Further Studies on Induction and Growth of Thyrotropic Pituitary Tumors in Mice

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The induction of tumors of the adenohypophysis in mice by radiothyroidectomy (Gorbman [8]) has been amply confirmed. These tumors are thyrotropic (4). They are initially transplantable only to hosts whose thyroid function has been depressed by either radiothyroidectomy or surgical thyroidectomy; but autonomous strains have been developed after subpassages of most of the originally dependent lines (2, 7). Whether or not radiation is essential to the induction of these tumors is controversial (2, 8). Our view that radiation is not essential is based on their having been induced by surgical thyroidectomy (2) and by sustained treatment with large dosages of propylthiouracil (10, 11). Induction of pituitary tumors by propylthiouracil has not been thus far confirmed, and the published experiments on their induction by surgical thyroidectomy (2) are not entirely conclusive. The thyroidectomized animals (2) received a diagnostic dose of radioiodine administered to ascertain the completeness of thyroidectomy. The dose used (5–9 μc. of I\(^{131}\)) is too small to induce pituitary tumors in normal mice, the threshold dose for tumor induction being 25 μc. in females and somewhat more in males. It is conceivable, however, that this dose of I\(^{131}\) exerted some co-carcinogenic effect on the pituitaries of mice whose thyroids were subtotally or completely resected. It seemed desirable, therefore, to repeat this experiment entirely avoiding the use of I\(^{131}\).

A closely related line of investigation involves the growth of transplanted dependent tumors. Since they had been shown to proliferate in surgically, as well as in radiologically, thyroidectomized hosts (2, 4), it seemed probable that they would also grow in hosts whose thyroids had been blocked with propylthiouracil, the essential stimulus for proliferation of thyrotropes being lack of thyroid hormone.

METHODS AND RESULTS

Surgical thyroidectomy.—The present experimental series includes fifteen surgically thyroidectomized mice kept, without any hormonal therapy or I\(^{131}\), until natural death (290 days or longer). Eleven well matched control animals were killed after the experimental animals had died. Spontaneous pituitary tumors or distinct pretumorous changes have not been seen by us in normal C57BL mice, either in this small control group or in earlier, larger series totaling well over 100 mice of comparable age. In contrast, two-thirds of the surgically thyroidectomized mice of the present series developed pituitary adenomas (Table 1).

At autopsy tumors were found to have replaced the normal pituitaries of two mice, minute tumor nodules were noted in another, and the pituitaries of three more animals were distinctly enlarged. On microscopic examination, it was discovered that the latter had well-developed microtumors such as have been illustrated in earlier publications (2). In addition, microscopic examination disclosed the presence of small tumor nodules in three more pituitaries which were not distinctly enlarged. The changes marking the course of tumor development have been described and illustrated earlier (2, 6, 9). The pituitaries were not weighed since they were fixed in toto with the sella for microscopic studies.

The pituitary glands of only four mice of the experimental series failed to show tumorous changes. Possibly, all four would have, in time, developed tumors. It might be suggested that tumor development was held back in two of these animals by regenerated thyroid remnants; however, it has been shown in earlier work (2) that the thyroid need be only depressed and not completely destroyed in order that tumors appear. In both the present series and the earlier one (2), adenomas of...
thyroid remnants were found to coexist with pituitary tumors. It is possible that the functioning of the residual thyroid was inadequate to check the tumor growth and that the thyroid remnant was minute immediately after operation, and became enlarged and adenomatous with the steadily increasing stimulation by thyrotropes; in other words, the balance was that of an uninterrupted excess of thyrotropic hormone (TSH). It is also conceivable that the adenomas of the pituitary are composed of somewhat altered (though fully dependent) cells which are not so responsive to physiologic stimuli as are normal cells.

Conditioning of tumor growth by administration of propylthiouracil.—The hypothesis that blocking proliferation of tumor implants was observed in normal hosts only in Exp. 4, indicating that the tumors gave rise to autonomous variants. Tumor growth occurred with propylthiouracil treatment and without low iodine in this experiment only. In Exps. 5 and 6 tumors failed to take even in the propylthiouracil-treated animals that were kept on low iodine diet. Hosts kept on a low iodine diet (without propylthiouracil) were not used; it is possible that such a treatment alone will condition mice to a thyrotropic tumor.

Pituitary changes in propylthiouracil-treated mice.—The pituitaries of propylthiouracil-treated animals exhibited the characteristic changes which follow thyroidectomy (2, 9). Most of these mice were not kept long enough for induction of primary pituitary tumors. In Exp. 2 mice were given this thyroid antagonist and were kept on a low iodine diet for 4 months before being grafted with tumors; thus, the total period of thyroid blockage was about 11 months. One of these mice had a gross pituitary tumor measuring 3-4 mm. in diameter and a grafted tumor of about 5 mm. in diameter. Microscopic examination indicated replacement of most of the normal pituitary with tumor cells exhibiting more cytologic abnormalities than the tumor graft which this animal carried. Three other mice similarly treated and autopsied at about the same time had pretumorous changes (9) in the pituitary gland. The thyroids of all four mice were greatly enlarged and contained numerous adenomas. Similar changes were noted in two mice of Exp. 1 which received the same antithyroidal treatment but only for a period of 4 months. These observations lend strong support

### TABLE 1

<table>
<thead>
<tr>
<th>No. AND LENGTH OF LIFE*</th>
<th>THYROID MICROSCOPIC</th>
<th>PITUITARY MICROSCOPIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ♀ 484</td>
<td>not identified</td>
<td>3×4 mm. T†</td>
</tr>
<tr>
<td>2 ♀ 577</td>
<td>not identified</td>
<td>5 mm. T</td>
</tr>
<tr>
<td>3 ♀ 360</td>
<td>not identified</td>
<td>adenoma</td>
</tr>
<tr>
<td>4 ♀ 348</td>
<td>not identified</td>
<td>normal micro T</td>
</tr>
<tr>
<td>5 ♀ 305</td>
<td>not identified</td>
<td>adenoma</td>
</tr>
<tr>
<td>6 ♀ 419</td>
<td>not identified</td>
<td>normal micro T</td>
</tr>
<tr>
<td>7 ♀ 436</td>
<td>not identified</td>
<td>normal micro T</td>
</tr>
<tr>
<td>8 ♀ 480</td>
<td>present</td>
<td>adenoma</td>
</tr>
<tr>
<td>9 ♀ 578</td>
<td>adenoma?</td>
<td>minute T</td>
</tr>
<tr>
<td>10 ♀ 538</td>
<td>not identified</td>
<td>normal pre-T</td>
</tr>
<tr>
<td>11 ♀ 390</td>
<td>l. lobe present</td>
<td>regeneration normal</td>
</tr>
<tr>
<td>12 ♀ 415</td>
<td>not identified</td>
<td>normal normal</td>
</tr>
<tr>
<td>13 ♀ 446</td>
<td>adenomas, regen.</td>
<td>normal normal</td>
</tr>
<tr>
<td>14 ♀ 446</td>
<td>r. lobe present</td>
<td>regeneration normal</td>
</tr>
<tr>
<td>15 ♀ 586</td>
<td>l. lobe present</td>
<td>not identified normal</td>
</tr>
</tbody>
</table>

Mice of C37 strain were 5-7 weeks old when thyroidectomized. The operative technic and other procedures were described (9).

* In days after surgical thyroidectomy.
† Abbreviations: T = tumor; r = right; l = left.

thyroid hormonal synthesis in the host will promote the proliferation of implanted, dependent, thyrotropic tumor cells was tested by sustained administration of propylthiouracil in the drinking water of mice kept on low iodine or on normal diet.

Six experiments were performed (Table 2). The first four were conducted on C57BL mice, the fifth or later subpassage of three different thyrotropic tumor strains being used. Of these strains, Nos. 19 and 101 have a tendency to give rise to autonomous variants; No. 82 has been transplanted in five consecutive passages and grew only in athyroid hosts. In Experiments 5 and 6, conditioned tumors of the second passage, carried in Rf mice, were used. These strains have not yet given rise to autonomous variants.

The results (Table 2) indicate that keeping mice on propylthiouracil and low iodine diet enables proliferation of dependent thyrotropic pituitary tumor cells of some strains but not of others. Pro-
TABLE 2
CONDITIONING HOSTS WITH PROPYLTHIOURACIL AND WITH RADIOTHYROIDECTOMY FOR GROWTH OF THYROTROPIC PITUITARY TUMOR GRAFTS

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Tumor Strain</th>
<th>No of Passage</th>
<th>Diet</th>
<th>Propylthiouracil treated</th>
<th>Latency*</th>
<th>Duration†</th>
<th>Tumor‡</th>
<th>No. positive</th>
<th>Radiothryoidectomy treated</th>
<th>Latency</th>
<th>Duration</th>
<th>Tumor</th>
<th>No. positive</th>
<th>Normal</th>
<th>Duration</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19-D</td>
<td>IXa</td>
<td>Low I</td>
<td>8/8</td>
<td>(24–66)</td>
<td>(24–106)</td>
<td>40</td>
<td>67</td>
<td>8/8</td>
<td>(42–65)</td>
<td>(72–119)</td>
<td>57</td>
<td>107</td>
<td>0/5</td>
<td>109</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td>Va</td>
<td>Low I</td>
<td>6/6</td>
<td>208</td>
<td>206</td>
<td>±</td>
<td>4/5</td>
<td>179</td>
<td>±</td>
<td>±</td>
<td>179</td>
<td>189</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>4</td>
<td>101-D</td>
<td>V1c</td>
<td>Low I</td>
<td>4/4</td>
<td>213</td>
<td></td>
<td>213</td>
<td>0</td>
<td>6/6</td>
<td>(78–109)</td>
<td>(78–192)</td>
<td>106</td>
<td>138</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>4/4</td>
<td></td>
<td></td>
<td>148</td>
<td>(128–158)</td>
<td></td>
<td>± to + +</td>
<td>6/6</td>
<td>(102–117)</td>
<td>(102–158)</td>
<td></td>
<td>± ± ±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5</td>
<td>RF1</td>
<td>IIa</td>
<td>Normal</td>
<td>0/2</td>
<td>316</td>
<td>2/3</td>
<td>192</td>
<td>259</td>
<td>± to ± ±</td>
<td>(174–210)</td>
<td>(246–271)</td>
<td>(272–298)</td>
<td>285</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>RF3</td>
<td>IID</td>
<td>Low I</td>
<td>0/2</td>
<td>299</td>
<td>4/4</td>
<td>117</td>
<td>169</td>
<td>± to ± ± ±</td>
<td>(164–177)</td>
<td>(164–177)</td>
<td>(342)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The low iodine diet was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Propylthiouracil, obtained through the courtesy of Dr. J. J. Olson, American Cyanamid Company, was given in distilled water (0.1 per cent) ad libitum. The normal mice were kept on normal diet.

*In days. The experimental animals were killed in extremis or died. The controls were killed at indicated days.

†Number of days from inoculation to death.

‡± = 0.5 cm., + = 1 cm., ++ = 3 cm. in approximate average diameter.

§Early death from pneumonia.
to those of Seifter et al. (11) and Moore et al. (10) indicating that blockage of TSH synthesis will induce pituitary tumors.

COMMENTS

Earlier experiments indicated an inverse relationship between rate of growth of dependent thyrotropic tumors and degree of destruction of the thyroid by $^{131}$I (5). The present findings show that propylthiouracil is not as effective as radiothyroidectomy in conditioning hosts for the growth of dependent tumors; this indicates either that the blocking of the thyroid with this compound was incomplete or that a nonhormonal thyrotrope-inhibitor was produced by the blocked thyroid epithelium.

There are several recorded failures of induction of pituitary tumors by antithyroidal-drugs, including our own. The work of Moore et al. (10) and, more recently, of Axelrad and Leblond (1) and our own experience suggest that lack of completeness of blockage of thyroxine synthesis explains most failures. Axelrad and Leblond (1) have shown that chronic deficiency of iodine alone will cause the development of pituitary tumors in the rat. Seven of 26 rats sacrificed after 9½ months or more on iodine-deficient diet had grossly visible tumors, and several others had clinical or other indication of having similar tumors (1).

SUMMARY

Pituitary tumors were induced in mice by surgical thyroidectomy and without any supplementary carcinogen (such as $^{131}$I or x-rays). Most surgically thyroidectomized mice which did not have tumors had regenerated thyroid tissue.

Propylthiouracil treatment of hosts maintained on a low iodine diet permitted the growth of implanted, dependent, thyrotropic pituitary tumor cells.

These findings support the hypothesis that mere disruption of the thyroid-pituitary feed-back mechanism is sufficient to initiate development of pituitary tumors.

ACKNOWLEDGMENTS

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REFERENCES

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