The prolonged administration of antithyroid compounds provides an experimental means by which the activity of endogenous pituitary thyrotropic hormone may be studied. The mechanism of action of these agents supposedly involves the inhibition of thyroid hormone formation, thus removing the controlling activity of the thyroid hormone upon the thyrotropic hormone of the anterior hypophysis (1, 2, 13, 14, 27, 34, 35).

A number of agents have been found which induce diffuse and nodular hyperplasia of the thyroid gland in rats by this mechanism. This is important because spontaneous thyroid tumors are rare in small laboratory animals. Recently, however, van Dyke found cystic papillary tumors in 56 per cent of his rats provided they were permitted to reach an age of 800 to 900 days. He related the development of spontaneous tumors in old rats to the ultimobranchial tissue and indicated the significance of involuting changes in the thyroid parenchyma adjacent to the tumors (49).

Among the agents which have been reported to induce adenomatoid hyperplasia of the thyroid gland in the rat were brassica seeds (42), thiourea (41, 42), thiouracil (5, 32), and acetyl aminofluorene with allylthiourea (3, 4). It has recently been shown, however, that 2-acetaminofluorene by itself produced thyroid tumors (6). Pulmonary metastases from malignant thyroid tumors have been described after thiourea (42).

In mice of several inbred and hybrid strains, marked hyperplasia of the thyroid glands without nodule formation has been described following prolonged feeding of goitrogens (8, 9, 17). Even though distinct tumor nodules failed to develop in the thyroid glands, thyroid tissue was found within the lung substance by these observers. Apparently species differences exist with respect to tumor formation in the thyroid gland.

The data to be reported summarize observations on the effect of thiouracil upon the thyroid gland of albino male and female rats, a preliminary communication having been published previously (32).

### MATERIAL AND METHODS

A total of 111 albino rats of the Stanford strain, 56 males and 55 females, were used in the experiments. They were placed on the experimental diet at an average body weight of 100 grams, which corresponds to an average age of 45 days for the male and 55 days for the female rat in this colony. The basic diet was ground Purina dog checkers to which thiouracil was added in a concentration of 100 mgs. per cent. The drug was blended with the diet in a mechanical mixer for 30 minutes. The rats were permitted unrestricted access to both food and tap water. Several experimental groups were inoculated intraperitoneally with a culture of the L4 strain of pleura-pneumonia-like organisms. They and the respective control groups were a part of a separate investigation reported elsewhere (48). No essential differences were noted in the response of the thyroid glands and hypophyses between the inoculated thiouracil treated animals and the rats treated with thiouracil alone, so the former were included in this report.

The thyroid and adrenal glands and the gonads were fixed in Bouin's solution, the hypophyses in Zenker's formol after weighing the organs on a torsion balance. Sections were stained with hematoxylin and eosin, and in addition azocarmine aniline blue stain was used on sections of the hypophyses.

Statistical procedures: Preliminary calculations showed that the thyroid weight in relation to the body weight in our rats closely paralleled the data published by Remington and collaborators (45) who used a strain of rats of the same ancestry and on the same diet. Hence, his data on 180 rats were combined with our data on 95 normal rats. From the combined data, the constants for the predicted thyroid weight were calculated according to the equation proposed by Huxley: \( \log y = \log b + k \log x \) (28). For convenience a nomogram was then...
set up from which the per cent deviation of the observed thyroid weight from the predicted thyroid weight was readily obtainable.  

The individual values for the per cent deviations were then subjected to the usual statistical methods and the means of all groups compared with one another (12). The analysis was indicated because a considerable variation in thyroid weight within the same group was apparent.

RESULTS AND DISCUSSION

Weight changes.—Thyroid gland: The weights of the thyroid glands with the respective standard deviations (S.D.) and the same weights calculated as per cent deviations from the predicted weight with the standard deviations are summarized in Table 1.

In males, the mean thyroid weight changed very little between 120 and 261 days of the experimental period, the values for P being consistently greater than 0.05. Hence, the difference between the means was not considered to be significant. However, a significant difference between the mean thyroid weights was found when the rats which had been on thiouracil for 261 and 312 days respectively were compared. The difference was minus 224.07 and the value for P was smaller than 0.01. It indicated that a significant decrease in mean thyroid weight had occurred, and the change in weight was emphasized by structural alterations to be described later.

A progressive increase in thyroid weights was apparent when the data obtained from the female rats were analyzed. Thus, comparison of the means of groups F and H (120 and 155 days) gave a value for P of 0.02, that of groups H and K (155 and 266 days) a value for P of 0.02, the difference between the means being significant in both cases. A group of rats corresponding to the 312 day period of the males was not run, and it is not known if a similar decrease would have resulted with further continuation of feeding.

Hypophysis: The mean weights of the hypophysis in mgs. per 100 gms. of body weight are included in the last column of Table 1. The data were treated by statistical methods as described for the thyroid gland.

Calculating the statistical constants for male rats, the animals in groups D were found to have the largest glands with a mean weight of 6.5 mgs. ± 1.22. Further continuation of feeding (group E) resulted in a decrease in pituitary weight to 5.00 mgs. ± 0.93. Comparison of the means between D and E gave a value for P smaller than 0.01, hence highly significant.

In the females, a progressive increase in mean weight of the hypophysis was apparent, a maximum being reached in the rats which had been on thiouracil longest. The mean of group K when compared with any of the other groups was found to be significantly larger, with values for P of less than 0.01. In addition, there was a significant and progressive increase in mean pituitary weight beginning with group H (155 days), values for P being smaller than 0.05 in the remaining two groups when compared with one another.

Summarizing the observations on the weight changes, an increase in the weight of the thyroid gland and hypophysis was apparent in female rats which was progressive during the period of observation. No significant changes were demonstrable in male rats over corresponding periods of experimentation. A diminution in the weight of both glands was apparent in those male rats which had received thiouracil for an additional period of 50 days.

Microscopic changes.—Thyroid gland: Many of the histologic changes were similar to those which have already been reported in animals after administration of antithyroid compounds. The principal response was a diffuse hyperplasia of the epithelial lining of the follicles accompanied by a nearly complete loss of colloid (Fig. 1). The individual epithelial cells were high columnar and usually possessed basally placed large vesicular nuclei. Hyperemia was severe. The parathyroid glands, usually situated at the lateral margins of the glands, were completely surrounded by thyroid tissue on occasions. The diffuse hyperplasia was nearly uniform in both sexes and in all groups which had received the experimental diet for as long as 261 and 266 days respectively. Thereafter, and coinciding with a decrease in thyroid weight, the degree of epithelial hyperplasia was more variable within the group and even within the same gland. Thus, the height of the epithelial cells was reduced in 8 of the 20 animals which had received thiouracil for 312 days. Patchy accumulation of colloid-like material was noted in the follicles of 11 of the 20 rats, and colloid was uniformly present throughout the glands in 6 more rats (Fig. 2).

In a little more than one third of all the animals, aggregates of tubules were found in the hyperplastic glands (Fig. 3). They were lined by a columnar epithelium which was lower than that lining the follicles in the adjacent portions of the gland. The tubules had definite lumina in which a little granular and homogenous material was seen.

I should like to thank Dr. A. E. Lewis of this Department for his help in many of the calculations and the preparation of the nomogram.

1
The nuclei of the epithelial cells were more compact and smaller than in the remaining gland. Short epithelial buds projecting slightly into the lumen were not infrequent. Groups of tubules traversed the width of the gland occasionally, although they were more frequent at the periphery. No definite relationship between the foci of tubular proliferation and the hyperplastic nodules to be described was evident.

In 37 of the 111 rats, or 33 per cent, areas of nodular hyperplasia were found. The lesions occurred as solitary or multiple nodules, the largest number in a single animal being four. The incidence is summarized in Table 2, in which the data are broken down with respect to sex, the number of rats having nodules, and the total number of nodules in each group. Small collections of acini fairly common. The majority of the nodules as well as the largest, which measured nearly 5 mms. in greatest diameter, had this histologic appearance.

A little different type of nodule which was seen in several instances is illustrated in Fig. 8. Such nodules, which were not encapsulated though well defined from the adjacent thyroid parenchyma, were composed of closely packed epithelial cells with a strongly acidophilic cytoplasm. The nuclei were hyperchromatic and located at the base of the cells, which formed small acini, often with a barely visible lumen. They were separated from one another by greatly distended endothelial lined channels containing blood. The largest of these nodules measured a little more than 2 mms. in greatest diameter.

No obvious explanation has been found for the variability in the appearance of the nodules. It was not unusual to find two different types of lesions in the same gland. Definite evidence of invasiveness was not demonstrated in any of the lesions and they were considered to be benign.

Because a relationship between the development of spontaneous thyroid tumors and ultimo-branchial cysts (49) had been suggested, a search for these structures was made in our material. The ultimo-branchial cyst was found in 14 animals, or 192.6 per cent, and in one of the animals each lobe contained the structure. The incidence is probably not a true one because serial sections were not made in the majority of the glands. However, when the animals bearing nodules in the thyroid were compared with those containing ultimo-branchial cysts, it was found that only 2 rats had both nodules and cysts. In both instances, the cysts were widely separated from the nodules. The remaining 12 rats which had cysts, had no nodules. This suggested that the experimentally produced

TABLE 1
WEIGHTS OF BODY, THYROID GLAND AND HYPOTHYSIS IN ALBINO RATS ON A DIET CONTAINING 0.1 PER CENT THIOURACIL

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Period</th>
<th>No. of</th>
<th>Av. BODY WT.</th>
<th>Thyroid gland</th>
<th>Mo. Hypophysis per 100 GMS. Body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GMS.</td>
<td>weight and S.D.</td>
<td>Mean deviation*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mgs. per cent mgs.</td>
</tr>
<tr>
<td>120</td>
<td>M</td>
<td>6</td>
<td>169.3</td>
<td>65.5 ± 16.15</td>
<td>345.17 ± 117.1</td>
</tr>
<tr>
<td>120</td>
<td>F</td>
<td>7</td>
<td>183.7</td>
<td>55.6 ± 13.33</td>
<td>345.17 ± 117.1</td>
</tr>
<tr>
<td>120</td>
<td>M</td>
<td>10</td>
<td>192.1</td>
<td>55.6 ± 15.07</td>
<td>345.17 ± 117.1</td>
</tr>
<tr>
<td>120</td>
<td>F</td>
<td>13</td>
<td>193.5</td>
<td>55.6 ± 15.07</td>
<td>345.17 ± 117.1</td>
</tr>
</tbody>
</table>
* Mean of per cent deviation of observed weight from predicted weight.
FIG. 1.—Diffuse hyperplasia of thyroid gland after 266 days of feeding thiouracil. Mag. ×220.

FIG. 2.—Accumulation of colloid in thyroid gland after 312 days of feeding thiouracil. Mag. ×220.

FIG. 3.—Area of proliferating tubules in hyperplastic thyroid after 142 days of feeding thiouracil. Mag. ×220.

FIG. 4.—Early focus of nodular hyperplasia after 266 days of feeding of thiouracil. Mag. ×220.
nodular hyperplasia occurring in young rats had little relationship to ultimo-branchial tissue.

Inflammatory reactions in the thyroid gland were fairly frequent but usually involved only the periphery, being most marked in the capsular fibrous tissue on the medial and posterior aspects of the glands. Among the cellular components, polymorphonuclear leucocytes with acidophilic granules, lymphocytes, and plasma cells were seen. No evidence of local vascular disease was found after special staining procedures for elastic and collagenous fibers. Further investigations supported the impression that the inflammatory reaction was not directly related to the effects of the drug because various degrees of inflammation of the tracheal mucosa and esophagus, with extension of the inflammatory reaction have been seen frequently in rats used for other purposes. Collections of lymphocytes such as are commonly seen in the human thyroid gland in Graves’ disease were never encountered.

The significance of the change in granulation of the anterior lobe cells is difficult to interpret. It seems important, however, that the cytologic changes in the hypophysis coincided with the alterations in the weight and cytology of the thyroid gland, suggesting that they had a common underlying mechanism.

Summarizing the morphologic changes in the thyroid glands, diffuse hyperplasia of the thyroid gland was noted in animals which had been on thiouracil for periods varying from 1920 to 9260 days. Thereafter involution of the hyperplastic glands was noted, and it was associated with accumulation of secretion within the lumina of the acini. The thyroid glands from about one-third of all animals contained areas of nodular hyperplasia, and the incidence increased somewhat as the period of feeding was prolonged. Foci of tubular proliferation were noted in a little more than one-third of the animals but no relationship between these foci of tubular hyperplasia and the development of nodules was evident.

The hypophyses contained many thyroidectomy cells and there were only occasional poorly granulated acidophiles between 1920 and 9260 days of the experimental period. Thereafter, well granulated acidophiles occurred in large numbers and granulated basophiles were more frequently seen than thyroidectomy cells.

**TABLE 2**

<table>
<thead>
<tr>
<th>Exper.</th>
<th>Male</th>
<th>No. of Rats</th>
<th>Nodular hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>No. of rats</td>
<td>No. nodules</td>
<td>period</td>
</tr>
<tr>
<td>190</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>169</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>223</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>361</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>312</td>
<td>20</td>
<td>11</td>
<td>19</td>
</tr>
</tbody>
</table>

**FEMALES**

<table>
<thead>
<tr>
<th>Exper.</th>
<th>No. of Rats</th>
<th>Nodular hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>No. of rats</td>
<td>No. nodules</td>
</tr>
<tr>
<td>190</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>142</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>135</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>233</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>256</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

Hypophysis: Little emphasis was placed upon detailed cytologic examination of the anterior lobe cells in the present study. The majority of the glands were prepared for assaying procedures, and the results of this study are being published separately. Those glands which were available for histologic observations showed various stages of transformation of granulated basophiles to thyroidectomy cells and a marked diminution in granulated acidophiles. The changes which have been described by many observers (13, 21, 23, 30, 33, 36) were seen fairly consistently in the hypophyses. The male and female rats which had been on the diet for 261 and 266 days respectively and the males which had thiouracil for 312 days, however, presented a different cytologic picture. Five hypophyses were available in each of these groups. They contained granulated acidophiles. In contrast to the other groups, after 260 days, they were still infrequent and widely separated, and the granules were scant. After 312 days, numerous well granulated acidophiles were present and the number of thyroidectomy cells was smaller, granulated basophiles being now more prominent.

Determinations of thyrotropic hormone which would elucidate quantitative changes in the blood and hypophyses, have not yet yielded uniform results in hypothyroid states. While thyroidectomy is followed usually by an increase in the hormone in serum and hypophyses, the hypothyroidism produced by antithyroid drugs has given more variable results. It seems quite possible that the
FIG. 5.—Border of thyroid nodule resembling adjacent thyroid parenchyma. Condensation of fibrous tissue at margin. 512 days of feeding. Mag. X220.

FIG. 6.—Central portion of nodule shown in Figure 5. Mag. X220.

FIG. 7.—Margin of thyroid nodule after 266 days of feeding thiouracil. Nuclei within nodule more hyperchromatic and epithelial height lower than in adjacent thyroid tissue. Mag. X220.

FIG. 8.—Margin of nodule after 266 days of feeding thiouracil composed of a vascular network and cords and small follicles of epithelial cells. Mag. X220.
LAQUEUR—Hyperplasia of Thyroid Glands and Thiouracil

removal of thyrotropic hormone from the circulation occurs in the presence of the thyroid gland, as has been suggested by Gordon and his collaborators (19). This mechanism does not exist in the thyroidectomized animal and the hormone may appear in detectable amounts in blood and urine.

Whatever the ultimate explanation of the quantitative changes in the production, release, and utilization of the thyrotropic hormone may be, our observations, and those of others, indicate that the thyrotropic hormone reaches the thyroid gland long enough and in such a quantity that active thyroid hyperplasia persists. Thus, our results show that the hyperplasia was maintained for about one-third of the life span of the experimental animal.

Diffuse hyperplasia is the earliest and most uniform response to antithyroid agents. Nodular hyperplasia may develop later in thyroid glands which still show diffuse hyperplasia in the remaining portions. In this respect, the experimentally induced thyroid nodules in young rats differ distinctly from the spontaneous thyroid tumors in old rats where involution of the thyroid parenchyma adjacent to the nodules has been described (49).

The observations on mice in which nodules do not develop in the thyroid gland even after prolonged exposure to these agents (8, 9, 17) are of importance. It seems reasonable that the cause for the development of nodules should be looked for in the thyroid gland itself. Since spontaneous tumors in that gland are rare in young rats, it may be suggested that the activity of the pituitary thyrotropic hormone provides the growth stimulus per se, and that the formation of nodules is one of the responses of which the thyroid is capable.

Although the nature of this particular mechanism is unknown, the focal or irregular type of response after periods of prolonged or, sometimes, exaggerated activity is not limited to the thyroid gland alone, and appears to be related in some way to age. In the hypophysis, for example, the incidence of nodular hyperplasia has been noted to be definitely increased in old rats (25, 40, 46, 51). Prolonged administrations of estrogens to rats, though first producing a diffuse type of cellular hyperplasia, have been found to be followed later by nodule formation in the hypophysis (11, 15, 16, 38, 47, 50, 52, 53). In man, the relation of aging to the incidence of nodular hyperplasia in hypophysis, thyroid glands and adrenals is frequently commented upon (7, 29). It is only possible, at this time, to point to the effects which aging or exaggerated stimulation may have upon the responses seen in various endocrine organs. It seems interesting to speculate on the possibility, however, that the chronic activity of growth stimulating substances upon endocrine organs may result in a state of reactivity in fairly young animals which, under normal circumstances, would have prevailed considerably later. That species differences may influence the responses, in addition, is possible and has been mentioned earlier.

While proliferative lesions in the thyroid gland are an undesirable side effect of the drug, Rawson and his collaborators (36) have shown that, in patients with Graves' disease treated with thiouracil, the hyperplasia of the thyroid gland can be restrained effectively by the simultaneous administration of iodine. Similar observations have been made in animals exposed to the combined activity of thiouracil and iodine for short periods of time (25, 32, 31). Further confirmation of the inhibitory effect of iodine upon thiouracil induced hyperplasia of the thyroid gland was obtained in our rats recently (16) where the animals had been exposed to both drugs for as long as 5 months. That the development of nodules may be prevented by this method is likely, although definite proof awaits continuation of such experiments for even longer periods than have been used thus far.

The cause or causes responsible for the structural and weight changes of the hypophyses and thyroid glands in rats which had been on thiouracil for 312 days were not evident. Attempts to demonstrate the mechanism should be made and include such possibilities as the development of a refractory state, the effect of aging, and exhaustion atrophy.

SUMMARY

The changes in weight and morphology of the thyroid gland and hypophysis following chronic administration of thiouracil in the diet have been described.

Analysis of the data has shown that a progressive increase in weight of both organs was limited to the female rats. There was evidence of involution of the thyroid gland in male rats which had received the diet for 10 months. The regressive changes in the thyroid gland were associated with cytologic alterations in the hypophyses, and these have been discussed briefly.

Diffuse hyperplasia of the thyroid gland was the most uniform response to the drug. In 33 per cent of all rats, definite nodular hyperplasia was found. The histologic structure of the nodules varied, and the same gland harbored nodules of different histologic appearance in several instances. All nodules were regarded as benign lesions.

Tubular proliferation of the epithelial cells lining the acini was seen in 40 per cent of the rats.
Examination of small foci of nodular hyperplasia failed to demonstrate a relationship between the tubules and the nodules, and both changes were seen in the same gland as distinctly separate structures.

Ultimo-branchial tissue was found in 12 per cent of the animals. Early lesions of nodular or tubular hyperplasia did not show a definite association with ultimo-branchial tissue such as has been found in spontaneous tumors of the thyroid gland in the rat.

Factors which may contribute to the development of nodular hyperplasia are discussed briefly in the light of comparable lesions in other endocrine organs and different species.

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Nodular Hyperplasia of Thyroid Glands Induced by Thiouracil

G. L. Laqueur


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