Growth of Pituitary Tumors Secreting TSH*

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Destruction of the thyroid gland by I's' in mice is followed by the development of TSH (thyroid stimulating hormone)-secreting pituitary tumors (5, 11, 13). These tumors can at first be grafted only on mice similarly radiothyroidectomized and not on normal animals (5); thus, they are initially conditioned or dependent growths. The tumor cells gain autonomy in the course of passages, as indicated by their ability to grow in normal mice (6). In further subpassages in normal hosts, they grow even better in normal than in radiothyroidectomized hosts. These findings are best explained by successive modification in cells of the pituitary from normal to autonomous type.

The mechanism of induction of pituitary tumor by radiothyroidectomy is not known with certainty. It has been postulated that depression of the thyroid gland with depression in TH (thyroid hormone) secretion stimulates those cells of the hypophysis which control thyroidal function. The possibility that injury to the pituitary by radiation, incidental to radiothyroidectomy, is a contributing factor in the induction of pituitary tumors has, however, not been excluded thus far (10, 12). This problem calls for studies of minimizing irradiation of the pituitary incidental to I's' treatment or avoiding irradiation altogether by performing surgical thyroidectomies. The quantitative study of the interrelationship between thyroid function and pituitary growth reported in this paper aims to contribute to the understanding of the mechanism of induction of pituitary tumors and maintenance of their growth. It will answer the question whether dependency and autonomy are absolute or relative characteristics of tumors.

Dependent and autonomous tumors.—The induction and transplantation and some salient characteristics of the transplantable pituitary tumors have been described (5–7). Dependent tumor cells (Fig. 2) (those requiring absence of the thyroid for their growth) resemble normal chromophobe pituitary cells but are probably related to basophiles. They exhibit no features of anaplasia, even though they usually metastasize to regional lymph nodes. These characteristics have changed but little in the 1½ years required for this work. The autonomous growths are more anaplastic and invasive. A conspicuous morphological difference between dependent and autonomous growths (Figs. 2, 3) relates to secondary changes in the tumors. Lack of secondary changes characterizes the dependent growth, while extensive necrosis, hemorrhage, and fibrosis are the rule with autonomous tumors. Anaplasia in the autonomous growths has increased markedly in the course of this study, and at its conclusion extensive lympathic metastases in draining superficial and abdominal lymph nodes are now the rule. Nevertheless, the ability to secrete TSH, as indicated by enlargement of the thyroid (Fig. 4) and its microscopic appearance (Fig. 8), was invariably retained. (A normal thyroid is shown in Fig. 5.) A more detailed description of the morphological features and secondary changes of these tumors will follow.

MATERIALS AND METHODS

Pituitary tumors were grafted on mice, 6–15 weeks in age, that had been given a single dose of 25, 50, 75, and 250 μc. of I131 by subcutaneous injection when 5–6 weeks of age. Approximately 200 μc. destroys the thyroid, 50 and 75 μc. depress it, and 25 μc. produces little or no damage to this organ. After 3–6 months, the functional capacity of the thyroids of these animals was assayed by the administration of a few μc. of I131.

C57BL mice of both sexes, either raised here or obtained from the Roscoe B. Jackson Memorial Laboratory, were used. Water and Purina chow, given ad libitum, were supplemented with carrots, lettuce, and hemp seeds. Tumor grafts were made by intramuscular injections of tumor particles in Tyrode solution. Carrier-free I131 solutions, stabilized with
NaHSO₃ and diluted with sterile 0.9 per cent NaCl, were injected in volumes of 0.2 ml. Following the injection of a near-tracer dose of I¹³¹ (~8 μc), the precise amount of I¹³¹ injected and its retention at various intervals after injection were measured by placing the animal in the 100 per cent geometry gamma chamber (19). This instrument was also used to measure the radioactivity in various tissues at autopsy. In vivo the amount of radioiodine localized in the thyroid and other sites was estimated by a scintillation counter with a thallium-activated sodium iodide crystal. The mouse was placed at a distance of 8.5 inches from the crystal. Localization and collimation of radiations were achieved using lead shielding, 2 inches in thickness, with a cylindrical hole of 0.5 inch in diameter. An aluminum filter was interposed between the source and the crystal to exclude the beta rays of I¹³¹. The error introduced by counting the activity in the tissues surrounding the thyroid was partially corrected by assuming that the volume of the thyroid region “seen” by the crystal was two-thirds that of the midbody region and that the activity not localized in the thyroid was equally distributed throughout the rest of the body. The scintillation counter was calibrated with I¹³¹ solutions standardized with the gamma chamber. All radioactivity measurements were adjusted for decay, with the time of injection being time zero.

Zenker-formol was the routine tissue fixative used. The pituitaries of tumor-bearing mice and pituitary tumors were stained with a combination of Martins and Mallory’s trichrome in addition to hematoxylin and eosin stains. Tissues to be autoradiographed were fixed in Bouin’s solution. The sections were placed on NTB plates 10 μ in thickness. Following exposure, development, and fixation, the sections were stained with Harris’ hematoxylin and fast green.

Success of Transplantation in Relation to Graded Depression of the Thyroid with I¹¹³

The relation between pretreatment with I¹¹³ and pituitary tumor growth was examined by grafting the tumors on mice that had received 25, 75, and 225 μc. of I¹¹³, respectively. Four separate experiments were performed with our oldest dependent strain (3-D) and two with more recently isolated strains of tumor (19 and 124). The results of these experiments are shown in Charts 1–6.

In the first and fourth experiments (Charts 1 and 4), the pituitary tumors of strain 3-D grew rapidly in the mice pretreated with 225 μc. and very slowly in those given 75 μc.; no tumor growth was observed in mice given 25 μc. In later experiments, indicated in Charts 2 and 3, the 3-D tumors appeared also in the 25-μc. group but only after a long latency period. This was probably due to the acquisition of autonomy, as indicated by earlier experiments (6). The tendency to gain autonomy was marked with strains 3-D and 19. Growth of strain 19 tumors was proportional to the I¹¹³ dose (Chart 5). Susceptibility of mice to tumor strain 124 was markedly enhanced by pretreatment with 225 μc., slightly by 75 μc., and not at all by 25 μc. (Chart 6).

The depression of thyroid function was not uniform in mice that were given the same dose of I¹¹³. Traces of thyroid were present in several mice in the 225-μc. groups and did not have an inhibitory effect on tumor growth. Great variations in thyroid depression in the 75 μc. groups were indicated by I¹¹³ uptake studies, histological examinations, and by enhancement of tumor growth. The function of these thyroid remnants was minimal, as will be shown later.

Functional Assay of the Thyroid Glands of Mice Given Graded Doses of I¹¹³

5-Day study.—The depression or destruction of thyroid function by I¹¹³ was quantitated by giving near tracer doses of I¹¹³ and measuring the radioactivity which remained in the body and in the thyroid at 24-hour intervals. Some of the mice carried grafted pituitary tumors; others served as controls. The radioactivity localized in the thyroid region is shown in Charts 7 and 8. The values are expressed in terms of the percentage of the total dose injected and in terms of the total activity retained in the body, respectively. Chart 9 shows the total retention of I¹¹³ in the body.

The highest retention of I¹¹³ in the thyroid (Chart 7) occurred in normal mice bearing autonomous pituitary tumors that were large and rapidly growing (Group III). The next highest retention was found in normal mice bearing minute, usually microscopic tumor grafts of dependent strains over a long period of time (Group II). The thyroids of mice pretreated with 25 μc. (Group X), which had minute tumor grafts, retained more than those of normal mice without tumor grafts (Group I). It is remarkable how extensive stimulation of the thyroid is produced by secretion of surviving pituitary grafts of microscopic size. Very low retention in the thyroid was found in mice that had received 225 μc. (Groups IV and V, respectively), and most of this activity can be attributed to nonthyroidal tissue.

Since the total body retention of I¹¹³ differed considerably among the various groups, it was of interest to compare the retention in the thyroid in
CHART 1.—The effect of pretreatment with 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 3-D). Each group was composed of four female mice.

CHART 2.—The effect of pretreatment with 0, 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 3-D). Each group was composed of five male mice.

CHART 3.—The effect of pretreatment with 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 3-D). Each group was composed of five male mice.

CHART 4.—The effect of pretreatment with 0, 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 3-D). Each group was composed of four female mice.

CHART 5.—The effect of pretreatment with 0, 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 19). Each group was composed of five female mice.

CHART 6.—The effect of pretreatment with 25, 75, and 225 μc. 1₂³I on growth of grafted pituitary tumors (Strain 194). Each group was composed of four female mice.

Charts 1–6 show the relation of pituitary tumor growth to intensity of 1₂³I pretreatment. The average tumor size in each group was plotted at various intervals from the time of grafting until the mice died with large tumors or were killed. In the event of death of animals within the group before the charted period, the tumor size at death was used thereafter.
relation to the total body retention (Chart 8). The curves representing the groups with pituitary tumor grafts fell in the same relative order as in Chart 7. In the normal and 25-μc. groups (I and X) the relative concentration of I\(^{131}\) in the thyroid increased until the fourth day following injection, after which it dropped. The curves for normal mice bearing tumors suggest a remarkable shift of I\(^{131}\) from the thyroid on the second day and to the thyroid on the third day. Further study would be required to establish fully the meaning of this shift.

The greatest 24-hour total body retention of I\(^{131}\) (Chart 9) occurred in radiothyroidectomized mice bearing large tumors, but this group (V) had also the most precipitous subsequent loss from the body. Radiothyroidectomy also enhanced the 24-hour retention of I\(^{131}\) (or reduced its elimination) in mice not given tumor grafts; this effect was, however, transient in these mice.

24-Hour study.—The ability of the thyroid hormone to inhibit the growth of pituitary tumors was further studied by microscopic examination of the thyroid glands following an assay of their functional capacity (Table 1). The mice were sacrificed 1 day following intraperitoneal injection of near tracer doses of I\(^{131}\), since the results of the previous experiment (Charts 7—9) indicated that thyroid function can be evaluated by determining the 24-hour retention of I\(^{131}\). Five experiments were per-

![Chart 7](chart7.png)

**Chart 7.**—Thyroid retention of a near tracer dose of I\(^{131}\).
- I. Normal nontumor-bearing mice.
- II. Normal mice bearing minute grafts of dependent pituitary tumors.
- III. Normal mice bearing large autonomous pituitary tumor grafts.
- IV. Radiothyroidectomized mice not grafted with tumors.
- V. Radiothyroidectomized mice bearing large dependent pituitary tumors.
- X. Mice pretreated with 25 μc. of I\(^{131}\) and bearing minute dependent pituitary tumor grafts.

The first number in the parentheses indicates the number of mice in the group, the second the number of tests in the pooled data. About half the mice received two consecutive doses at an interval of 10 days.

![Chart 8](chart8.png)

**Chart 8.**—Localization of a near tracer dose of I\(^{131}\) in the thyroid. See legend of Chart 7 for explanation.
All data are combined in Table 1. There was some variability in values, but the trend was the same in all experiments.

The mean total body retention in 24 hours in normal mice was 19.2 per cent of the tracer dose, and, of this, 70.5 per cent was in the thyroid gland (Group I). In contrast, the radiothyroidectomized mice (Group IV, Figs. 9, 10) retained 36.6 per cent of the injected dose in the body, but only a trace or none of the activity was in the thyroid region. The mean total-body retention in 24 hours in radiothyroidectomized mice bearing grafted tumors was 75.0 per cent of the dose (Group V), with only a trace or none at the site of the thyroid. The enhancing effect of grafted pituitary tumors on the uptake of radioiodine was thus confirmed.

The total-body 24-hour retention of I\(^{131}\) was almost as much in normal mice bearing minute grafted dependent pituitary tumors (Group II) as in normal mice bearing large autonomous tumors (Group III). In Group II most of the radioiodine retained was in the thyroid gland (89.0 per cent), and most of this was presumably hormonal (TH) iodine. The tumor nodules in mice of this group were rarely larger than a normal pituitary gland at autopsy performed 4½—9 months after grafting. The rapidly growing autonomous tumor grafts in the mice of Group III weighed at autopsy 2—5 gm., a thousand times that of a normal pituitary gland. Secretion of TSH by minute tumor grafts over a long period of time produces a striking stimulation with great enlargement of the thyroid gland.

The effects of pretreatment with 75 μc of I\(^{131}\) on thyroid function of mice bearing tumor grafts were variable. There was, however, an inverse relationship between morphological appearance and function of the thyroid gland on the one hand and size of the tumor grafts on the other. This is clearly indicated by subdividing this group according to the quantity of I\(^{131}\) retention. In none of the four mice in which the 24-hour total-body I\(^{131}\) retention was above 50 per cent of the dose (Group VIIIB) was the thyroid gland identified on gross examination, and in these mice the grafted tumors were large (>25 mm. in average diameter). In Group VIIIA the tumors were in general much smaller and the thyroids larger than in Group VIIIB. While more I\(^{131}\) was retained in the body of the mice in Group VIIIB (71.6 per cent) than in that of Group VIIIA (43.7 per cent), only 4.7 per cent of that retained was in the thyroid in Group VIIIB, as contrasted with 62.1 per cent in that of Group VIIIA. These values indicate that 75 μc injures the thyroid of different mice to a highly varying degree. The thyroids of all animals of this group had morphological changes indicative of stimulation by TSH and of varying grades of antecedent injury (Figs. 11—14). One was markedly fibrosed. A feature of the 75-μc. group was the co-existence of apparently resting and hyperstimulated follicles; similarly, the autoradiographs revealed a highly variable uptake of I\(^{131}\) in different follicles.

The behavior of the mice in the 25-μc. group bearing minute tumors was less variable than that of the 75-μc. group. The two mice with the lowest I\(^{131}\) uptake by the thyroid (Group XB, Fig. 16) had larger tumor grafts than those of XA, so that it is probable that 25 μc. of radioiodine injured the thyroid gland of these mice. If the remaining thirteen of the fifteen mice receiving 25 μc. (Group XA) are compared to the 0-μc. group bearing minute tumors (IIA), it is noted that the I\(^{131}\) retention was about the same in both (60.8 and 61.9 per cent, respectively), and most of the I\(^{131}\) retained was in the thyroid (86.1 and 89.0 per cent). Thus, the conclusion is warranted that in these thirteen mice 25 μc. did not noticeably alter the thyroid.

![Chart 9](chart9.png)
Whether or not 25 μc. had injured the thyroid could also be evaluated by comparing normal mice (Group I) to those given 25 μc. but not grafted with tumor (Group IX, Fig. 15). The 1131 retention in the body and the thyroid was only slightly greater in Group IX. This can be explained by assuming that this dose slightly depresses the thyroid function of some mice.

The possibility that the enhanced total body retention of 1131 in tumor-bearing radiothyroidectomized animals was due to specific localization in the intestine, but the site of most retained in radiothyroidectomized animals was in the eviscerated carcass.

The activity retained was found in the intestine, but 75 hours after injection was also small. In four radioiodine injected dose.

The site of most retained in radiothyroidectomized animals was in the eviscerated carcass.

The 1131 uptake in tumors following the administration of near tracer doses. The data show that the 1131 uptake in tumors following the administration of near tracer doses. The data show that the uptake of 1131 by different organs indicates that the uptake of 1131 by tumors is within the range of that of tumors of similar size. Mice of Group A had minute tumors not detectable by palpation and those of Group B had small palpable tumors. The 75-μc. group was subdivided according to the 1131 retention: Group V is composed of animals retaining less than 50 per cent and Group VI more than 50 per cent of the 1131.

Total Body and Thyroid Retention of Near Tracer Doses of 1131 in Normal and 1131 Pretreated Mice Bearing Tumors and in Controls

<table>
<thead>
<tr>
<th>No. of Mice</th>
<th>Type of Tumor</th>
<th>Body Retained</th>
<th>Thyroid Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10</td>
<td>0</td>
<td>Dependent Minute</td>
</tr>
<tr>
<td>Group II</td>
<td>5</td>
<td>0</td>
<td>Dependent Small</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>0</td>
<td>Dependent Large</td>
</tr>
<tr>
<td>Group IV</td>
<td>10</td>
<td>0</td>
<td>Autonomous Minute</td>
</tr>
<tr>
<td>Group V</td>
<td>8</td>
<td>200—300</td>
<td>Dependent Large</td>
</tr>
<tr>
<td>Group VI</td>
<td>9</td>
<td>100</td>
<td>Autonomous Minute</td>
</tr>
<tr>
<td>Group VII</td>
<td>4</td>
<td>75</td>
<td>Dependent Minute</td>
</tr>
<tr>
<td>Group VIII</td>
<td>3</td>
<td>75</td>
<td>Dependent Minute</td>
</tr>
<tr>
<td>Group IX</td>
<td>2</td>
<td>25</td>
<td>Dependent Small</td>
</tr>
</tbody>
</table>

*Minute = measuring 1-5 mm. in average diameter; small = approximately 1 cm.; large = 2-3 cm. The subdivision of Groups II and X was made on basis of tumor size: Mice of Group A had minute tumors not detectable by palpation and those of Group B had small palpable tumors. The 75-μc. group was subdivided according to the 1131 retention: Group VII is composed of animals retaining less than 50 per cent and Group VIII more than 50 per cent of the injected dose.

Table 2: Assay of Iodine Retention of TSH-Secreting Pituitary Tumors with Near-Tracer Doses of 1131

<table>
<thead>
<tr>
<th>Original</th>
<th>Type of Tumor</th>
<th>Weight of Tumor</th>
<th>Per cent of Tracer Dose Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of 1131 (μc.)</td>
<td></td>
<td></td>
<td>Body</td>
</tr>
<tr>
<td>0 Autonomous</td>
<td>1.9</td>
<td>69.8</td>
<td>0.5</td>
</tr>
<tr>
<td>0 Dependent</td>
<td>0.3</td>
<td>82.0</td>
<td>0.1</td>
</tr>
<tr>
<td>0 Autonomous</td>
<td>0.4</td>
<td>84.5</td>
<td>2.2</td>
</tr>
<tr>
<td>75 Dependent</td>
<td>2.5</td>
<td>85.5</td>
<td>2.4</td>
</tr>
<tr>
<td>225</td>
<td>3.6</td>
<td>57.1</td>
<td>3.0</td>
</tr>
<tr>
<td>400</td>
<td>1.0</td>
<td>70.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

To provide controls for the tumor-bearing mice pretreated with graded doses of 1131, similar mice without tumors, available 7 months after the administration of graded doses of 1131, have been included in the present study (Table 1). The thyroids of the controls (Group IX) that were given 25 μc. exhibited subtle changes which could be detected only by a close comparison with normal thyroids (Figs. 6, 7). There was a slight increase in
size of the thyroid cells, notably at the periphery of the gland, with some resolution of the colloid (Fig. 15). The uptake of I\(^{131}\) by thyroids of mice pretreated with 25 \(\mu\)c. averaged 28.1 per cent of the injected dose, as contrasted with 14.5 per cent in normals. In animals that had received 25 \(\mu\)c. and bore pituitary tumor grafts, injury was indicated by slight fibrosis and occasional slight atrophy, and stimulation by swelling of the epithelial cells. The latter contained many intracytoplasmic vacuoles and colloid droplets and exhibited some features of anaplasia. With tumors of long duration, adenoma formations were common (Figs. 17, 18).

Fifty and 75 \(\mu\)c. of I\(^{131}\) produced conspicuous changes consisting of slight to moderate fibrosis, occasional atrophy, moderate to marked variability in the size and shape of the cells and acini and in quantity and staining quality of the colloid (Figs. 12, 18). The lining cells of some acini were flat; others were greatly swollen, and frequently the same follicle was lined with cells of greatly varying sizes. There were marked differences in size and chromatophilia of the nuclei. Some were small, others "giants," some normochromic or hyperchromic with respect to affinity for hematoxylin. The cytoplasm of many cells was filled with colloid droplets varying greatly in size and number. Most cells were intensely vacuolated.

One hundred \(\mu\)c. of I\(^{131}\) produced a marked reduction in the number of cells of the thyroid so that the organ could not be identified at a magnification of 5 \(\times\) at autopsy. Microscopic examination disclosed a more intense change in the residual thyroid than in mice of the 50- and 75-\(\mu\)c. groups. Anaplasia as concerns nuclear size, shape, and chromatophilia was particularly marked. Note that 50–100 \(\mu\)c. markedly depressed the thyroidal uptake of I\(^{131}\), but, in the presence of tumor, I\(^{131}\) uptake was fairly high (75-\(\mu\)c. group). The presence of tumor markedly increased the total body retention of I\(^{131}\) (estimated 19–50 per cent of the injected dose), and this enabled a greater uptake of I\(^{131}\) by the small residual thyroid tissue that had been stimulated by the TSH secreted by the pituitary tumors.

Two hundred and fifty to 400 \(\mu\)c. of I\(^{131}\) destroyed the thyroid almost completely. Its site was identified by a marked stenosing "radiation arteritis," often with perivascular infiltrations by lymphoid cells and hemosiderin-filled macrophages in an area of fibrosis adjacent to the parathyroid (Figs. 9, 10). Frequently, with a 200-\(\mu\)c. dose, a few isolated acini remained, exhibiting the regressive changes already described, anaplasia being particularly marked. The presence of these rudimentary thyroid cells can be readily detected in vivo by the scintillation counter following administration of a tracer dose of I\(^{131}\). Thus, they function, but are unable to undergo compensatory hyperplasia when constantly stimulated by the tremendous quantities of TSH discharged by the grafted tumor. In mice receiving 300 \(\mu\)c. or more, such rudimentary thyroid tissue was but rarely identified.

Thus, the results of the morphological and iodine uptake studies are in agreement. The threshold dose of I\(^{131}\) for depressing the thyroid of mice appears to be in the neighborhood of 25 \(\mu\)c. This dose might cause a slight depression or even an apparent stimulation in some animals. Correspondingly, this is also the threshold dose for rendering some animals susceptible to tumor growth; in others the grafts are merely held in situ as they are in normal hosts.

**DISCUSSION**

While it is felt that the prime aim of this study has been attained, several deficiencies in technic were noted post hoc; pointing these out will be helpful in future research on related problems. Not anticipating the importance of knowing precisely the size of the thyroid, this organ was not weighed. Careful inspection under the binocular microscope might identify thyroid remnants. Weighing and serially sectioning this organ as well as weighing the tumors and the pituitary post mortem will add to the accuracy of such work. Autoradiography should be performed on tissues dehydrated in the frozen state in order to minimize loss of I\(^{131}\).

**Correlation of function with tumor growth.**—One objective of this study is to correlate thyroid function with growth of TSH-secreting pituitary tumors. The 24-hour uptake of I\(^{131}\) by the thyroid is a good index of the functional capacity of the thyroid in mice. Hyperthyroidism (TSH excess) increases the thyroid uptake of I\(^{131}\) in mice as it does in man (4); hypothyroidism reduces it, and zero values are obtained in athyroidism. There is an excellent correlation between depression of thyroid function and growth of grafted dependent pituitary tumors. Individual differences are evident in all groups, and are greatest in the 75-\(\mu\)c. group (Figs. 11–14). These variations can be attributed in part to technical inaccuracies in amounts injected, and in part to individual variations in response. Whatever its cause, this variability was an aid in correlating thyroid function with tumor growth; e.g., all mice of the 75-\(\mu\)c. group in which the uptake was more than 50 per cent had gross tumors, and those with an uptake of I\(^{131}\) of less than 50 per cent had no tumors.

A correlation between morphology and functional capacity of the thyroid was usually evident; e.g., in the 75-\(\mu\)c. group the mouse with the highest I\(^{131}\) uptake had, on microscopic examination, the
largest and most stimulated thyroid of the group. There are ample data on the quantitative relationship between TSH and thyroid stimulation (1, 9, 15); the present study supplements these by correlating thyroid function with stimulation of growth of the pituitary cells secreting TSH. A complete review of the thyroid-pituitary relationship is beyond the scope of this paper; there are many excellent recent articles on this subject (cf. 1, 2, 4, 9, 15).

The present studies and those of Rugh (16) indicate that complete destruction of the thyroid is not necessary for induction of pituitary tumors. There is no clear-cut evidence of new formation of follicles after partial radiothyroidectomy; however, there is ample evidence that some cells surviving partial radiothyroidectomy are hyperfunctioning.

The failure or tardiness of the thyroid gland partially destroyed by small doses of I\(^{131}\) to undergo fully compensating hyperplasia deserves emphasis and further study. This may be a radiation effect; in man, partial removal by surgery is known to be followed by regeneration. One noteworthy difference between surgical and radiothyroidectomy is the stenosing arteritis and fibrosis caused by irradiation, which alone may explain failure of regeneration. Comparative studies on regeneration after surgical and radiothyroidectomy are desirable.

No close correlation could be made between the results of tests on the uptake of I\(^{131}\) and the autoradiographs of the same thyroids. This partial failure can be attributed to the loss of I\(^{131}\) in the course of processing, as pointed out by Holt and Warren (14), and might be avoided by the use of the freeze-dry technic (14). Nevertheless, the loss in wet processing was not great, and the autoradiographs furnished some confirmatory data on the degree of I\(^{131}\) retention and served to establish the uniformity of distribution of I\(^{131}\) or the lack of it.

It is well known that morphologic appearances may be misleading. Follicles in thyroid may contain colloid yet be functionally inactive; tumors may appear well differentiated yet highly invasive (2). Autoradiographs have indicated the heterogeneity of acini in thyroids injured by I\(^{131}\). Adenomas caused by sustained stimulation of the thyroid by TSH-secreting tumors failed to take up demonstrable amounts of I\(^{131}\) in the presence of normal-appearing acini which accumulated I\(^{131}\). In mice given large doses of I\(^{131}\) thyroid remnants composed of vacuolated atypical cells could be definitely identified as such by their effect on the photographic emulsion.

If the growth stimulus of pituitary tumors is lack of TH, blocking TH synthesis with thiouracil might also be expected to render normal mice susceptible to pituitary growth; administration of thyroid hormone ought to abolish susceptibility caused by radiothyroidectomy. Studies to be reported later indicate both to be true. Thiouracil has proved, however, to be much inferior to complete radiothyroidectomy, and its effect is equivalent to partial radiothyroidectomy as caused by approximately 50 \(\mu\)c. of I\(^{131}\). Induction of pituitary tumors by administration of thiouracil in mice has also been reported (18) but not confirmed. That administration of TH will prevent the induction of primary pituitary tumors has already been shown by Gorbman (13) and Goldberg and Chaikoff (10); similarly, tumors grafted in radiothyroidectomized mice can be restrained by TH. Thus, there is some parallelism between conditions influencing induction of pituitary tumors and their transplantability.

**Hormonal secretion of the pituitary tumors and its relation to tumorigenesis.**—Knowledge of the type and quantity of secretions is essential for the understanding of the problems under consideration. Several investigators have independently established by bioassays the secretion of TSH by these tumors (Dent,1 D'Angelo,2 Halmi3), and their findings have indicated the discharge of large quantities of TSH by the tumors. Most valuable are the assays of Anderson4 in hypophysectomized mice which likewise indicate secretion of TSH by the tumors and, in addition, absence of changes indicative of secretion of other hormones in significant amounts. Normal mice bearing autonomous pituitary tumor grafts have tremendously enlarged and stimulated thyroids. Changes in the thyroid of mice that received 50 to 100 \(\mu\)c. of radioiodine without tumor grafts were similar to those seen in normal mice bearing autonomous pituitary tumors. It can be assumed that partial radiothyroidectomy causes stimulation of TSH secretion by the pituitary, with secondary hyperfunction of the thyroid cells whose proliferative capacity is impaired by the irradiation. In mice that had been given 100 \(\mu\)c. and larger doses 7 months earlier, microscopic examination of the hypophyses showed nodular hyperplasia of chromophobe cells, which is known to precede tumor development. Similar alterations were absent in the hypophyses of mice given 25 and 50 \(\mu\)c.

The relationship between the development and growth of pituitary tumors and thyroid function

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1 Personal communication with Dr. James N. Dent.
2 Personal communication with Dr. S. A. D'Angelo.
3 Personal communication with Dr. N. S. Halmi.
4 Personal communication with Dr. Evelyn Anderson.
applies only to those pituitary tumor cells which secrete TSH. It is possible that a similar relationship exists between pituitary cells secreting other types of hormones and the function of the stimulated organ. The problem of tumor induction is how to disturb the specific normal balance between the pituitary and its target organ. Pituitary tumors have been described in gonadectomized mice (3), but the functional capacity of these tumors and their specific relationship to the gonads have not been adequately studied. Compensation by the adrenal gland for lack of gonadal hormone doubtless interferes with the development of pituitary tumors following gonadectomy. Adrenalectomized mice have not to our knowledge been maintained long enough for the development of tumors in the pituitary gland. Pituitary tumors have also been induced in C57BL mice by long continued administration of estrogens (cf. 8). This and other related pioneer research are being surveyed and discussed in a review on conditioned and autonomous neoplasms.5 It is noteworthy that all work on the induction of pituitary tumors by radiothyroidectomy reported thus far was done with C57BL mice.

Genetically controlled strain and species differences in susceptibility may be additional factors influencing the development of pituitary tumors following radiothyroidectomy. The possible role of irradiation as an accessory factor has been suggested by Gorbman (12). By keeping mice on an iodine-free diet he could reduce to 30 μc the quantity of radiiodine needed to destroy the thyroid. However, reducing iodine in the diet will enhance the uptake of radioiodine in the thyroid, and consequently the dose of irradiation received by the thyroid will equal that of animals kept on a normal diet and given much larger doses of radioiodine. The statement that thyroidectomy is not followed by the development of pituitary tumors (16) has to our knowledge never been documented; we found no report in the literature on chronic effects of thyroidectomy in mice. Thus, it is uncertain whether or not irradiation of the pituitary coincidental to radiothyroidectomy is essential to induction of pituitary tumors.

Enhancement of body retention of I\textsuperscript{131} after radiothyroidectomy.—An explanation is wanted for the enhanced total body retention of I\textsuperscript{131} in radiothyroidectomized mice. This procedure nearly doubled I\textsuperscript{131} retention, and the tumor graft further doubled this under our experimental conditions. It is known that TSH decreases the excretion rate of I\textsuperscript{131} (9), but in normal hosts this can be attributed to increased synthesis of TH. Studies on the uptake of I\textsuperscript{131} indicate that the tumors have no special affinity for I\textsuperscript{131}. The exact localization of I\textsuperscript{131} in radiothyroidectomized mice as well as the chemistry of compounds carrying it remain to be studied. It is probable that radiothyroidectomized mice have an increased amount of circulating TSH. Pituitary tumor grafts raise the TSH blood level, and it is perhaps this hormone itself which modifies the iodine retention capacity of the body. These assumptions call for quantitative studies of levels of TSH in relation to iodine retention in the body and partition of substances containing io-

All sections were stained with hematoxylin and eosin. The magnifications are approximate.

Fig. 1.—Gross appearance of a mouse with large dependent pituitary growth (Strain 3). Arrows point to large seminal vesicle, cystic dilatation of the biliary tract, and metastases in iliac lymph node.

Fig. 2.—Dependent pituitary tumor of Strain 3. Note uniformity of cells and absence of anaplasia. \( \times 240 \).

Fig. 3.—Autonomous pituitary tumor of Strain 3. Note the variations in size, shape, and staining intensity of the cells, and regressive changes. \( \times 100 \).

Fig. 4.—Gross appearance of an enormously enlarged thyroid gland of a noniodinated mouse bearing a small pituitary tumor 10 months after graft (Strain 3; late autonomy). \( \times 4.5 \).

Fig. 5.—Normal thyroid shown for comparison with Figure 4; arrow points to the left lobe of the thyroid. \( \times 4.5 \).

Figs. 6 and 7.—Microscopic appearance of the normal thyroid of the strain of mice under study. \( \times 100 \) and \( \times 240 \), respectively.

Fig. 8.—Hyperstimulated thyroid of a normal mouse carrying a large autonomous pituitary tumor graft (Strain 3). Note the swelling of cells, resolution of colloid, and intracytoplasmic globules of colloid, the latter indicated by arrows. \( \times 970 \).
Fig. 9.—The site of the thyroid in a mouse that had been given 269 μc. of P31 14 months before death is indicated by the parathyroid, which is undamaged, a stenosed artery ("radiation arteritis"), and a nerve bundle. ×75.

Fig. 10.—The site of the thyroid in a mouse that had been given 375 μc. of P31 3½ months before death indicated by thick-walled stenosed arteries, chronic diffuse and perivascular inflammation, fibrosis, and degeneration with ossification of the adjacent part of tracheal cartilage. ×100.

Fig. 11.—Residual thyroid tissue with fibrosis of the surrounding area in a mouse that had been given 75 μc. This animal had the smallest thyroid of mice so treated, and the grafted pituitary tumor grew to be large. ×240.

Figs. 12 and 13.—Small thyroid of a mouse that had been given 75 μc. Note the fibrosis, moderate anaplasia, and intracytoplasmic colloids. The grafted tumor (Strain 3D) measured 10 mm. in average diameter. Fig. 12, ×240; Fig. 13, ×970.
dine. Little, if any, of the I\textsuperscript{131} retained in thyroidectomized mice is expected to be in thyroid hormone; even if extrathyroidal TH synthesis occurs, the total amount of hormone produced is evidently below that required to compensate for the absence of the thyroid.

**SUMMARY AND CONCLUSIONS**

The relationship of growth of TSH-secreting pituitary tumors to thyroid function was studied by destroying or depressing the thyroid gland of mice with graded doses of I\textsuperscript{131}, grafting tumors on such animals, and correlating tumor growth with thyroid function. The results indicate that proliferation of grafted TSH-secreting pituitary tumor cells depends on the secretion of TH. Near complete or complete destruction of the thyroid follows administration of a single dose of \(200\) or more \(\mu c\) of I\textsuperscript{131}. This provides the maximum stimulus to pituitary growth. A lower dose, about \(75\) \(\mu c\), produces a lasting depression of the thyroid, some follicles of which, although normal in appearance, are incapable of undergoing fully compensating hyperplasia. In most mice \(25\) \(\mu c\) interferes little, if at all, with the thyroid function, with ability of this gland to be stimulated by TSH, and with its capacity to inhibit the growth of pituitary tumors. The inverse relationship between thyroid function, as indicated by its ability to take up I\textsuperscript{131}, and stimulation of the pituitary tumor grafts is almost quantitative.

Cells of thyroid glands which escaped destruction by large doses of I\textsuperscript{131} do not have the ability to undergo a fully compensating hyperplasia. This may be due in part to the stenosing "radiation arteritis" and diffuse fibrosis in the thyroid region caused by I\textsuperscript{131}. Radiothyroidectomy causes an increased total-body retention of I\textsuperscript{131} during the first 24 hours following injection. Pituitary tumor grafts secreting TSH further enhance total-body retention of I\textsuperscript{131}. The sites of this I\textsuperscript{131} are not precisely known. In radiothyroidectomized mice (with and without tumor) the elimination of the retained I\textsuperscript{131} is rapid. In normal mice bearing grafted tumors most of the retained radiiodine is in the thyroid, and its retention is lasting.

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**REFERENCES**

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Quantitative Relationship between Thyroid Function and Growth of Pituitary Tumors Secreting TSH

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