Studies with Mouse Pituitary Thyrotropic Tumors
I. Survival of Implants during Prolonged Suppression by Thyroid Hormone*

SIDIEMY C. WERNER AND RAUL GRINBERG

(Deportment of Medicine, Columbia Universify College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N.Y.)

SUMMARY

Implants of dependent mouse pituitary thyrotropic tumors survived prolonged suppression of growth with thyroid hormone. With one dependent strain, 3052, no tumor became palpable after implantation in control mice with intact thyroid to 17 months. However, tumors regularly appeared in implanted radiothyroidectomized mice. The latent period to appearance of the tumor decreased with increasing time of suppression with thyroid hormone prior to thyroid ablation. The findings were similar with those of another radiothyroidectomized dependent strain, 4183, kept suppressed by thyroid feeding, then discontinued.

With an autonomous responsive L24 strain, no tumors appeared in control mice with an intact thyroid, up to 10 months after implantation. Tumors appeared upon radiothyroidectomy, and the latent period was progressively less with more prolonged suppression. After 15 months, however, growths became palpable, even with an intact thyroid.

The pioneer work of Furth has led to the identification and isolation of a variety of hormonally active mouse pituitary tumors (2). By destruction of the thyroid with I131, Furth induced and maintained by serial transplantation, tumors of this type, secreting thyrotropin and not other pituitary hormones.

Some of these strains, "dependent," are responsive to thyroid hormone in that they do not grow or may recede in the presence of circulating thyroid hormone. Others, "autonomous responsive," respond when the tumor is small, but not later. Still other strains are completely non-responsive, "autonomous non-responsive." Representative strains were made available to this laboratory in June to September, 1958.

Studies were initiated of the fundamental biology of the three tumor strains and of possible mechanisms responsible for variations in responsiveness to thyroid hormone. As part of the work, it was determined whether dependent, and autonomous responsive tumor implants survived when kept under thyroid hormone suppression over prolonged periods of time. At the same time, studies of the effect of thyroxine and of a series of available analogs of thyroxine on tumor growth and function were initiated. These results are being reported in this and in subsequent papers.

MATERIALS AND METHODS

Four tumor strains maintained in LAF1 mice have been available: two dependent strains, 3052 and 4183; one autonomous, responsive strain, L24; and an autonomous, nonresponsive strain, L23. The donor mice were killed, and the tumors growing in thigh muscles at the site of previous implantation, were dissected free. The tumor was immediately minced in Eagle's solution with a fine scissors. Some of the mince was implanted through a 2-inch #18-gauge needle into the right lateral thigh muscles of healthy LAF1 mice, or with the thyroid destroyed by I131. Female mice, 1 month old and weighing approximately 18 gm. at the time of implantation, were used throughout.

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From 1958 to early 1959, the mice were fed a Rockland Farm diet containing 44 μg iodine/100 gm food. It was discovered that the diet maintained the serum protein-bound iodine (PBI) at over 35 μg. per cent (5). Ken-L-Kibble medium low-iodine ration has been used since, containing 0.25 gm. per cent iodized salt. However, no evident difference in experimental results developed with the change in diet.

Before the thyroid was destroyed with I\(^{131}\), the mice were placed on a Remington (7) low-iodine diet (Nutritional Biochemicals, Inc.) containing 0.45 gm. per cent iodized salt and distilled water for 10 days. They were then given 50 μc. I\(^{131}\) intraperitoneally. Implantation was performed 10 days after this with the mice still on the same low-iodine diet and distilled water. Terramycin was given postoperatively for 48 hours. The animals were returned to the stock diet immediately after implantation was performed. With the responsive tumor strains, tumors developed at the site of implantation only in thyroidectomized animals. The tumors first appeared approximately 5 months after implantation with the 3053 and 4183 strains; after approximately 4 months with the L34 strain; and after 3 months with the L3S strain.

When thyroid was fed, the concentration in the diet was 0.13 gm. per cent.

RESULTS

Dependent strains.—Experiments were conducted with the two dependent tumor strains, as follows: in the first series, A, the tumors were subjected to the effect of endogenous thyroid hormone from an intact thyroid in the host; in the second, B, the effect was observed of exogenous thyroid hormone upon tumors growing in mice with the thyroid destroyed by I\(^{131}\).

A dependent strain of tumor, 3052, was implanted (October, 1958) into 64 normal LAF mice. The animals were maintained on a Rockland Farm diet. The results are shown in Table 1. No tumors became palpable in any mouse with an intact thyroid, even as late as 1 year and 5 months after implantation in the ten mice remaining for this part of the experiment.

The other 54 mice were given I\(^{131}\) to destroy the thyroid, at intervals of from 2 months to 1 year and 2 months following implantation of the tumor. In every instance, when thyroid destruction was accomplished by a 50-μc. dose of I\(^{131}\), tumors ultimately appeared. However, only one of ten animals developed tumor when a smaller, 5-μc., dose of I\(^{131}\), was employed for the purpose.

The latent period for tumors to appear was strikingly different when thyroid destruction was performed after 3 months of suppression by endogenous thyroid hormone or when the thyroid was destroyed later. With destruction after 2 months of suppression, only two of thirteen ani-

<table>
<thead>
<tr>
<th>Months after tumor injection until radiothyroidectomy</th>
<th>Dose (μc.)</th>
<th>NO. TUMORS/TOTAL NO. MICE INJECTED (MONTHS AFTER RADIOTHYROIDECTOMY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>5</td>
<td>0/10 0/10 0/10 0/10</td>
</tr>
<tr>
<td>9.5</td>
<td>50</td>
<td>1/10 1/10 1/10 1/10</td>
</tr>
<tr>
<td>7.5</td>
<td>50</td>
<td>0/10 2/13 9/15 12/15</td>
</tr>
<tr>
<td>9.0</td>
<td>50</td>
<td>2/10 6/10 9/10</td>
</tr>
</tbody>
</table>

mals had developed tumors by 4 months later; and it required 6 months before virtually all the mice had tumors (twelve of thirteen). With ablation after 14 months of suppression, four of eight mice developed palpable tumors 1½ months later. When the thyroid was destroyed at 7 and 11 months after implantation, the latent period until tumors appeared was between the two extremes, 3 and 2 months, respectively.

Twenty LAF mice, with thyroids destroyed by I\(^{131}\) administration 1½ weeks previously, were given implants of a responsive strain of tumor, 4183 (Table 2). Of these, seventeen were placed on Ken-L-Kibble medium, a relatively low-iodine diet, only. Tumors appeared uniformly in eleven of eleven surviving for approximately 6 months thereafter.

A second group of three mice was maintained after implantation on thyroid hormone in addition to the Ken-L-Kibble diet, 0.12 gm/100 gm food, for approximately 9 months. No palpable tumors appeared. Thyroid was then removed from the

TABLE 1

EFFECT OF DELAYED RADIOTHYROIDECTOMY ON GROWTH OF RESPONSIVE TUMOR, 3052
diet, and within 2 months three of the three had evident tumors.

Autonomous, responsive strain.—The two types of experiments conducted with the dependent tumor strains were repeated with the autonomous responsive L24 strain of tumor. The first experiment (A) was begun June 23, 1958; the second (B), January 19, 1959.

A: Fourteen intact LAF mice were given implants of L24 tumor and maintained on Ken-L-Kibble medium ration. The subsequent events are depicted in Table 3. Tumors appeared in all when the thyroid was destroyed with $^{131}$I at 4½ and 10 months after implantation of the tumor. The latent period between thyroid destruction and appearance of tumor was 5 months when the thyroid was destroyed 4½ months after tumor implantation but only 2½ months when the suppression by the thyroid was ended at 10 months after implantation. In contrast, no tumors appeared in any of the animals with an intact thyroid, up to the 10-month period when the

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### Table 2

**Effect of Thyroid Hormone Therapy upon Growth of Responsive Tumor, 4183, in Radiothyroidectomized Mice**

<table>
<thead>
<tr>
<th>Dietary Period (months after implantation)</th>
<th>Time on Diet (months after implantation)</th>
<th>Tumors/mice injected</th>
<th>Av. tumor size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ken-L-Kibble</td>
<td>Thyroid hormone+ Ken-L-Kibble</td>
<td>3½</td>
<td>8/17</td>
</tr>
<tr>
<td>0–12</td>
<td>5½</td>
<td>11/11</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11/11</td>
<td>++++</td>
</tr>
<tr>
<td>0–3</td>
<td>3½–12</td>
<td>5½</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2/4*</td>
<td>+</td>
</tr>
<tr>
<td>10–12</td>
<td>0–10</td>
<td>5½</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3/3*</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3/3</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Two of the original six mice died between 8 and 12 months after implantation.

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### Table 3

**Effect of Thyroid Hormone upon Growth of Semi-Responsive Tumor, L24**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Dietary Period (months after radiothyroidectomy)</th>
<th>Months after radiothyroidectomy</th>
<th>Tumors/total no. mice injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 1.</td>
<td>Ken-L-Kibble</td>
<td>Thyroid hormone + Ken-L-Kibble</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0–7</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>2.</td>
<td>0–10</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0–4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>B. 1.</td>
<td>0–4½</td>
<td>1½–4½</td>
<td>4½</td>
</tr>
<tr>
<td>2.</td>
<td>0–1½</td>
<td>4½</td>
<td>0/14</td>
</tr>
<tr>
<td></td>
<td>4½–6½</td>
<td>5</td>
<td>7/14</td>
</tr>
<tr>
<td>3.</td>
<td>0–1½</td>
<td>1½–6½</td>
<td>6½</td>
</tr>
<tr>
<td></td>
<td>6½–7½</td>
<td>7½</td>
<td>6/6</td>
</tr>
</tbody>
</table>

* In Experiment A, tumors were implanted in intact mice, and the thyroid was subsequently destroyed; in Experiment B, the thyroid was destroyed, desiccated thyroid added to the diet for varying periods of time, and therapy discontinued.
A second group of twenty mice was implanted with L24 tumor and maintained on a Rockland Farm diet. This group, not shown in the table, was left undisturbed, except for periodic examination for tumors, for approximately 13 months. Ten mice survived this long, and tumors were first noted at this time in three of these ten, despite the presence of the healthy thyroid of the host.

B: Twenty-nine mice were radiothyroidectomized and given implants of L24 tumor in July, 1958. Two months later thyroid was mixed with the feed of 22 of these mice, 0.12 gm/100 gm food; seven were kept without added thyroid, as controls (Table 3). After 2½ months of thyroid feeding, the hormone was removed from the diet of fourteen mice but was continued in the diet of the other eight mice. Tumors appeared in all fourteen mice off hormone by 5 weeks after stopping medication. No tumors appeared in any of the eight mice kept on treatment. Thyroid was then discontinued in these latter, approximately 2½ months after the hormone therapy was stopped in the first group. Tumors became palpable in 4 weeks (Table 3) in six of six surviving mice, two dying in this interval. The short latent period for tumors to appear is in contrast to the 4½-month latent period seen in the seven control implanted mice not receiving thyroid (Table 3).

DISCUSSION

The capacity of the experimental tumor cell to lie dormant in the host after implantation was first noted by Gardner and co-workers (4), when estrogenic stimulation was not provided for a testicular tumor which required such stimulation for growth. The phenomenon of “dormancy” has since been confirmed with this type of testicular tumor (6) and with other tumors (1).

The present study adds the further finding that dependent tumor cells, in this case from thyrotropic pituitary tumors, can survive prolonged suppression of growth by the hormonal agent to which they are responsive. In the present experiments, the implanted tumor cells remained viable despite suppression of as long as 14 months by thyroid hormone. With an autonomous responsive tumor, escape from suppression occurred at 13 months after implantation of the tumor into normal host mice. Furth had previously observed such escape from suppression with a responsive thyrotropic tumor strain implanted in normal host mice. Since implants from this growth had become nonresponsive, the term “late autonomy” was coined (3).

In line with the tendency of thyrotropic tumors to escape ultimately from suppression by thyroid hormone is the progressively shorter latent period between removal of thyroid hormone suppression and appearance of tumor, the longer suppression had been maintained. This was true of both the dependent and autonomous responsive tumor strains. The decrease in latent period was most marked in the experiment with autonomous responsive tumor in which fed thyroid hormone instead of endogenous hormone was used as the suppressive agent. When treatment was subsequently discontinued 3 and 6 months after implantation, tumors became palpable within a month. Similar implants in control animals not given thyroid did not develop into palpable tumors until approximately 4 months after implantation and did not appear for 2½ months when thyroid destruction was delayed for 2 months after the implantation was performed.

The shorter latent period with increasing duration of the suppression by thyroid hormone may simply reflect less efficient suppression by exogenous hormone compared with endogenous hormone. The importance of the available thyroid hormone level in tumor suppression has been stressed by Furth and group (3), but the amounts fed in the present experiments were more than adequate to replace and exceed daily endogenous hormone secretion. The data suggest that the tumor was growing through the period of suppression, but at a greatly slowed rate. This is also suggested by the ultimate growth of the autonomous responsive L24 implants after more than a year of suppression by the host's own thyroid. Another likely explanation is that a change in the character of the tumor occurs with aging (3), despite, or because of, suppression by thyroid hormone. The possibility of a change in the host, either from decreased rate of secretion of thyroid hormone by the thyroid, when present, or from some systemic change, seems less likely, inasmuch as the altered behavior of the tumor was maintained in subsequent passages in at least one experiment (3). Hence, the alteration presumably is present in the tumor cell.

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Sidney C. Werner and Raul Grinberg


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