The Role of Sex and Iodine Deficiency in the Growth and Function of the Rat Thyroid Transplantable Tumor


Departments of Internal Medicine (Nuclear Medicine) and Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48104

SUMMARY

In a study of the role of sex and iodine deficiency in the growth and function of thyroid carcinoma in rats, half of the male and female litter rats were surgically gonadectomized at the age of two months. One month later, all animals were implanted with a transplantable, autonomous, follicular thyroid carcinoma and one-half of all animals were given an iodine-deficient diet. Six weeks later, the animals were injected with $^{131}$I and thymidine-$^3$H and sacrificed.

The tumor was derived from the male rat thyroid gland by transplantation in the male, inbred isologous rat. In castrated male, iodine-deficient rats the tumor grew faster (greater tumor weight, thymidine-$^3$H-DNA specific activity, and cell nuclei labeling) than in castrated male rats on the regular diet and in normal male rats on the regular or iodine-deficient diet. The growth of the tumor in all female rats was several times slower and was not affected by diet and/or gonadectomy.

The tumor $^{131}$I uptake was equal in the male and female rats. The tumor $^{131}$I uptake was higher in normal rats of both sexes on an iodine-deficient diet than in any other rats. When expressed per unit of weight of tissue, the tumor $^{131}$I uptake was equal in the male and female rats. The tumor $^{131}$I uptake was decreased by gonadectomy possibly as a consequence of an associated decrease in thyrotropin secretion.

The dominant factor in increased tumor growth was a lack of testicular hormones combined with an increased secretion of thyrotropin (resulting from iodine deficiency). Each separately contributed little to the growth of the tumor. The enlarged and presumably overactive adrenals could have influenced the tumor growth in the castrated male iodine-deficient host. The immune response of the female host to the male Y-chromosome of the tumor may have had an adverse effect on the tumor growth.

INTRODUCTION

The incidence of thyroid carcinoma in man is about two times higher in females than in males of comparable age (4, 9, 12, 22, 28, 31). The female/male ratio varies with the cell type of the thyroid tumor, and it is higher in the well-differentiated than in the anaplastic carcinoma.

The observations on the relation of the incidence of thyroid carcinoma to the prevalence of iodine-deficient goiter are conflicting. Saxen and Saxen (20) and Pendergast et al. (18) reported no correlation, while Wegelin (28), Zimmerman and Wagner (32), and Wahner et al. (26) found high incidence of thyroid carcinoma in the areas of severe endemic goiter. At present the role of the "soil" in thyroid carcinoma development was not determined with certainty. McDermott et al. (11) reported the preexistence of the goiter in 32% of the papillary, 60% of the follicular, and 22% of the undifferentiated carcinoma in an area without endemic goiter (Boston).

Therefore, an understanding of the role of sex-linked factors and iodine deficiency in the development of thyroid carcinoma are of importance in the therapy and prevention of the disease.

The investigation of this problem in man is difficult because only a small number of statistically comparable observations are available. Fortunately, the transplantable rat thyroid tumor offers a relatively good model for such an investigation.

In laboratory rats and mice, transplantable thyroid tumors are produced by maintaining prolonged high secretion of thyrotropin in the isologous, inbred donor and recipient of thyroid tissue. Iodine-deficient goiter is grown by feeding the animals thiourea drugs and/or iodine-deficient diet. Thereafter the iodine-deficient thyroid tissue is transplanted in the normal or thyroidectomized animals maintained on the above regimen (1—3, 6, 19, 30). The transplantable thyroid tumors were also produced in mice by grafting hyperplastic thyroid tissue with an autonomous thyrotropin-secreting pituitary tumor (21, 25).

The thyroid tissue of the donor is severely hyperplastic, but infrequently contains malignant elements. The first generation transplantable tumors are usually differentiated and functional; they are relatively benign and dependent on high thyrotropin secretion. The tumors of the later generation are often poorly differentiated and less functional; they are malignant, thyrotropin-independent, and grow in hypophysectomized animals.

The growth rate and iodine metabolism of the tumor vary greatly with different tumor lines and with different generations of the same tumor line.

Morris et al. (17) observed no effect of high and low iodine diet on two lines of autonomous transplantable mouse thyroid tumors. In one tumor line, ingestion of propylthiouracil in the...
diet increased the rate of growth and caused hyperplasia of the tumor but was without effect on two other lines. The growth rate of one tumor line was slightly increased by prolonged administration of thyrotropin, but an opposite effect was observed in another tumor line. The increased secretion of thyrotropin in partially 131I-thyroidectomized mice produced similar results. Neither administration of thyrotropin nor increased endogenous thyrotropin secretion affected the concentration of radioactive iodine by the tumor.

Money and Rawson (14) reported that administration of thiouacil (0.1% in drinking water), thyrotropin (10 units a day) and triiodothyronine (5 μg a day) to the rat for 100 days was without effect on the growth of the autonomous thyroid transplantable tumor. The tumor concentration of 131I was increased, but the 131I-labeled tyrosines and thyronines were decreased in rats given thiouacil. The 131I-labeled monooiodothyrosine (MIT) fraction in tumor tissue was increased after rats were treated with thyrotropin. The administration of triiodothyronine caused a decrease in the tumor 131I-labeled diiodothyrosine (DIT) fraction.

Similarly, no effect of thyrotropin on the tumor concentration of radioactive iodine in vitro was observed by Wolff et al. (29).

The role of the genetic sex and secretion of the sex endocrine glands in growth and function of the transplantable thyroid tumor was not investigated.

The experiments reported here were carried out to examine the effect of the iodine deficiency and some genetic and hormonal sex factors on the growth and functions of the rat thyroid transplantable tumor. Groups of normal and gonadectomized male and female rats on regular and iodine-deficient diets were implanted with a differentiated thyroid transplantable tumor. Tumor weight, desoxyribonucleic acid specific activity, and percentage of tumor nuclei labeled with thymidine-3H, as indices of tumor growth, were significantly greater in the castrated male iodine-deficient rats than in the other rats. Tumor function, as represented by 131I uptake, was higher in normal male and female rats on iodine-deficient diet than in the tumors of the other animals.

Materials and Methods

To examine the effect of gonadectomy and of iodine deficiency on the tumor growth and function, it was necessary to establish their roles in normal homeostasis and thyroid function of the host free of tumor. Therefore, one experiment with groups of five litter animals without tumor was carried out in the same manner as the experiment with groups of seven litter gonadectomized and iodine-deficient animals with the tumor.

Animal

Fischer strain #344 inbred male and female littermate rats three months old were used. Animals were kept in large cages, and by random distribution seven and five animals formed a group. They were fed a regular diet (2.5 μg iodine/gm) and tap water ad libitum. A constant temperature (28°C) and 12 hours of light were maintained.

Gonadectomy

At the age of two months, half of the male and female animals were castrated under chloral-hydrate anesthesia by a midline incision. Testes and epididymis, and ovaries were removed.

Transplantation of the Tumor

One month later, an autonomous, 10th generation rat thyroid transplantable tumor was implanted subcutaneously in the animals. Half of the male and female animals were started on the same day on Remington iodine-deficient diet (wheat gluten 18%, brewers' yeast 10%, yellow corn 70.0%, calcium carbonate 1.0%, sodium chloride 1.0%, and iodine 0.02–0.05 μg/gm of food) and distilled water ad libitum. The other half of the animals remained on the regular diet.

Thyroid transplantable tumor #2 was produced in the inbred Fischer #344 rat by implantation of small bits of 18-month iodine-deficient thyroid tissue from a male rat under the skin of both lumbar areas of the 131I-thyroidectomized iodine-deficient rat. Thereafter, the tumor was carried through 9 generations of 131I-thyroidectomized iodine-deficient and control male rats on a regular diet. The grey, firm, encapsulated, autonomous differentiated follicular tumor grows relatively slowly (17.1 ± 5.6 gm during 5.3 months in the normal rat on regular diet). Lung metastases are formed by 4- to 6-month-old tumors. The tumor does not concentrate radioactive iodine over the serum level,

\[
\frac{\text{tumor iodide }^{131}\text{I}}{\text{serum iodide }^{131}\text{I}} = 0.66
\]

determined by the method of Wollman (30), but it forms small amounts of iodothyrosines with high, (8:1) MIT/DIT ratios, and minute amounts of iodothyronines. The endocrine function of the tumor does not affect the thyroid weight and iodine metabolism. Its contribution of thyroid hormone to the iodine-deficient host is insignificant.

Except for sections for histologic examination, one whole tumor was minced with scissors in 0.90% sterile saline to a very fine brie. The brie was thoroughly mixed, and within one hour, 0.1 ml (equivalent to 25 mg of tissue) was randomly injected from the same syringe under the skin of both lumbar areas of all animals.

The "takes" of the tumors were detected by daily palpation.

Sacrifice

One and one-half months after tumor implantation, all animals were sacrificed on the same day. Twelve hours before the animals were injected intraperitoneally with 100 μc of carrier-free 131I and three hours before the animals were injected with 1 μc of thymidine-3H (specific activity 6.7 mc/mnmole, diluted with distilled water to 500 μc/ml) per 1 gm of body weight. Animals were anesthetized with 0.6 ml of 1% pentothal and bled from the aorta. The tumors were immediately removed and put on ice, cleaned from surrounding tissue, and weighed. Sections of tumors were put in 10% formalin for histologic studies.
examination. The rest of the tumor was immediately frozen in dry ice and alcohol. The thyroid glands were treated in the same way. One piece of the right gluteal muscle was weighed and frozen. In all animals the pituitary and adrenals were removed and weighed.

Iodine Metabolism

The tumors and thyroid glands were homogenized in ice-cold physiologic saline solution. The radioactivity of the aliquots of muscle, tumor, and thyroid tissue were determined in a well-type scintillation detector. The calculation of the 12-hour tumor and thyroidal $^{131}$I uptake was done according to the method described previously (10). The radioactivity of an aliquot of muscle was adjusted and subtracted from the radioactivity of the tumor.

Nucleic Acid Metabolism

The metabolic activity and growth rate of the tumors and thyroid glands were evaluated by the chemical analyses of RNA, DNA (according to the Schmidt-Tannhauser-Schneider procedure), and by determination of the specific activity of the DNA labeled with thymidine-$^{3}$H as described previously (11). These analyses were carried out shortly after the sacrifice of the animals. Then the aliquots of extracts of DNA were kept at $-20^\circ$C until the $^{131}$I was decayed. Tritium counting of aliquots of DNA extracts was done in the Tri-Carb liquid scintillation spectrometer. The quenching was corrected by repeat counting after the addition of a known number of counts of thymidine-$^{3}$H. The specific activity of DNA is expressed as counts per minute per $\mu$g of DNA.

$^{3}$H Radioautography

The radioautography of $4\mu$ thick tissue sections was done by the dipping technic with a Kodak NTB emulsion according to the method of Baserga (personal communication). After the development the tissue slides were stained with hematoxylin-eosin. The percentage of labeled nuclei was estimated per 3000 tumor or thyroid cell nuclei counted randomly in widely separated areas of the tissue sections.

Statistical Analysis

Probability ($P$) values were derived by the Student “t” test (23).

RESULTS

Effect of Gonadectomy and Iodine Deficiency on Normal Homeostasis, Thyroid and Tumor Weight, and Function

Body Weight. All animals were healthy, and their skin and hair were normal. Iodine-deficient animals appeared euthyroid. All gonadectomized rats were less active than corresponding normal animals. The initial and final body weights of all groups of male rats were higher than of their respective female groups, but their weight gains were proportionally similar (Table 1).

All animals receiving an iodine-deficient diet gained more weight than corresponding animals on a regular diet. Normal male rats on regular and iodine-deficient diets weighed more initially and gained more than the castrated male animals on a corresponding diet. The initial weight of the spayed female rats on both diets was lower, but their final weight was greater than the weight of the respective normal female animals.

The accumulation of the fat was especially increased in spayed female animals. In none of the animals were macroscopic organ abnormalities or tumor metastases observed.

Weight of Thyroid, Pituitary, and Adrenals and Thyroid Function. In male rats the weight and $^{131}$I uptake of the thyroid tended to decrease after gonadectomy (Table 2). The thyroid gland of castrated male rats on the iodine-deficient diet was smaller, less hyperplastic, and concentrated less radioactive iodine than the thyroid in the normal rats on an iodine-deficient diet (Table 2; Figs. 1A, B).

Table 1

<table>
<thead>
<tr>
<th>Host, diet</th>
<th>Body weight (gm)</th>
<th>Weight gain (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Castration time</td>
<td>Sacrifice time</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, regular</td>
<td>245.7 ± 5.5</td>
<td>294.4 ± 5.6</td>
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<tr>
<td>Castrated, regular</td>
<td>228.9 ± 7.5</td>
<td>267.5 ± 6.8b</td>
</tr>
<tr>
<td>Normal, iodine-deficient</td>
<td>244.6 ± 4.7</td>
<td>332.3 ± 4.8d</td>
</tr>
<tr>
<td>Castrated, iodine-deficient</td>
<td>236.1 ± 4.1</td>
<td>282.7 ± 6.9</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, regular</td>
<td>156.4 ± 3.4</td>
<td>189.4 ± 4.1</td>
</tr>
<tr>
<td>Spayed, regular</td>
<td>143.3 ± 2.9b</td>
<td>193.1 ± 3.4</td>
</tr>
<tr>
<td>Normal, iodine-deficient</td>
<td>157.7 ± 3.4</td>
<td>189.7 ± 3.1</td>
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<tr>
<td>Spayed, iodine-deficient</td>
<td>147.3 ± 5.9</td>
<td>217.3 ± 10.8a</td>
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</table>

Transplantable thyroid tumor #2. Groups of 7 rats. Mean ± S.E.

$^aP < 0.05-0.02$, compared with normal rat of each sex on regular diet.

$^bP < 0.02-0.01$, compared with normal rat of each sex on regular diet.

$^cP < 0.01-0.001$, compared with normal rat of each sex on regular diet.

$^dP < 0.001$, compared with normal rat of each sex on regular diet.
Sex, Iodine Deficiency, and Tumor Growth

Table 2

<table>
<thead>
<tr>
<th>Host, diet</th>
<th>Thyroid Host</th>
<th>131I at 12 hr (uptake % dose)</th>
<th>Pituitary weight</th>
<th>Adrenals weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, regular</td>
<td>17.5 ± 0.2</td>
<td>7.4 ± 0.4</td>
<td>9.63 ± 0.06</td>
<td>40.6 ± 1.1</td>
</tr>
<tr>
<td>Castrated, regular</td>
<td>15.8 ± 0.6</td>
<td>6.5 ± 0.3</td>
<td>11.3 ± 0.8</td>
<td>45.5 ± 1.2</td>
</tr>
<tr>
<td>Normal, iodine-deficient</td>
<td>96.4 ± 2.0e</td>
<td>64.3 ± 0.5d</td>
<td>10.3 ± 0.8</td>
<td>41.6 ± 2.7</td>
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<tr>
<td>Castrated, iodine-deficient</td>
<td>32.3 ± 2.2e</td>
<td>48.3 ± 3.1d</td>
<td>12.1 ± 0.1e</td>
<td>45.5 ± 1.5d</td>
</tr>
<tr>
<td>Females</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Normal, regular</td>
<td>14.9 ± 1.2</td>
<td>8.4 ± 0.8</td>
<td>9.6 ± 0.6</td>
<td>39.9 ± 1.2</td>
</tr>
<tr>
<td>Spayed, regular</td>
<td>12.3 ± 0.5</td>
<td>6.4 ± 0.2e</td>
<td>10.0 ± 0.4</td>
<td>40.7 ± 0.9</td>
</tr>
<tr>
<td>Normal, iodine-deficient</td>
<td>31.5 ± 3.5e</td>
<td>65.7 ± 2.5e</td>
<td>11.0 ± 0.2b</td>
<td>42.5 ± 3.7e</td>
</tr>
<tr>
<td>Spayed, iodine-deficient</td>
<td>20.8 ± 1.5b</td>
<td>60.1 ± 2.7e</td>
<td>12.4 ± 0.6c</td>
<td>42.4 ± 1.7e</td>
</tr>
</tbody>
</table>

Transplantable thyroid tumor #2. Groups of 7 rats. Mean ± S.E.

*P < 0.05—0.02, compared with normal rat of each sex on regular diet.

bP < 0.02—0.01, compared with normal rat of each sex on regular diet.

P < 0.01—0.001, compared with normal rat of each sex on regular diet.

dP < 0.001, compared with normal rat of each sex on regular diet.

In the female rats the thyroid weight and 131I uptake was decreased by gonadectomy, but this phenomenon was less pronounced in the spayed female, iodine-deficient animals than in the castrated male rats on the same diet.

In all animals the pituitary was larger after gonadectomy and was further enlarged when the animals were given an iodine-deficient diet. Relative to body weight, the pituitary in the female was larger than in the male animals. Only in the males were the adrenal glands enlarged after castration, and diet was without effect upon the adrenal weight.

Thyroid Nucleic Acids and Cell Proliferation. The concentration of the thyroidal RNA and DNA were similar in comparable groups of animals of both sexes. Iodine deficiency caused a greater increase in the concentration of thyroidal RNA of the normal than of the gonadectomized animals. The concentration of DNA in the thyroid was increased only in the normal male rats receiving an iodine-deficient diet. In all iodine-deficient animals the specific activity of thyroidal DNA was increased; it was greater in male than in female rats and in normal than in gonadectomized animals (Table 3).

The autoradiographic evidence for thymidine-3H uptake by thyroid cells of all normal and gonadectomized rats on a regular diet was very small (less than one labeled nucleus per 2000 thyroid cell nuclei), and it was barely detected in animals fed an iodine-deficient diet (0.2—0.3% nuclei were labeled). The percent of thymidine-3H-labeled thyroid cell nuclei corresponded with the values for thymidine-3H incorporation into DNA (Table 3).

Tumor Growth. Most of the animals had tumor "takes" 21 days after implantation. In castrated male iodine-deficient rats, the "takes" of tumors were detected about five days earlier than in all other rats. The mean tumor weight in the castrated male, iodine-deficient host was about 50% greater than that of the tumors in other male rats (Table 4). The tumor weight per 100 gm body weight was equally higher in these rats than in other male animals.

Table 3

<table>
<thead>
<tr>
<th>Host, diet</th>
<th>Thyroid</th>
<th>RNA (µg/mg wet wt.)</th>
<th>DNA (µg/mg wet wt.)</th>
<th>DNA specific activity counts/min/µg</th>
<th>% of thymidine-3H labeled cell nuclei</th>
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<tr>
<td>Males</td>
<td></td>
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</tr>
<tr>
<td>Normal, regular</td>
<td>3.96 ± 0.11</td>
<td>4.37 ± 0.13</td>
<td>1.33 ± 0.15</td>
<td></td>
<td></td>
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<tr>
<td>Castrated, regular</td>
<td>3.89 ± 0.09</td>
<td>4.17 ± 0.12</td>
<td>0.99 ± 0.13</td>
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<tr>
<td>Normal, iodine-deficient</td>
<td>5.32 ± 0.15c</td>
<td>4.70 ± 0.10</td>
<td>3.02 ± 0.26c</td>
<td>0.29 ± 0.07</td>
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<tr>
<td>Castrated, iodine-deficient</td>
<td>4.42 ± 0.16a</td>
<td>4.00 ± 0.13</td>
<td>2.13 ± 0.16b</td>
<td>0.21 ± 0.06</td>
<td></td>
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<tr>
<td>Females</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal, regular</td>
<td>3.94 ± 0.11</td>
<td>4.13 ± 0.13</td>
<td>1.17 ± 0.22</td>
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<tr>
<td>Spayed, regular</td>
<td>3.88 ± 0.12</td>
<td>4.20 ± 0.10</td>
<td>1.03 ± 0.08</td>
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<tr>
<td>Normal, iodine-deficient</td>
<td>4.77 ± 0.22c</td>
<td>4.33 ± 0.19</td>
<td>2.67 ± 0.24b</td>
<td>0.20 ± 0.06</td>
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<tr>
<td>Spayed, iodine-deficient</td>
<td>4.24 ± 0.18</td>
<td>3.85 ± 0.11</td>
<td>2.01 ± 0.18a</td>
<td>0.21 ± 0.09</td>
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</tr>
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</table>

Transplantable thyroid tumor #2. Groups of 7 rats. Mean ± S.E.

*P < 0.02—0.01, compared with normal rat of each sex on regular diet.

bP < 0.01—0.001, compared with normal rat of each sex on regular diet.

cP < 0.001, compared with normal rat of each sex on regular diet.

dP < 0.05%.
radioactive iodine. In female rats, the tumor 131I uptake was approximately equal, but per unit of weight, concentration in the normal and castrated rats receiving an iodine-deficient diet was about ten times higher in both normal and castrated iodine-deficient male rats. The tumors in female rats weighed about four times less than the tumors in the castrated animals did not differ significantly. The tumors in female rats on the regular diet were not significantly different. The tumors in male rats were not affected by castration and the iodine-deficient animals than the 131I uptake by the tumors of regular diet.

The mean tumor weights in control and experimental female animals did not differ significantly. The tumors in female rats weighed about four times less than the tumors in the castrated male iodine-deficient rats.

**Tumor Function.** In male rats, the tumor 131I uptake was about ten times higher in both normal and castrated iodine-deficient animals than the 131I uptake by the tumors of respective animals on a regular diet (Table 5). The tumor 131I concentration in the normal and castrated rats receiving an iodine-deficient diet appeared equal, but per unit of weight, the tumor of the gonadectomized male rats concentrated less radioactive iodine. In female rats, the tumor 131I uptake was about six times higher in the normal iodine-deficient than in the normal animals on regular diet. In iodine-deficient females, the tumor 131I uptake was also decreased by gonadectomy. The 131I uptake per unit weight of tumor tissue in male and female iodine-deficient rats was not significantly different. The tumor 131I uptake in both male and female rats on the regular diet was too low for evaluation of the effect of gonadectomy.

**Tumor Nucleic Acids and Cell Proliferation.** The tumor RNA concentration in male rats was not affected by castration and diet. In the castrated male, iodine-deficient rats, the tumor DNA concentration was slightly higher than in other animals (Table 6).

The tumor DNA-3H specific activity was most increased in castrated male, iodine-deficient rats. It was also higher in the tumor in the castrated animals on the regular diet than in the normal animals on the iodine-deficient diet, but the difference was not significant.

The percentage of thymidine-3H-labeled nuclei varied moderately from tumor to tumor, and from section to section of the same tumor. The percentage of 3H-labeled tumor nuclei in castrated male, iodine-deficient rats was greater than in tumors of other animals (Table 6; Figs. 1E, F).

In female rats the concentration of tumor RNA and DNA, the tumor DNA-3H specific activities, and the 3H-labeling index of the tumor nuclei did not differ significantly. In female rats the DNA-3H specific activities of the tumors and the 3H-labeling index of the tumor nuclei were three times lower than in the tumor of the castrated male, iodine-deficient animals.

**Histologic Examination.** The thyroids of the castrated male iodine-deficient rats were less hyperplastic than the thyroids of

In Table 4, we present the mean tumor weights in control and experimental female animals. The mean tumor weight in control and experimental female animals did not differ significantly. The tumors in female rats weighed about four times less than the tumors in the castrated male iodine-deficient rats.

In Table 5, we present the 131I uptake per unit weight of tumor tissue in male and female animals. The 131I uptake per unit weight of tumor tissue in male and female iodine-deficient rats was not significantly different. The tumor 131I uptake in both male and female rats on the regular diet was too low for evaluation of the effect of gonadectomy.

In Table 6, we present the DNA concentration in male rats. The DNA concentration in male rats was not affected by castration and diet. In the castrated male, iodine-deficient rats, the tumor DNA concentration was slightly higher than in other animals.

The tumor DNA-3H specific activity was most increased in castrated male, iodine-deficient rats. It was also higher in the tumor in the castrated animals on the regular diet than in the normal animals on the iodine-deficient diet, but the difference was not significant.

The percentage of thymidine-3H-labeled nuclei varied moderately from tumor to tumor, and from section to section of the same tumor. The percentage of 3H-labeled tumor nuclei in castrated male, iodine-deficient rats was greater than in tumors of other animals.
all other male and female iodine-deficient animals (Figs. 1A, B).

The tumor was a follicular carcinoma with a tendency for solid growth. It was more cellular in castrated male, iodine-deficient rats than in the other animals (Figs. 1C, D).

Effect of Gonadectomy and Iodine-deficient Diet on Normal Homeostasis and Thyroid Function in Animals without Tumor

The final mean body weight of the castrated male, iodine-deficient rats without tumor was higher than that of comparable rats with tumor. The weights of the thyroid, pituitary, and adrenals, and the thyroid $^{131}$I uptake of comparable groups of animals without and with tumor were similar.

**DISCUSSION**

The growth of the tumor derived from male, iodine-deficient thyroid was significantly larger in the castrated male, iodine-deficient rat than in all other animals of both sexes (Table 4). In contrast, the thyroid glands of the castrated male, iodine-deficient rats with the largest tumors were one-third as enlarged as the thyroid of the normal male rats on iodine-deficient diet, which developed much smaller tumors (Table 2). The growth of the tumor was not directly related to the body growth of the animals. The gain in weight and the final body weight of the castrated male iodine-deficient rats with the larger tumors were 41 gm less than in normal male iodine-deficient animals with the smaller tumors (Tables 1, 4).

The largest tumor $^{131}$I uptake was observed in the normal iodine-deficient rats of both sexes (Table 5). The tumor $^{131}$I uptake was higher in the male than in the female rats, but this difference did not exist when $^{131}$I uptake was expressed per unit weight of tumor tissue. Therefore, these observations suggest that: (a) the castration of the male, iodine-deficient rats promoted the growth of the tumor, but partly suppressed the enlargement of the thyroid gland, and (b) both tumor and thyroid $^{131}$I uptake was decreased by gonadectomy of both male and female iodine-deficient rats.

The differences in the response of the tumor (increased growth, decreased function) and the thyroid (both inhibition of growth and function) to castration of the male iodine-deficient rat suggest that the growth and function of the tumor are affected separately and by different factors.

Similar to the thyroid, the function of the tumor was probably stimulated by the thyrotropin. The serum level of thyrotropin was presumably most elevated in the normal, iodine-deficient rats, and therefore the $^{131}$I uptake of their tumor and the thyroid were greater than in the tumors and thyroids of all other animals. Since the castrated male iodine-deficient rats were smaller than the comparable normal animals, their energy expenditure and daily requirements of thyroid hormone presumably were smaller. Therefore, the lower secretion of thyrotropin was probably the cause for the tumor and thyroid $^{131}$I uptakes in the castrated male iodine-deficient rats being lower than in normal male iodine-deficient animals. However, it is possible that gonadectomy, by virtue of lack of estrogen or androgen respectively, directly (and by different magnitude) affected the pituitary secretion of thyrotropin and even thyroidal and tumor response to it. It is of interest that recently Moon and Turner (15) and Kumarsen and Turner (8) showed that the thyroid hormone secretion is more decreased in the female than in the male rat by gonadectomy, but their experiments were conducted with animals on a regular diet.

The faster growth of the tumor in the castrated male, iodine-deficient rat than in all other animals suggests that the effects of castration and iodine deficiency are interrelated and synergistic, because (a) the castration of the rat on a regular diet did not promote the tumor growth, and (b) the tumor growth rate was not increased if normal male rats were given an iodine-deficient diet. However, each factor (castration, i.e., lack of testicular hormones and iodine deficiency, i.e., high thyrotropin) per se stimulated the tumor growth, because the specific activities of the tumor DNA were slightly, but significantly higher in both castrated male rats on regular diet, and normal male, iodine-deficient rats than the DNA specific activity of the tumor in the normal rat on regular diet (Table 6). The growth of the tumor in the castrated, male, iodine-deficient rat was probably more promoted by a lack of testicular hormones than by a less than maximal secretion of thyrotropin, the thyroidal weight and $^{131}$I uptake of these rats being less increased than in the normal male, iodine-deficient rats growing a smaller tumor. A more convincing support for this impression is lacking, because the tumor DNA-$^{3}$H specific activity and the tumor $^{3}$H-labeling index of cell nuclei were higher, but not significantly higher in the castrated male rat on regular diet than in the tumor of the normal male rat on iodine-deficient diet (Table 6).

The tumor in the castrated male, iodine-deficient rat was more cellular and less follicular than the tumors in all other animals (Figs. 1D, E). The greater cellularity of this tumor was reflected in a higher concentration of DNA than in other tumors. It is of interest that both DNA and RNA concentrations of the tumor tissue were about two times higher than in the thyroid tissue. As observed previously, the concentration of thyroidal RNA varied directly with the level of thyrotropin, while the DNA was quite thyrotropin-independent (11). The concentration of RNA was practically the same in all tumors, while the concentration of tumor DNA was slightly higher in the castrated male iodine-deficient rats (Table 6).

There is no explanation for the slower growth of the tumor in the female rats, and the lack of effect of combined gonadectomy and iodine-deficient diet on the growth rate of this tumor is not understood. The differences in tumor growth in the male and female rat could have been due to the male origin of the tumor. The immune response of the female rat to the tumor Y-chromosome could have had adverse effect on tumor growth. The antigenic effect of the Y-chromosome in the stroma of the mammary carcinoma, reticulum cell sarcoma, liposarcoma, and fibrosarcoma implants was suggested as a tumor growth-inhibiting factor. In this case the tumors were developed in the female mice, then passed several times through the male rats before their growth rates were again tested in the female mice (3).

The effect of the enlarged and presumably hyperactive adrenal glands on the growth of the tumor of the castrated male iodine-deficient rats was not decisive, because the growth
of the tumor was significantly slower in the castrated male rat on the regular diet with equally enlarged adrenals. According to Kitay (7), castration of the male rat slows the growth of the animal and produces enlargement of the adrenals. Plasma corticosterone concentration, and response of the adrenal to exogenous ACTH are not affected by gonadectomy. However, the biologic half-life of the corticosterone is shortened. The response of the female rat to gonadectomy is different. The animal body weight increases, but the adrenal weight decreases. The plasma concentration of corticosterone is decreased, and its turnover is prolonged. Obviously, the role of the adrenal in the tumor growth is complex, because Houssay et al. (5) reported that castration of the male rat results in increased secretion of adrenal estrogens due to an increased secretion of pituitary gonadotropins (24). Gonadectomy affects, also, the secretion of pituitary growth hormone (6) which may influence the tumor growth.

The role of other homeostatic factors was only partly analyzed. The state of nutrition as reflected in body weight probably did not contribute to tumor growth, because the castrated male, iodine-deficient rat with the largest tumor gained less weight than the normal male rats on the same diet but with much smaller tumors. It is possible that due to the large tumor the body weight of the castrated male iodine-deficient male rats was lower than that of the comparable animals without tumor. However, the weight of the endocrine organs and thyroid function in comparable animals with and without tumors were equal. Therefore, the general homeostasis of the animals with tumor was probably not different enough to account for the observed difference in tumor growth.

Finally, the similarities and differences in the response of the transplantable thyroid tumor and the thyroid to environmental factors are illustrated in this experiment. The function of the transplantable thyroid tumor and the thyroid was similarly increased in the iodine-deficient rat, and relatively decreased in the castrated, iodine-deficient rat. The growth responses under the same environmental conditions were quite different. The growth of thyroid in the iodine-deficient rat was maximally stimulated, but the growth rate of the tumor was not affected. The tumor growth was increased in the castrated, iodine-deficient rat, while the growth rate of the thyroid was relatively decreased.

The correlation of these observations with the problems in human thyroid carcinoma must await further studies. Tentatively, it appears that in normal males the internal environment is adverse to growth of thyroid carcinoma. This is in keeping with clinical observations. Rawson (unpublished observations) has suggested that physiologic hypogonadism favors the development of thyroid carcinoma. Iodine deficiency, i.e., increased secretion of thyrotropin in conjunction with lack of androgen, could contribute to the growth of differentiated types of thyroid carcinoma in man.

REFERENCES


Fig. 1A. Thyroid gland of the intact male rat on iodine-deficient diet. × 425.
Fig. 1B. Thyroid gland of the castrated male rat on iodine-deficient diet. × 425.
Fig. 1C. Rat thyroid transplantable tumor in intact male rat on regular diet. × 425.
Fig. 1D. Rat thyroid transplantable tumor in castrated male rat on iodine-deficient diet. × 425.
Fig. 1E. Radioautograph of rat thyroid transplantable tumor in intact rat on regular diet, showing the number of tumor nuclei labeled with thymidine-3H. × 1420.
Fig. 1F. Radioautograph of rat thyroid transplantable tumor in castrated male rat on iodine-deficient diet, showing the number of tumor nuclei labeled with thymidine-3H. × 1420.
J. Matovinovic, R. H. Nishiyama, H. C. Hill, and G. R. Poissant
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