Formation of Adenomata in Hypophyses of Rats Subjected to both Subtotal Thyroidectomy and Administration of $^{131}$I, and its Prevention by Feeding of Desiccated Thyroid

W. E. Griesbach, I. L. Chaikoff, C. W. Nichols, Jr., and Robert C. Goldberg

Department of Physiology, University of California, Berkeley, California

SUMMARY

The tumorigenic effect of subtotal thyroidectomy on the pituitary was studied in 212 Long-Evans female rats. Of the 133 pituitaries that contained adenomata after 2 years, 76% were of the thyrotropic cell type. This finding confirms earlier work on the effect of long-term thyroxine deficiency.

The feeding of desiccated thyroid reduced the percentages of thyrotropic cell adenomata from a mean of 60 to a mean of 33. Thyroid feeding reduced the percentages of all adenomata from a mean of 72 to a mean of 53.

A single administration of 1 μc of $^{131}$I did not increase the number of, or tendency towards, anaplastic changes in pituitary adenomata of subtotal thyroidectomized rats fed a stock diet. The same dose of $^{131}$I did not increase the incidence of pituitary adenomata in unoperated animals fed either the stock diet or the stock diet supplemented with desiccated thyroid. Two rats that developed malignant thyroid carcinomata after receiving 1 μc of $^{131}$I did not show cell changes or adenomata in the pituitary. The absence of cytologic signs of thyroxine deficiency in the pituitary cells of these rats supports the view that irradiation per se might well have been the cause of the neoplasms found in their thyroid glands.

The pituitary adenomata found in 124 unoperated rats, treated in the same way as the subtotal thyroidectomized groups with respect to $^{131}$I dosage and thyroid feeding, closely resembled those found in the normal control group in number as well as in cell type.

It has been known for almost 2 decades that hyperplasia as well as benign and malignant neoplasms develop in thyroid glands of mice and rats in which, for prolonged periods, a state of thyroxine deficiency has been established by any one of the following means: the feeding of a low-iodine diet (1, 2); the administration of anti-thyroid substances (5, 15, 25); irradiation of the gland by $^{131}$I, astatine ($^{211}$At), or X-rays (7, 12, 14, 19); and subtotal thyroidectomy (6, 14). There is good evidence for the belief that the neoplastic changes should be attributed to the hyperplasia of thyrotropic hormone (TSH)-producing cells in the anterior pituitary glands of the animals suffering from long-term thyroxine deficiency. Assays of TSH contents of the hypophysis and blood, as well as cytologic study of the TSH-producing cells in the pituitary, lend support to this interpretation. The literature dealing with this subject has been reviewed by Bielschowsky (2) and Bielschowsky and Horning (3).

A matter of some interest as well as practical importance arose in the case of experiments in which irradiation was applied to the rodents' thyroid gland, either alone or in combination with chemical or surgical means, for inducing the thyroxine deficiency. In most cases, especially in the early work with large doses of $^{131}$I (up to 875 μc), the irradiation had been sufficiently strong to destroy much or all of the thyroid tissue (11, 12). In later experiments the $^{131}$I was given in a considerably reduced dose (25–40 μc). It was shown that hereby the number of experimentally induced malignant thyroid neoplasms was definitely higher (22). It could further be seen that hyperplasia of the thyroid epithelium surrounding the neoplasms was a constant feature of these glands. It was assumed that this was significant for the strong and permanent action of thyrotropic hormone. No signs of radiation damage were found. The question whether irradiation per se can induce thyroid tumors was discussed in another study in which either both lobes or a single lobe of the thyroid gland had
TABLE 1
ADENOMATA IN PITUITARIES OF INTACT FEMALE RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Pituitaries with adenomata (Total %)</th>
<th>Cytologic classification of adenomata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thyrotrophs</td>
</tr>
<tr>
<td>I</td>
<td>None</td>
<td>47</td>
<td>21 (45%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>VI</td>
<td>1 µCi ¹³¹I injected</td>
<td>34</td>
<td>10 (29%)</td>
<td>3 (90%)</td>
</tr>
<tr>
<td>VII</td>
<td>Desiccated thyroid in diet</td>
<td>57</td>
<td>23 (40%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>VIII</td>
<td>1 µCi ¹³¹I plus desiccated thyroid in diet</td>
<td>33</td>
<td>17 (51%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>171</td>
<td>71 (42%)</td>
<td>9 (13%)</td>
</tr>
</tbody>
</table>

TABLE 2
ADENOMATA IN PITUITARIES OF SUBTOTALLY THYROIDECTOMIZED FEMALE RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Pituitaries with adenomata (Total %)</th>
<th>Cytologic classification of adenomata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thyrotrophs</td>
</tr>
<tr>
<td>II</td>
<td>Subtotal thyroidectomy only</td>
<td>67</td>
<td>42 (63%)</td>
<td>36 (86%)</td>
</tr>
<tr>
<td>III</td>
<td>Subtotal thyroidectomy plus 1 µCi ¹³¹I injected</td>
<td>49</td>
<td>40 (82%)</td>
<td>33 (83%)</td>
</tr>
<tr>
<td>IV</td>
<td>Subtotal thyroidectomy, 1 µCi ¹³¹I injected plus desiccated thyroid fed</td>
<td>60</td>
<td>32 (53%)</td>
<td>22 (69%)</td>
</tr>
<tr>
<td>V</td>
<td>Subtotal thyroidectomy plus desiccated thyroid fed</td>
<td>36</td>
<td>19 (53%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>212</td>
<td>133 (63%)</td>
<td>101 (70%)</td>
</tr>
</tbody>
</table>

been irradiated (21). There, most of the carcinomata were found in rats in which both lobes had been irradiated, but 2 carcinomata also appeared in singly-irradiated lobes and 1 in an opposite, shielded lobe. Hence, it could not be decided whether thyroid carcinogenesis resulted from prolonged TSH stimulation or was a direct consequence of the irradiation. In a more recent paper, Goldberg et al. (14) reported finding a papillary and a follicular carcinoma in intact thyroid glands of rats that received but a single µCi of ¹³¹I. The present report deals with the 383 pituitaries excised from the rats whose thyroids had been described in this latter study (14). In these pituitaries, many adenomata were found; they are described in detail, particularly with regard to the hormonal conditions that may have influenced their development.

MATERIALS AND METHODS

A total of 876 female, Long-Evans rats were randomly selected at 5–6 weeks of age, divided into 8 groups, and treated as shown in Tables 1 and 2. After weaning, all rats were maintained on a nutritionally adequate diet (Diablo Double-Check Labration) containing 3 µg of iodine/gm on July 19, 2017. © 1965 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from
The number of gonadotroph adenomata was not changed by thyroid feeding, but that of chromophobe adenomata was increased—29%, as compared to 12% in rats fed the stock diet.

EFFECTS OF $^{131}$I ADMINISTRATION

In Groups VI and VIII (unoperated rats, Table 1), in which the majority of adenomata were of the chromophobe cell type, no significant effect of the $^{131}$I injection was detected. Among the subtotally thyroidectomized rats, Groups III and IV, comprising 109 animals (Table 2), received $^{131}$I, whereas Groups II and V (103 animals) received no $^{131}$I. Groups IV and V (96 rats) were fed the desiccated thyroid-containing diet. The incidence of thyrotropic cell adenomata was not significantly altered in Group III by the $^{131}$I administration as compared with Group II, which received no $^{131}$I. Of all adenomata found in Group III, 83% were thyrotrropic cell adenomata. The corresponding figure for Group II was 86%. In the thyroid-fed rats, the higher percentage (69%) of all adenomata belonged to Group IV ($^{131}$I-injected). Group V ($^{131}$I not injected) contained the lower percentage of 53%. Anaplasia was found more frequently in the adenomata of the rats fed the stock diet than in those of rats fed the desiccated thyroid-containing diet—21% versus 13% (Table 2).

The 2 unoperated rats in which Goldberg et al. (14) found malignant thyroid neoplasms following the administration of 1 μc of $^{131}$I did not show pituitary adenomata or any deviation from the normal cell picture in their anterior pituitary glands (Table 3).

HISTOLOGY OF NEOPLASMS FOUND IN ANTERIOR PITUITARY GLANDS

Neoplasms were found in the pituitaries of all 8 groups of rats. These neoplasms contained primarily 1 type of cell, and each neoplasms was therefore classified according to its main cell type. Difficulties in classification arose in a few cases because of anaplastic areas in some of the neoplasms.

Chromophobe cell adenomata.—These were found in 30% of the anterior pituitaries of all unoperated rats and in 12%
of all thyroidectomized rats at the end of 2 years. They were composed of small cells that did not contain stainable granules. In many neoplasms of this type, the cells had a follicular arrangement. The follicles were filled with blood. Grossly visible hemorrhages were often found; these, in combination with rapid growth (mitoses), compressed the surrounding areas of otherwise intact pituitary tissue. Almost regularly, macrophages containing hemosiderin (clasmatoocytes) were seen in the chromophobe adenomata, and these macrophages were stained with PAS. So typical were the hemosiderin-containing cells that this type of adenoma was easily recognized in unstained sections by the presence of brown pigment (27).

**Thyrotroph and thyroidectomy cell ("T"-granulated cell) adenomata.**—Neoplasms composed of thyrotrophs were found in 9 pituitaries of the unoperated rats (5%) and in 101 pituitaries of the thyroidectomized groups (48%). In the former, the neoplasms were small and never anaplastic. The thyrotroph cells, which were multangular and irregular, sometimes bizarre, in shape, stained well with aldehyde fuchsin; the sections had not been previously oxidized by permanganate (Figs. 1—3, 11). The intensity of staining was not constant throughout the adenomata (Fig. 10), probably because the degree of granulation in the cells varied.

The pleomorphic cells of partially anaplastic neoplasms often contained aldehyde fuchsin-positive granules (Figs. 7, 8). Such neoplasms occasionally contained large hypertrophic cells with mitoses of unusual size (Fig. 17). The type of thyrotroph adenoma most frequently encountered, however, contained relatively uniform, tightly packed cells that stained intensely with aldehyde fuchsin and had vesicular, round or oval nuclei with 1 nucleolus (Figs. 4, 5, 14).

The adenomata varied greatly in size and shape. Usually they were multiple, and as many as 6 discrete foci have been noted in a single gland. Since these would not be expected to grow at the same rate or to possess identical degrees of neoplastic change, it is not surprising that quite different structures were seen in a single pituitary or even in a single adenoma (Figs. 5—8). The presence in such growths of aldehyde fuchsin stained cells having the angular shape characteristic of thyrotrophs simplified the differential diagnosis of such neoplastic alterations (Figs. 12, 15, 18). The area in the anterior lobe with the highest incidence of thyrotroph adenomata was the posterior edge near the pars intermedia.

The pituitaries of the subtotally thyroidectomized rats contained a large number of hyalinized basophil cells, the so-called thyroidectomy cells, which are known to be thyrotrophs that were transformed during the state of thyroxine deficiency. According to Purves and Griesbach (24), such cells, found in the pituitaries of rats in which thyroxine deficiency has been established for at least 2 weeks, contain coarse PAS-stainable granules ("T" granules) that differ from normal thyrotrophic granules in being insoluble in water and not implicated apparently in thyrotrophic hormone production. "T"-granules may also be stained by intense aldehyde fuchsin treatment (24), especially after preoxidation with permanganate. Recently, it has been shown by Doniach and Williams (6) that "T"-granulated cells can form adenomata. In the present study we found, in nearly 50% of adenomata, "T"-granulated cell nodules adjacent to typical thyrotroph adenomata in a single pituitary gland. The fact that the "T" granules seen here always stained with AF helped avoid confusion with the gonadotrophs (Fig. 13; see also Ref. 24).

Fully hyalinized cell areas were found in 14% of the pituitaries with thyrotroph adenomata. Such hyalinized cells resembled "thyroidectomized" cells and sometimes formed large parts of the neoplasms. Even mitoses were found among them (Fig. 9; see Ref. 18).

**Gonadotroph adenomata.**—The number of gonadotroph adenomata was small and was not affected by subtotal thyroidectomy. The cell type was the pale central variety (luteinizing hormone) described by Purves and Griesbach (17, 23). These cells are round or oval and have dark-red-stained Golgi areas near the nuclei, surrounded by pale cytoplasm (after PAS). Rapidly growing neoplasms of this type with many mitoses and compression of the neighboring area were noted (Fig. 16). In contrast to the thyrotroph adenoma, the gonadotroph adenoma always preserved its acinar structure and manifested strongly PAS-positive vascular membranes. This helped to differentiate the 2 adenoma types, even at low magnification.

**DISCUSSION**

**GENERAL CONSIDERATIONS**

This paper deals with the cytology of the anterior hypophyses of the rats used in the experiments of Goldberg et al. (14). A large number of pituitary adenomata were found in these rats and were classified according to their morphologic appearance as thyrotropic, gonadotropic, or chromophobe. Their size was small, measuring 1–2 mm in diameter, and they could not be separated from the surrounding pituitary tissue even when their presence was known before autopsy. This fact prevented an assay of the neoplastic tissue for its hormone content by biologic means. The presence of thyrotropic cells in the adenomata therefore had to be based on their characteristic multangular form and their specific stainability with aldehyde fuchsin without previous oxidation of the section.

Goldberg et al. (14), 2 years after starting the experiments, found, in addition to many adenomata, 5 carcinomata in the thyroid glands of their rats. One papillary and 1 follicular carcinoma developed in the intact thyroid glands of their rats. One papillary and 1 follicular carcinoma developed in the intact thyroids of rats that received only 1 μc of 131I, and it was believed possible that these neoplasms were induced solely by the radiation. A single papillary carcinoma developed in a rat that had been subjected to subtotal thyroidectomy, given an injection of 1 μc 131I, and fed the desiccated thyroid-containing diet. The question arose whether the tumor-producing mechanism could be deduced from a study of the pituitary glands (Table 3).

It is now widely acknowledged that enhanced and prolonged thyrotropin secretion from the pituitary gland may provoke hyperplasia of thyroid cells, adenoma formation in the thyroid gland, and provided a state of thyroxine deficiency is established for a prolonged period, even carcinogenesis of the thyroid gland (1, 2, 6, 25).
increased thyrotropin secretion is manifested in the pituitary by greater numbers of thyrotropin-secreting cells (thryotrophs) and by the appearance of thyroidectomy cells. It would, therefore, have been of interest if this had happened in the pituitaries of the 2 rats bearing carcinomata in their otherwise intact thyroid glands. This was not the case, however. The dose of 131I administered was too small to have been destructive to the rats' thyroid glands, and this accounts for the absence of thyrotroph hyperplasia and adenoma formation in their hypophyses. We found no evidence that the injected 131I increased the effect of subtotal thyroidectomy on the pituitaries (see Groups II and III). The numbers of pituitary adenomata in these groups of operated rats, regardless of whether the thyroid glands were irradiated, were not significantly different. We therefore have no reason to believe that the administered 131I had induced neoplastic changes in the pituitary cells.

CONCERNING THE MECHANISM OF PITUITARY TUMOR INDUCTION

Old rats of either sex have been shown to contain a large number of spontaneous pituitary adenomata (27). These vary from microscopic to grossly visible tumors, and they probably arise because of hormonal changes due to aging. Chromophobe adenomata were found in 30% of the pituitaries of the unoperated rats in the present study. In another paper for which the same rat strain (Long-Evans) was used, Van Dyke et al. found a similar incidence (26). Wolfe et al. (27) reported an occurrence of 29% and 68% of pituitary tumors in Vanderbilt and Wistar rats, respectively, and Griesbach and Purves (17), using a Wistar strain, found that of a total of 31% adenomata, 23% were of the chromophobe type.

Cramer and Horning (cited in Ref. 3) were the first to produce neoplastic lesions in the rat pituitary experimentally by the administration of estrogen. These neoplasms consisted of chromophobe cells and manifested a strong tendency to hemorrhage; they thus resembled the many chromophobe tumors reported in the present study. Deficiency of sex hormones, induced by gonadectomy in mice and some strains of rats, may also cause the development of chromophobe cum acidophil and of gonadotroph cell adenomata. Griesbach and Purves (17) reported that rats with such neoplasms secrete milk and that this is observed occasionally even in males.

Bielschowsky (2) and Axelrad and Leblond (1) showed in rats that a diet almost free of iodine could in the course of time lead to adenoma formation in the thyroid gland and the pituitary. These tumors were prevented from developing by the feeding of about 22 μg of KI per day. It is also well known that an inadequate supply of thyroxine following the administration of anti-thyroid substances or subtotal thyroidectomy may cause neoplastic reactions in the thyroid and pituitary glands of rats (3, 6, 16). This is confirmed again here in the subtotally thyroidectomized rats. It is doubtful that complete surgical thyroidectomy leads to the formation of thyrotropic tumors in the pituitary. Purves and Griesbach (unpublished observation) did not find that type of pituitary neoplasms in rats after complete surgical thyroidectomy. The experiences of Goldberg and Chaikoff (11, 12) and Furth and his co-workers (7–10), as well as the recent report on the action of astatine-211 (28), leave no doubt that after the destruction of the thyroid by radiation, thyrotropic neoplasms are not formed in the pituitaries of rats. It seems possible that the rat hypophysis needs a very small amount of thyroxine to form neoplasms. This would be produced by the thyroid-rest, which remains in the animal after the subtotal thyroidectomy applied in this series of experiments.

Between 1950 and 1952, Goldberg et al. (11–13) dealt with the effects of 131I (in doses from 18 μc to 875 μc) on the structure and viability of the thyroid and pituitary cells of the rat. They showed that (a) a dose of 18 μc of 131I produced no demonstrable cytologic or functional changes in either gland; (b) 300 μc of 131I caused mild chronic inflammation and fibrous proliferation in the thyroid, as well as a loss of acidophils and an increase of basophils in the pituitary; (c) between 5 and 8 months after the latter injection, both glands were similar to normal controls. With doses of 525–875 μc of 131I, the damage to the thyroid gland was much greater. Although 525 μc did not destroy the thyroid cells entirely, "atypical" epithelial cells with little follicular organization and scarce colloid appeared. After 6-8 months, atypical cells in greater numbers and hypertrophic epithelium with follicular organization were found. The almost complete degranulation of the pituitary acidophils proved that thyroxine production in the damaged thyroids was quite inadequate (11). A dose of 875 μc of 131I destroyed the thyroid cells almost completely in 72 hr, and after 6 and 8 days...
Fig. 7, 8.—The same tumor shows more atypical cells stained with AF. Group II, AF. × 400.

Fig. 9.—Adenoma with area of hyalinized thyroidectomy cells. Mitoses near center. Group III, PAS. × 400.

Fig. 10.—TSH cell adenoma. Generally small cells varying greatly in shape and uptake of AF. Group III, AF. × 400.

Fig. 11.—TSH cell adenoma showing the “stretched out” appearance not infrequently seen. Group III, AF. × 150.

Fig. 12.—This pituitary contains adenomata, some with “T” granules and others, like the one depicted, with a tendency towards anaplasia. Group III, AF. × 400.
FIG. 13.—Higher magnification of "T"-granulated cells giving a good picture of the coarse granules. Group III, PAS. × 900.

FIG. 14.—Very large adenoma with uniformly small cells. Group IV, AF. × 400.

FIG. 15.—Large adenoma showing unusual arrangement of the TSH cells. Group III, PAS. × 400.

FIG. 16.—PAS-positive, AF-negative adenoma of "pale cells" with intensely PAS-stained Golgi bodies, unoperated, 131I treated. Group VI, PAS. × 900.

FIG. 17.—Adenoma with very large AF-positive and PAS-positive anaplastic cells, one showing a large mitotic figure. Group IV, PAS. × 900.

FIG. 18.—Anaplastic part of otherwise typical AF-positive TSH cell adenoma. The cells seen are not densely granulated. Golgi rings visible. Group III, PAS. × 900.
The present experiment with 1 mc of 131I

The thyroid glands of the 3 groups of unoperated rats (VI—VIII) described by Goldberg et al. (14) showed a histologic pattern similar to that of their control group I. The suppression of thyroid activity in Groups VII and VIII by feeding the thyroid-containing diet was to be expected. In all 4 groups, including the control Group I, a certain number of alveolar or lobular thyroid carcinomas were found (24—36%), as previously described in normal Long-Evans rats (14, 20, 21). However, 1 papillary and 1 follicular carcinoma developed in the otherwise intact thyroid glands of rats that received only 1 mc of 131I. These malignant neoplasms were possibly induced by the 131I irradiation. A single papillary carcinoma was found in a subtotal thyroidectomized rat (Group IV) that had been given 1 mc of 131I in addition to the thyroid-containing diet. This neoplasm could have been the result of either prolonged thyrotropic hormone stimulation or 131I irradiation (14).

In the present paper, possible cytologic changes in the pituitaries have been chosen as indicators of thyroxine deficiency caused by inadequacy of thyroid function. Subtotal thyroidectomy in all instances provoked the appearance of such cytologic changes, but it was impossible to decide whether the changes had been aggravated by the administration of 131I (the number of thyrotropic cell adenomas in Groups II and III were not significantly different). On the other hand (Table 3), the absence of thyrotroph hyperplasia in the pituitaries of the irradiated unoperated rats is considered to prove that no thyroxine deficiency was present in these animals. The finding of 2 malignant thyroid neoplasms in rats treated with 1 mc of 131I (Groups VI and VIII) and showing no sign of pituitary thyrotropic stimulation lends support to the view that irradiation per se can be an exciting cause of carcinogenesis in the thyroid gland of the rat.

REFERENCES

6. Doniach, I., and Williams, E. D. Development of Thyroid and Pituitary Tumors in the Rat Two Years after Partial Thyroidectomy. Ibid., 16: 222—31, 1962.
20. La Roche, G., Carpenter, D., and Coxworth, A. Isolation and Estimation of Serum Organically-Bound Iodine. II. Semi Annual Report, Biology and Medicine, Donner Laboratory and Donner Pavilion, Lawrence Radiation Laboratory, UCRL 11857, 1964.
21. Lindsay, S., Sheline, G. E., Potter, G. D., and Chaikoff, I. L.


Formation of Adenomata in Hypophyses of Rats Subjected to both Subtotal Thyroidectomy and Administration of $^{131}$I, and its Prevention by Feeding of Desiccated Thyroid

W. E. Griesbach, I. L. Chaikoff, C. W. Nichols, Jr., et al.

*Cancer Res* 1965;25:1804-1816.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/25/10/1804

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.