uptake was high (83.8 ± 2.7% or 19.7 ± l.2%/100 mg tissue). Most of the 131I was bound to protein (89.1 ± 3.2%). The mean thyroid tissue 131I-moniodotyrosine/diiodotyrosine and 131I-triiodothyronine/thyroxine ratios were elevated (68.4%/7.6% and 13.1%/4.9%, respectively). Serum protein bound iodine (PB127I) was low (0.5 ± 0.1 μg/ml) while the serum 131I-T3/T4 ratio was elevated (32.9/53.5).

Transplantable Thyroid Tumors Developed from the Implants of ID-G and ID-G + Tu. Five thyroid glands were
Table 1

Iodine-deficient goiter

Animals were fed an iodine-deficient diet (0.02 to 0.05 μg iodine/g food) for 18 months. Mean values are ± S.E.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Thyroid weight (mg)</th>
<th>Whole gland Thyroid 131I uptake (% dose)</th>
<th>Tissue Thyroid PB 131I % uptake</th>
<th>Serum PB 127I (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320.7 ± 18.2</td>
<td>433.9 ± 31.4</td>
<td>83.8 ± 2.7</td>
<td>19.7 ± 1.2</td>
<td>89.1 ± 3.2</td>
</tr>
<tr>
<td>433.9 ± 31.4</td>
<td></td>
<td></td>
<td></td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Thyroid 131I compounds (% total)

<table>
<thead>
<tr>
<th>I</th>
<th>MIT</th>
<th>DIT</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 ±</td>
<td>68.4 ±</td>
<td>7.6 ±</td>
<td>13.1 ±</td>
<td>4.9 ±</td>
</tr>
<tr>
<td>0.7</td>
<td>0.4</td>
<td>1.3</td>
<td>0.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Serum 131I compounds (% total)

<table>
<thead>
<tr>
<th>I</th>
<th>MIT</th>
<th>DIT</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1 ±</td>
<td>2.9 ±</td>
<td>1.6 ±</td>
<td>32.9 ±</td>
<td>53.5 ±</td>
</tr>
<tr>
<td>0.7</td>
<td>1.3</td>
<td>0.8</td>
<td>4.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>

The mean 131I uptake by the tumor varied greatly (37.2 ± 9.2%), but it was low when expressed per unit weight of tissue (1.32% dose/100 mg tumor tissue). The protein bound fraction of 131I was uniformly high (90.1 ± 1.8%). The mean tumor 131I-MIT/DIT ratio was elevated (86.9%/4.5%) and the fraction of 131I thyronines was low. This group of tumors produced a small amount (1.4 ± 0.6%) of an unidentified 131I-labeled compound with an RF of 0.36 in the 1-butanol-acetic acid solvent system.

The tissue of these tumors was simultaneously implanted into groups of Tx-ID rats and N-RD rats. The implants in Tx-ID rats developed only papillary-follicular tumors free of nonneoplastic elements. These tumors grew more rapidly and were less active in metabolizing iodine than the first generation tumors.

Similar papillary-follicular tumors developed in N-RD rats. Growth was slower and function was less than in tumors in the Tx-ID rats. One N-RD rat developed a papillary-follicular tumor with large aggregates of normal-appearing thyroid follicles.

Transplantable Thyroid Tumors Developed from the Implant of ID-G and ID-G + Tu Rats. Tumors developed in all Tx-ID rats implanted with either ID-G or ID-G + Tu tissue. The “takes,” growth rates, gross and microscopic structure, and iodine metabolism in these tumors were similar; therefore, they were treated as one group (Table 2 and Figs. 1A, E, and F).

The “takes” were observed in 3 months. At autopsy, 9 months after implantation, the tumors were moderately large (4.4 ± 0.7 g), dark red, encapsulated, and soft. Microscopically, they were papillary-follicular tumors with a predominance of papillary elements. In some, small microscopic fragments of hyperplastic tissue were mixed with tumor tissue.

The mean 131I uptake by the tumor varied greatly (37.2 ± 9.2%), but it was low when expressed per unit weight of tissue (1.32% dose/100 mg tumor tissue). The protein bound fraction of 131I was uniformly high (90.1 ± 1.8%). The mean tumor 131I-MIT/DIT ratio was elevated (86.9%/4.5%) and the fraction of 131I thyronines was low. This group of tumors produced a small amount (1.4 ± 0.6%) of an unidentified 131I-labeled compound with an RF of 0.36 in the 1-butanol-acetic acid solvent system.

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Implant of ID-G + Tu Tissue in N-RD Rats. The animals were sacrificed at 10 months. There were no “takes” of the implants of ID-G tissue into the N-RD rats. (The implantation sites were microscopically examined.)

"Takes" of implants of ID-G + Tu tissue into N-RD rats were observed in all animals in 6 to 8 months. Growth was slow and small. Encapsulated tumors were found at autopsy at 10 months. No metastases were found.

The mean weight and histological structure of the thyroid and pituitary glands of the N-RD hosts did not differ from the same organs in comparable normal animals (Fig. 1C). Histologically, 2 types of tumors developed. One tumor was composed of pure papillary, pure follicular elements, and normal-appearing thyroid follicles. The papillary part of the tumor was characterized by papillary projections within large, distended follicles. The follicular areas of the tumor had small and irregular follicles with cuboid epithelium and little colloid. The normal-appearing thyroid follicles varied in size and consisted of flat epithelium with thin colloid (Figs. 1G, H, and I). In 1 rat a similar combination of the tumor and normal-appearing follicles was present in 1 lumbar area, while in the other the tumor was present. The other tumor was composed of purely follicular elements and normal-appearing thyroid follicles. (Figs. 2G, H, and I). The irregular follicles of the follicular tumor were lined with cuboidal epithelium and contained no colloid. The islets of normal-looking thyroid tissue consisted of aggregates of 5 to 10 follicles (Figs. 2G, H, and I).

In 1 rat both transplants developed into pure follicular tumors without normal-appearing thyroid follicles.

Metabolism of iodine was studied in 1 tumor composed of papillary and follicular elements and normal thyroid follicles. The tumor was small (270.3 mg). \(^{131}I\) uptake was relatively high (7.5%), and \(^{131}I\) concentration of \(^{131}I\) was high (88.0%). The \(^{131}I\)MIT/DIT ratio was elevated (43.3%/20.3%) while the fraction of \(^{131}I\)-thyriones was relatively high (14.6%) (Table 3).

The first-generation tumors, composed of tumor elements and normal-appearing thyroid follicles, were transplanted into N-RD rats. All animals developed tumors but without the accompanying normal-appearing thyroid follicles. The growth rate of the tumors increased during the subsequent 2 to 3 generations. The histological structure did not change. The metabolism of iodine remained constant.

Iodine-deficient, Thyroxine-involuted Goiters and Transplantable Thyroid Tumors Developed from Them

Iodine-deficient, Thyroxine-involuted Goiter. The animals were healthy and euthyroid. The thyroid glands were enlarged (143.7 ± 14.8 mg) and moderately nodular (Table 4, Chart 1, and Fig. 3A). The thyroid glands were composed of moderately large follicles with flat or low cuboidal epithelium and thin colloid. β-Nodules were rare and no γ-nodules were found in these glands. The thyroidal \(^{131}I\) uptake was within normal range (9.8 ± 1.5%). The concentration of \(^{131}I\) in thyroid tissue was normal, but it was decreased when expressed per unit weight of tissue (6.3 ± 1.1%/100 mg tissue). The composition of \(^{131}I\)-labeled compounds was normal (\(^{131}I\)-MIT/DIT = 32.0%/39.6% and \(^{131}I\)-T\(_3\)/T\(_4\) = 4.7%/13.9%). Serum protein iodine (PI) (3.6 ± 0.2 μg/100 ml) and the distribution of the serum \(^{131}I\)-labeled compounds were normal.

Transplantable Thyroid Tumors Developed from the Implant of ID-T\(_4\)G into Tx-ID Rats. Five (ID-T\(_4\)G) were separately minced and implanted into groups of 3 to 4 Tx-ID rats. Two types of transplantable tumors were developed: transplantable thyroid tumors which grew in 3 of 5 groups of rats, and transplantable nodules of hyperplastic tissue which developed in all rats of the other 2 groups of animals.

Transplantable Thyroid Tumors Developed from the Implant of ID-T\(_4\)G into Tx-ID Rats. "Takes" were noted with 3 months in 3 groups of animals. The animals were sacrificed after 9 months (Table 5, Chart 1, and Fig. 3B). The mean tumor weight was 4.3 ± 2.0 g. The tumors were deep red and encapsulated with partly solid and cystic areas. Microscopically, they were papillary-follicular tumors. In a few tumors severely hyperplastic tissue adjacent to neoplastic

<table>
<thead>
<tr>
<th>Implant sacrifice interval month</th>
<th>Rat body weight (g)a</th>
<th>Tumor weight (g)</th>
<th>Tumor (^{131}I) 12 hr uptake (% dose)</th>
<th>Tumor (^{131}I) in Tissue (100 mg)</th>
<th>Tumor PB(^{131}I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary-follicular tumor(b)</td>
<td>9</td>
<td>271.0 ± 10.3c</td>
<td>4.4 ± 0.7</td>
<td>37.2 ± 9.2</td>
<td>1.32 ± 0.36</td>
</tr>
</tbody>
</table>

\(a\)15 animals.
\(b\)Thyrotropin-dependent tumor, first generation.
\(c\)Mean ± S.E.

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**Table 2**

Transplantable thyroid tumor developed from iodine-deficient goiters in thyroidectomized, iodine-deficient rats
Table 3

Transplantable thyroid tumor developed from iodine-deficient goiter in normal rat on regular diet

<table>
<thead>
<tr>
<th>Implant sacrifice interval month</th>
<th>Rat body weight (g)</th>
<th>Tumor weight (g)</th>
<th>Tumor $^{131}I$ 12-hr uptake (% dose)</th>
<th>Tumor PB $^{131}I$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Whole tumor (100 mg)</td>
<td>Tissue (100 mg)</td>
</tr>
<tr>
<td>Papillary-follicular, and follicular tumors with normal-appearing follicles$^b$</td>
<td>10</td>
<td>320.0 ± 13.0$^c$</td>
<td>1.0 ± 0.4</td>
<td>7.5$^d$</td>
</tr>
</tbody>
</table>

Tumor $^{131}I$ compounds (% total)$^d$

<table>
<thead>
<tr>
<th>I</th>
<th>MIT</th>
<th>DIT</th>
<th>T$_3$</th>
<th>T$_4$</th>
<th>U_T</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>43.3</td>
<td>20.3</td>
<td>5.6</td>
<td>9.0</td>
<td>8.4</td>
</tr>
</tbody>
</table>

$^a$5 animals.
$^b$Autonomous tumor, first generation.
$^c$Mean ± S.E.
$^d$One tumor weighing 270.3 mg.

Table 4

Iodine-deficient, thyroxine-involuted goiter

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Thyroid weight (mg)</th>
<th>Thyroid $^{131}I$ 12-hr uptake (% dose)</th>
<th>Serum $^{131}I$ compounds (% total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole gland (100 mg)</td>
<td>Tissue (100 mg)</td>
</tr>
<tr>
<td>6 rats</td>
<td>285.6 ± 11.2</td>
<td>143.7 ± 14.8</td>
<td>9.8 ± 1.5</td>
</tr>
</tbody>
</table>

Thyroid $^{131}I$ compounds (% total)

<table>
<thead>
<tr>
<th>I</th>
<th>MIT</th>
<th>DIT</th>
<th>T$_3$</th>
<th>T$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.4 ± 1.9</td>
<td>32.0 ± 39.6</td>
<td>4.7 ± 13.9</td>
<td>6.3 ± 1.3</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Animals were fed an iodine-deficient diet (0.02 to 0.05 μg iodine/g food) for 18 months; then they were given the regular diet and sodium L-thyroxine, 5 μg/day i.p. for 2 months. Mean values are ± S.E.

Table 5

Transplantable thyroid tumor developed from iodine-deficient, thyroxine-involuted goiter in thyroidectomized, iodine-deficient rats

<table>
<thead>
<tr>
<th>Implant sacrifice interval month</th>
<th>Rat body weight (g)$^d$</th>
<th>Tumor weight (g)</th>
<th>Tumor $^{131}I$ 12-hr uptake (% dose)</th>
<th>Tumor PB $^{131}I$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Whole tumor (100 mg)</td>
<td>Tissue (100 mg)</td>
</tr>
<tr>
<td>Papillary-follicular tumor$^b$</td>
<td>9</td>
<td>295.5 ± 17.1$^c$</td>
<td>4.3 ± 2.0</td>
<td>16.8 ± 10.4</td>
</tr>
</tbody>
</table>

Tumor $^{131}I$ compounds (% total)

<table>
<thead>
<tr>
<th>I</th>
<th>MIT</th>
<th>DIT</th>
<th>T$_3$</th>
<th>T$_4$</th>
<th>U_T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ± 0.7</td>
<td>87.8 ± 8.7</td>
<td>3.3 ± 1.8</td>
<td>1.0 ± 0.5</td>
<td>1.4 ± 0.6</td>
<td>5.3 ± 2.3</td>
</tr>
</tbody>
</table>

$^a$5 animals.
$^b$Thyrotropin-dependent tumor, first generation.
$^c$Mean ± S.E.
tissue was present. The mean $^{131}$I uptake by the tumors was low (16.8 ± 10.4%). This variability in function was probably related to the varying amounts of hyperplastic tissue within the tumors. The low $^{131}$I uptake is accentuated when expressed per unit weight of tumor (0.37 ± 0.22%/100 mg tissue). The organization of $^{131}$I by the tumors was uniformly high (94.3 ± 1.2%). The mean $^{131}$I-MIT/DIT ratio was high (87.8%/3.3%) and the fraction of $^{131}$I-thyronines was small (2.4%). In some tumors small fractions (5.3 ± 2.3%) of unidentified iodinated compounds with a RF of 0.36 in the 1-butanol-acetic acid solvent system were detected. The tumors secreted an insignificant amount of thyroid hormone as reflected by moderate hyperthyroid state of the hosts. These tumors grew rapidly when transplanted into N-RD rats. However, in normal rats the tumor was functionally less active.

Transplantable Nodules of Hyperplastic Tissue Developed from the Implant of ID-T$_4$G into Tx-ID rats. In 2 other groups of Tx-ID rats no “takes” were palpated. At autopsy, 10 months after implantation, small (120.0 ± 30.0 mg) deep red, encapsulated, grapelike nodules of severely hyperplastic thyroid tissue were found in all animals (Table 6, Chart 1, Figs. 3C and D). The $^{131}$I uptake by the nodules were relatively high (35.3 ± 8.3%, 29.3 ± 4.3% per 100 mg tumor tissue) and the protein bound fraction of $^{131}$I was high (94.3 ± 1.2%). The tissue $^{131}$I-MIT/DIT ratio was elevated and the fraction of $^{131}$I-thyronines was small (about 1.0%). The function of the nodules was low as suggested by a moderate hypothyroidism of the hosts.

Transplantable nodules were separately minced and implanted in 2 groups of 2 Tx-ID rats. Papillary-follicular tumors grew within 3 months and these tumors were similar to the first generation tumors developed directly from implants of ID-T$_4$G into Tx-ID rats described above.

**DISCUSSION**

Furth (3) has suggested that thyrotropin in high concentrations can promote the development of thyroid tumors by stimulating proliferation of thyroid cells which increases the chances of replication errors by the DNA code or enhances the breaking of the DNA code by environmental carcinogens. This possible role of TSH in thyroid carcinogenesis was further developed in the present experiments.

The implants of both ID-G and ID-G + Tu into Tx-ID rats grew similar papillary-follicular tumors. They were grossly, histologically, and functionally similar. In both groups “takes” were noted 3 months after implantation and at autopsy, 9 months after implantation, their weights were equal. As one explanation for this phenomenon we must consider the possibility that the benign ID-G implants also harbored tumors. For exclusion of the presence of hidden tumors serial sections of the whole thyroid gland would be necessary, thus leaving no tissue for transplantation. The failure of transplants of ID-G tissue to grow in N-RD rats is an argument against this possibility. In the N-RD rat the serum level of TSH was probably too low to stimulate tumor development from the ID-G implants because the same implants in the Tx-ID rat, presumably with elevated TSH levels, grew into tumors in all animals.

The most probable cause for failure of growth of the normal thyroid tissue in N-RD rats has been suggested by the studies of Taptildis (12). He observed that injected isolated, normal thyroid cells remain dormant for 1 year in the lungs of isologous mice. These cells formed follicles with colloid when the secretion of thyrotropin was increased by the administration of methylthiouracil to the hosts. The successful autotransplants of rat thyroid glands by Wollman (16) and autografts of 1 thyroid lobe in hypophysectomized rats by Williams and Doniach (15) may be due to species differences. Therefore, it is suggested that prolonged increased TSH stimulation is responsible for the similar growth response of the ID-G and ID-G + Tu implants in the Tx-ID rats.

The transplant of ID-G + Tu tissue into N-RD rats regularly produced autonomous tumors combined with normal-appearing thyroid follicles. The origin of the normal-appearing follicles is unknown. It is doubtful that they developed from the hyperplastic thyroid tissue in the implants. The neoplastic origin of the normal-appearing follicles is suggested by the fact that their mass was several times larger than that of the original implant and the thyroid gland of the host. These follicles were functionally less active than the follicles of the hosts’ thyroid glands. Because

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**Table 6**

<table>
<thead>
<tr>
<th>Implant sacrifice interval month</th>
<th>Rat weight (g)$^a$</th>
<th>Tumor weight (g)</th>
<th>Tumor $^{131}$I 12-hr uptake (% dose)</th>
<th>Whole tumor (100 mg)</th>
<th>Tumor PB$^{131}$I$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodule of hyperplastic thyroid tissue$^b$</td>
<td>10</td>
<td>312.4 ± 16.1$^e$</td>
<td>0.12 ± 0.03</td>
<td>35.3 ± 8.3</td>
<td>29.3 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor $^{131}$I compounds (% total)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>3.1</td>
<td>83.0 ± 3.9</td>
</tr>
</tbody>
</table>

$^a$5 animals.

$^b$Thyrotropin-dependent nodule, first generation.

$^c$Mean ± S.E.

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tumors were small (1.0 ± 0.4 g) their function did not affect the weight and structure of the thyroid and pituitary glands of the hosts.

Differentiated transplantable thyroid tumors are “conditioned neoplasms” which like hyperplastic thyroid tissue respond to thyrotropin stimulation. The transplantable tumors derived from ID-G + Tu tissue in Tx-ID and N-RD rats belong in the category of “conditioned neoplasms” with “autonomous responsive” regulation of growth. i.e., their growth rates in normal rats and thyroidectomized rats are related to serum thyrotropin levels (4, 5)

The development of differentiated tumors containing normal-appearing follicles may be explained by the concept of the multipotentiality of tumor cells (7). By accepting the ability of thyroid tumor cells to differentiate into normal thyroid follicular cells, one is confronted with the problem of whether this multipotentiality is characteristic of a single cell or a population of thyroid tumor cells. From these experiments it is not possible to answer this question. The cloning of thyroid tumor cells in vivo could clarify this problem. By the cloning of a single embryonal carcinoma cell Kleinsmith and Pierce (7) have demonstrated its multipotentiality. They have also proved the heterogeneity of the cells of the embryonal carcinoma. The present experiments indicate that at a certain stage in the development of rat thyroid tumor cells, differentiation into normal-appearing thyroid follicular cells does occur and probably the serum level of thyrotropin influences this process. Huggins et al. (6) in 1959 reported similar observations by developing a breast tumor in the rat.

The implants of ID-T₄G into Tx-ID rats produced papillary-follicular tumors with small foci of hyperplastic tissue. Other implants of the same ID-T₄G tissue gave rise to transplantable nodules of only hyperplastic tissue. In the first group of transplants, despite a complete involution of the goiter implant, some of its cells were already altered and tumors developed promptly on resumption of high thyrotropin stimulation. These tumors were similar in every respect to those grown from ID-G and ID-G + Tu transplanted into Tx-ID rats. However, the development of tumors from the ID-T₄G implants was relatively faster considering that is started from thyroxine-involved tissues. At the time of transplantation the ID-T₄G tissue was 2 months older than the ID-G implant, but both donors were iodine-deficient for an equal period of time. It seems paradoxical, but an additional 2 months of involution by thyroxine may have contributed to a relatively faster rate of growth of the tumors. Werner and Grinberg (13) have observed this phenomenon with transplantable pituitary thyrotropic tumors in mice. They noted that the longer a tumor implant was suppressed by thyroid hormone the shorter was the latent period between the withdrawal of suppression by thyroid hormone and the eventual appearance of the tumor.

Finally, the development of nodules of hyperplastic tissue in Tx-ID rats suggests that interruption of thyrotropin stimulation may postpone the development of tumors, possibly because of the variation in time required for malignant alteration of thyroid cells.

The formation of transplantable nodules of hyperplastic tissue offers some insight into the development of tumors. In their slow growth the benign histological appearance and increased capacity to concentrate ¹³¹I these nodules resemble simple goiters. Like some differentiated thyroid tumors they form little iodothyronines, have low ¹³¹I T₃/T₄, despite iodine deficiency, and like true tumors are easily transplantable. In common with goiters and transplantable differentiated tumors they are characterized by high organic binding of iodine with a high MIT/DIT ratio and a dependence on thyrotropin stimulation.

The correlation of observations on rat transplantable thyroid tumors with similar phenomena in human thyroid carcinoma is difficult. Histological and biochemical characteristics of premalignant nodules in human thyroid glands have not been described. The transplantable papillary-follicular tumors with normal-appearing follicles in normal rats in this study are similar to the papillary-follicular carcinomas found in the thyroid glands of young persons. They also resemble some papillary carcinoma with well-differentiated follicular metastases in lymph nodes. These findings raise a question as to the nature of normal-appearing thyroid follicles adjacent to such a carcinoma in the human. However, the solution of such problems must be accomplished by studies directly in man.

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REFERENCES

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Fig 1. A, iodine-deficient goiter with a papillary tumor ID-G + Tu in the rat, X 25; B, ID-G + Tu detail from A, X 125; C, thyroid gland of the normal rat fed regular diet N-RD which was host of the tumor in G, X 150; D, transplantable papillary-follicular tumor developed from ID-G + Tu in the Tx-ID rat, X 125; E, papillary-follicular tumor. Detail from D, X 175; F, hyperplastic thyroid tissue. Detail from D, X 200; G, autonomous papillary and follicular tumor developed from ID-G + Tu seen at A in N-RD rat, X 50; H, autonomous follicular tumor with normal-appearing thyroid follicles. Detail from G, X 130; I, thyroid follicles with and without papillation. Detail from G, X 130.

Fig. 2. A, iodine-deficient goiter with a follicular tumor in the rat ID-G + Tu, X 50; B, detail from ID-G + Tu, X 80; C, detail from ID-G + Tu, X 150, D, transplantable follicular tumor developed from ID-G + Tu in the Tx-ID rat, X 75; E, follicular tumor. Detail from D, X 150; F, hyperplastic thyroid tissue. Detail from D, X 150; G, autonomous follicular tumor developed from ID-G + Tu, seen at A, in N-RD rat, X 50; H, autonomous follicular tumor. Detail from G, X 150; I, normal and involuted thyroid follicles. Detail from G, X 150.

Fig. 3. A, iodine-deficient, thyroxine-involuted goiter ID-T4G of a rat, X 200; B, transplantable thyroid tumor developed from the implant of ID-T4G in thyroidecotomized, iodine-deficient Tx-ID rat, X 200; C, ID-T4G of another rat, X 200; D, transplantable nodule of hyperplastic tissue developed from the implant of ID-T4G in Tx-ID rat, X 250.

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