Induction of Neoplasms in Thyroid Glands of Rats by Subtotal Thyroidectomy and by the Injection of One Microcurie of I\textsuperscript{131}*

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SUMMARY

Female Long-Evans rats were subjected to: (a) subtotal thyroidectomy, (b) subtotal thyroidectomy plus injection of 1 \( \mu \text{c.} \) of I\textsuperscript{131}, (c) subtotal thyroidectomy plus injection of 1 \( \mu \text{c.} \) of I\textsuperscript{131} plus feeding of a diet containing desiccated thyroid, (d) subtotal thyroidectomy plus feeding of a diet containing desiccated thyroid, (e) injection of 1 \( \mu \text{c.} \) of I\textsuperscript{131}, (f) feeding of a diet containing desiccated thyroid, and (g) injection of 1 \( \mu \text{c.} \) of I\textsuperscript{131} plus feeding of a diet containing desiccated thyroid.

Single and multiple adenomas were found in rats subjected to subtotal thyroidectomy and in those subtotally thyroidectomized and given injections of 1 \( \mu \text{c.} \) of I\textsuperscript{131}. In rats subjected to these same treatments but, in addition, fed the thyroid-containing diet, significantly fewer adenomas were encountered.

Four papillary carcinomas and one follicular carcinoma were found in rats subjected to subtotal thyroidectomy and/or given injections of 1 \( \mu \text{c.} \) of I\textsuperscript{131}. No carcinoma was observed in control rats. Two papillary carcinomas were found in glands following subtotal thyroidectomy alone, a finding suggesting that thyrotropic hormone stimulation may cause the development of both benign and malignant thyroid neoplasms. One papillary and one follicular carcinoma developed in the intact thyroid glands of rats that received only 1 \( \mu \text{c.} \) of I\textsuperscript{131}. These malignant neoplasms were possibly induced solely by the I\textsuperscript{131} irradiation. One papillary carcinoma developed in a rat that had been subjected to subtotal thyroidectomy, given an injection of 1 \( \mu \text{c.} \) of I\textsuperscript{131}, and fed the desiccated thyroid-containing diet. This neoplasm appeared to be the result of either prolonged thyrotropic hormone stimulation or I\textsuperscript{131} irradiation.

In 1951 Goldberg and Chaikoff (10, 11) described benign and malignant thyroid neoplasms in the rat 1.5–2 years after a single injection of a large dose of I\textsuperscript{131}. Doniach (3–6) also produced benign and malignant thyroid tumors in rats treated with I\textsuperscript{131} alone or in combination with methythiouracil and acetylaminofluorene. Interestingly, the highest incidence of thyroid tumors was observed by Lindsay et al. (13, 14) and by Potter et al. (16) in rats that received lower doses of I\textsuperscript{131}—in the range of 25–40 \( \mu \text{c.} \). The failure of other workers (Frantz et al. [9], Field et al. [8]) to induce thyroid carcinomas in Long-Evans rats by injections of I\textsuperscript{131} can be ascribed to their using extremely large doses of I\textsuperscript{131}, which almost completely destroyed the thyroid glands. Thyroid carcinomas were also induced in rats by x-radiation of the glands (Frantz et al. [9], Doniach, [5, 6], Lindsay et al. [15]). Whether prolonged stimulation of benign nodules by thyrotropic hormone caused development of the carcinomas or whether irradiation was the primary cause is still undecided.

Doniach and Williams (7) recently reported that thyroid neoplasms develop in rats after partial thyroidectomy and that these tumors are identical with those observed after irradiation. This finding led us to compare the pathogenesis of thyroid neoplasms induced by: (a) subtotal thyroidectomy, (b) irradiation with I\textsuperscript{131}, and (c) a combination of these two procedures. We have attempted to assess the relative carcinogenic importance of irradiation per se and of prolonged thyrotropic hormone stimulation of the thyroid gland.

MATERIALS AND METHODS

A total of 876 female, Long-Evans rats were randomly selected at ages of 5–6 weeks, divided into eight groups, and treated as shown in Table 1. These rats had been maintained after weaning on Diablo Double Check Laboratory of the following percentage composition: protein, 24.65; fat, 5.18; carbohydrate, 46.50; fiber, 3.95; ash, 9.10; and moisture, 10.62. The diet contained 3 \( \mu \text{g.} \) of iodine per gm.

Subtotal thyroidectomy was performed under light ether anesthesia. Both lateral lobes of the gland were removed, leaving only the isthmus, which weighed ca. 0.1 mg. Each rat given an injection (Table 1) received

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* This work was supported by a contract from the United States Atomic Energy Commission.
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Received for publication June 17, 1963.
intraperitoneally 1 μc. of carrier-free I\(^{131}\) in 0.1 ml. of a 0.9 per cent NaCl solution. The rats that had been subjected to subtotal thyroidectomy received the I\(^{131}\) injection 1 week after the operation. Preliminary experiments had shown that, 24 hours after administration of 1 μc. of I\(^{131}\), the isthmus contained ca. 2 per cent of the radioactivity.

Desiccated thyroid powder (Lilly Thyroid, U.S.P., powder no. 58) was added to the stock diet to yield a concentration of 250 mg/kg. The feeding of this thyroid-supplemented diet was begun 1 week after the injection of I\(^{131}\) or the subtotal thyroidectomy. The uptake of I\(^{131}\) by the thyroid glands of the rats fed this diet was almost completely suppressed.

The rats were maintained for 2 years, and the survivors were killed with ether. The remainder died of chronic respiratory infections, and autopsies were not done on these animals. The thyroid glands of the rat of Groups I, VI, VII, and VIII were excised and weighed. In the other rats the trachea and paratracheal tissues were removed en bloc. All tissues were fixed in 10 per cent neutral formalin. Multiple horizontal blocks were prepared from each specimen of the trachea with attached thyroid tissue and from the separated thyroid lobes, and sections were stained with hematoxylin and eosin. Blocks of other tissues, including cervical lymph nodes and lungs, were sectioned and stained in a similar manner. The pituitary glands were removed, fixed in sublimate-formol (9:1), dehydrated with ethanol, and cleared in cedarwood oil.

**RESULTS**

**GROSS DESCRIPTION**

The rats of Group I (Table 1), which served as controls, had thyroid glands of normal size and weight. Sixty-eight of the rats that had been subtotally thyroidecmtomized (Group II) survived the 2-year period. Varying amounts of lateral thyroid tissue were found in the majority of these rats.

Forty-nine rats of Group III (subjected to subtotal thyroidecmtom and given injections of 1 μc. of I\(^{131}\)) survived the 2-year period. Four rats had a moderately abundant amount of lateral thyroid tissue; 38 showed small lateral thyroid remnants.

Sixty-one rats of Group IV survived for 2 years. The thyroid glands of 56 were of moderate size. Small thyroid remnants were observed in three rats, and in two rats thyroid tissue was not grossly visible.

Among the 41 survivors of Group V, 29 had varying amounts of pale lateral thyroid tissue, and ten showed abundant thyroid tissue. No thyroid tissue was detected in two rats.

The thyroid glands of the rats of Groups VI, VII, and VIII were of normal size, weight, and appearance.

**MICROSCOPIC DESCRIPTION**

**Thyroid Parenchyma**

*Group I (controls).*—The follicles varied in size. The larger peripheral follicles were lined by flattened cuboidal epithelium; the smaller central follicles had slightly larger cuboidal cells.

*Group II (subtotal thyroidectomy).*—The thyroid glands in about half of the rats closely resembled those of the control group. The lobular patterns were normal, suggesting that these lobes represented the original thyroid tissue rather than tissue that had regenerated after subtotal thyroidectomy. In the remainder of the rats, smaller amounts of thyroid tissue were found, either confined to the isthmus or extended into what appeared to be small segments of lateral lobes. In these rats the residual tissue was distinctly hyperplastic (Fig. 1).

*Group III (subtotal thyroidectomy followed by injection 1 μc. of I\(^{131}\)).*—In seventeen rats the appearance of thyroid tissue differed little from the normal. In the remainder the glands were hyperplastic and closely resembled those of the control group. The lobular patterns were normal, suggesting that these lobes represented the original thyroid tissue rather than tissue that had regenerated after subtotal thyroidectomy. In the remainder of the rats, smaller amounts of thyroid tissue were found, either confined to the isthmus or extended into what appeared to be small segments of lateral lobes. In these rats the residual tissue was distinctly hyperplastic (Fig. 1).

*Group IV (subtotally thyroidecmtomized, given injections of 1 μc. of I\(^{131}\) and fed the desiccated thyroid-containing diet).*—The histologic pattern of the thyroid tissue of the majority of these rats was essentially normal. Fewer hyperplastic glands appeared. In some of the glands hyperplasia was
less pronounced than that observed in Groups II and III, and the epithelium was tall or cuboidal rather than columnar.

**Group V (subtotal thyroidectomy and fed the desiccated thyroid-containing diet).**—The glands of most rats had normal follicular patterns; large lateral lobes were present in only two. Five glands were hyperplastic, similar to those observed in Group IV and usually less than those in Groups II and III.

**Group VI (given injections of 1 μc. of I^{131}).**—The histologic pattern of the glands of all rats was identical with that of the control group. The thyroid cells were uniform and did not display Askanazy changes. No evidence of radiation injury was apparent.

**Group VII (fed the desiccated thyroid-containing diet).**—The glands of the majority of rats were normal and identical to those of the control group. In twelve rats, however, the follicles were generally larger than normal and lined by flattened, cuboidal epithelial cells. The colloid was abundant and uniform. These changes suggested suppression of thyroid activity.

**Group VIII (given injections of 1 μc. of I^{131} and fed the desiccated thyroid-containing diet).**—The majority of glands had normal histologic patterns. The pattern was identical with that of Group VII.

**Thyroid Neoplasms**

**Adenomas.**—No adenomas were found in the normal control rats. Single or multiple adenomas were encountered in eight rats of Group II (subtotal thyroidectomy), usually in glands that showed hyperplastic parenchyma. These adenomas consisted of follicles ranging in size from normal to very large and lined by flattened, cuboidal epithelial cells. The colloid was abundant and highly staining. These changes suggested suppression of thyroid activity.

**Alveolar or lobular carcinomas.**—Thirteen of the normal control rats contained alveolar or lobular carcinomas identical to those previously described in normal, Long-Evans rats (Fig. 3). In the glands of the rats subjected to subtotal thyroidectomy (Groups II–V), only three and two follicular adenomas, respectively, were found. The incidence of adenomas in Groups IV and V appeared significantly lower than that in Groups II and III. No adenomas were found in the glands of rats in Groups VI, VII, or VIII.

**Papillary and follicular carcinomas.**—Four papillary carcinomas and one follicular carcinoma were observed in the rats subjected to subtotal thyroidectomy and/or given injections of I^{131}. None was found in control rats or in the glands of rats in Groups III, V, and VII.

In Group II the thyroid tissue of one rat contained a large neoplasm that occupied an entire lobe and consisted of papillary and follicular structures with sparse, pale colloid (Fig. 4). The neoplastic cells had pale, round or oval, pleomorphic nuclei and contained delicate chromatin particles. In a second rat of Group II a similar neoplasm had caused enlargement of a lateral thyroid lobe, and several satellite neoplastic nodules were found outside the thyroid capsule (Fig. 5). The neoplastic cells were arranged mainly in a micro- and macrofollicular pattern, but few papillary structures were also present.

A single papillary carcinoma was found in the gland of a rat of Group IV. Approximately half of a lateral lobe was occupied by a circumscribed but incompletely encapsulated neoplasm that extended through the capsule into the adjacent parenchyma (Fig. 6). The neoplastic cells formed papillary and microfollicular structures that contained sparse, pale colloid.

In the thyroid gland of one rat of Group VI a large, circumscribed, and unencapsulated neoplasm had infiltrated the parenchyma and almost completely replaced a lateral lobe. This neoplasm consisted of sheets of cells arranged in poorly defined microfollicles almost devoid of colloid. The neoplastic cells were oval or round, and their nuclei were pleomorphic and hyperchromatic. The lumens of two large, extracapsular veins were filled by neoplastic epithelial cells (Fig. 7).

In one rat of Group VIII, approximately two-thirds of a lateral lobe was occupied by a circumscribed, unencapsulated, and locally infiltrating epithelial neoplasm. The neoplastic cells had formed papillary and follicular structures filled with deeply staining, uniform colloid. The cells were pleomorphic, with pale, opaque nuclei containing delicate chromatin (Fig. 8).

Metastases were not found in the regional lymph nodes or in the lungs of any of the rats with malignant thyroid neoplasms.

**DISCUSSION**

In most cases subtotal thyroidectomy was followed by compensatory thyroid hyperplasia, presumably the result of increased thyrotropic hormone stimulation. In those instances in which hyperplasia was not observed, abundant thyroid tissue, appearing as lateral lobes, was found at autopsy, indicating either that the lobectomies had been incomplete or that significant quantities of thyroid tissue had regeneraded after the operation.

In most cases the follicular adenomas found in the residual thyroid tissue after subtotal thyroidectomy arose apparently from hyperplastic foci. The histogenesis of these benign follicular adenomas seemed identical to that which has been described in rats subjected to irradiation by I^{131} or x-rays (13-16).

The majority of rats of Group III (subtotal thyroidectomized and given injections of 1 μc. of I^{131}) showed a similar compensatory thyroid hyperplasia. Although neither normal nor hyperplastic thyroid cells showed definite Askanazy changes as a result of the irradiation with I^{131}, presumably some irradiation injury had occurred, since
In our previous studies we concluded that benign follicular adenomas resulted from excessive thyrotropic hormone stimulation of hyperplastic thyroid glandular foci and that at least some of the malignant neoplasms were associated with cytologic evidence of increased thyrotropic hormone activity in the pituitary glands.1

In the rats of Group IV (subtotally thyroidectomized, given injections of I¹³¹, and fed desiccated thyroid) and of Group V (subtotally thyroidectomized and fed desiccated thyroid) the incidence of follicular adenomas was distinctly lower than in rats whose diets did not contain desiccated thyroid (Group III). Also, fewer animals in Groups IV and V showed pituitary cytological evidence of increased thyrotropic hormone secretion.1 In Groups VI and VIII (given injections of 1 μc. of I¹³¹), no Askaniay effects on thyroid epithelial cells that could be ascribed to injury by I¹³¹ were detected. None of the glands in the rats of Groups VI and VIII was hyperplastic, no adenomas developed, and no increased thyrotropic hormone activity was suggested by cytological changes in the pituitary glands.

Malignant thyroid neoplasms (papillary and follicular carcinomas) were found in five rats in the present study. These neoplasms appeared identical with those induced previously in rat thyroids by I¹³¹ or X-radiation (13–16) and with those of corresponding neoplasms in the human being. Such neoplasms were not encountered in our previous studies of old, normal, Long-Evans rats. Since none of the malignant neoplasms in the present study seemed to have arisen in pre-existing, benign thyroid neoplasms, they presumably were malignant from their inception. Only one of the papillary carcinomas arose in a hyperplastic gland.

In our previous studies we concluded that benign follicular adenomas resulted from excessive thyrotropic hormone stimulation of hyperplastic thyroid glandular foci and that at least some of the malignant neoplasms originated in previously benign nodules or adenomas that had been exposed to long-standing thyrotropic hormone stimulation (13). However, the possibility that irradiation was primarily responsible for the malignant changes in the rat thyroid gland could not be ruled out (15).

In the present experiment two papillary carcinomas were found in glands 2 years after subtotal thyroidectomy. This finding is in accord with a previous report indicating that thyrotropic hormone stimulation may cause the development not only of benign thyroid neoplasms but also of malignant papillary carcinomas (7). One papillary and one follicular carcinoma developed in the intact thyroid glands of rats that received 1 μc. of I¹³¹. Since rats treated in this manner did not develop benign thyroid neoplasms and showed no evidence of irradiation injury in their thyroid glands (Askaniay cells) or of increased thyrotropic hormone activity in their pituitary glands, these thyroid neoplasms apparently were induced solely by irradiation. A fifth rat, which had been subtotally thyroidectomized, treated with I¹³¹, and fed desiccated thyroid, also developed a papillary carcinoma—the result presumably of either prolonged thyrotropic hormone stimulation, or I¹³¹ irradiation, or both.

The production of tumors in the rat thyroid by a variety of methods (2) led Hall to postulate the existence of initiating and promoting factors in the development of benign and malignant thyroid neoplasms in the rat (12). It has been concluded in the present study that thyrotropic hormone stimulation is a promoting factor in the development of both benign and malignant thyroid neoplasms in the rat and that it may indeed initiate the development of some malignant neoplasms. It also seems clear that 1 μc. of I¹³¹ alone may initiate the development of malignant thyroid neoplasms. In this case the promoting factor may be normal thyroid growth, as suggested by Doniach (6). The possibility that other initiating and promoting factors exist should not be disregarded because of the consistently high incidence of naturally occurring, low-grade malignant thyroid carcinomas in old, Long-Evans rats.

ACKNOWLEDGMENTS

Mr. Hal Strong assisted in the preparation of the photomicrographs.

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Fig. 1.—Hyperplasia of thyroid tissue following subtotal thyroidectomy. The thyroid follicles are enlarged and lined by columnar epithelial cells that have granular eosinophilic cytoplasm. The colloid is generally pale and sparse. Hematoxylin-eosin (H. & E.) stain, X230.

Fig. 2.—Macrofollicular adenoma in remaining thyroid tissue in a ratsubtotaly thyroidectomised, given an injection of 1 μc. of I¹³¹, and fed a diet containing desiccated thyroid. The residual parenchyma is normal and not hyperplastic. H. & E., X75.

Fig. 3.—Naturally occurring lobular or alveolar carcinoma in control rat. The neoplasm is invading thyroid follicles. H. & E., X125.

Fig. 4.—Papillary carcinoma in thyroid isthmus following subtotal thyroidectomy. The pattern is papillary and micro follicular. Note characteristic pale nuclei. H. & E., X125.
Fig. 5.—Papillary carcinoma in thyroid isthmus following subtotal thyroidectomy. The pattern is mainly follicular, but papillary structures are present. Note invasion of the neoplasm beyond the thyroid capsule. H. & E., X125.

Fig. 6.—Papillary carcinoma in thyroid tissue of a rat subtotally thyroidectomized, given an injection of 1 µc. of I¹³¹, and fed a diet containing desiccated thyroid. The neoplasm is circumscribed but unencapsulated and is invading adjacent thyroid parenchyma. The pattern is mainly microfollicular, but papillary structures are present. H. & E., X125.

Fig. 7.—Follicular carcinoma in thyroid gland of rat given an injection of 1 µc. of I¹³¹. A trabecular pattern predominates, but a few microfollicles containing colloid are present. The cells have small, hyperehromatic nuclei. Note large capsular veins filled with neoplastic tissue. H. & E., X75.

Fig. 8.—Papillary carcinoma in thyroid gland of rat given an injection of 1 µc. of I¹³¹ and fed desiccated thyroid-containing diet. The pattern is follicular and papillary. Note pale, opaque nuclei. H. & E., X125.
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Cancer Res 1964;24:35-43.

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