Basophil Adenomata in the Pituitary Glands of 2-year-old Male Long-Evans Rats

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SUMMARY

Pituitaries of male and female Long-Evans rats were examined two years after internal or external thyroid radiation.

Treatment with radioiodine significantly increased the numbers of thyrotropic pituitary cell adenomata in both sexes. No increase in the numbers of such adenomata followed the X-irradiation of the male thyroid glands in the doses used. Gonadotropic cell adenomata were found in 74% of male rat pituitaries. The incidence was not affected by thyroid irradiation. In the female rat pituitaries, the incidence of gonadotropic cell adenomata was only 3%.

On the basis of cytoplasmic granule characteristics, most of the gonadotropic cell adenomata could be homologized with follicle-stimulating hormone cells or with luteinizing hormone cells.

INTRODUCTION

This paper reports observations on the pituitary glands of rats which had been used in experiments concerned with the carcinogenic effects of irradiation of the thyroid gland. The total investigation comprised experiments with 131I administration or local X-irradiation of the thyroid in glands of male and female Long-Evans rats. The effects on the thyroid glands have been reported elsewhere (7, 8). Observations on the pituitary glands of female rats receiving 131I have also been reported (3, 4). This paper deals mainly with the pituitary glands of male rats receiving 131I or local X-irradiation of the thyroid gland. A small group of female rats, the results from which have not been previously reported, are also included.

MATERIALS AND METHODS

The rats were of the Long-Evans strain. Treatment with 131I or X-rays was given at about 8-12 weeks of age. The rats together with the controls were killed two years later.

The pituitary glands were fixed in formal-sublimate, embedded in paraffin, and sectioned (5). The sections were distributed between four slides for each gland. Two slides were stained by the PAS1 method as used by Purves and Griesbach (9), one by Grossmen's (1) modification of the Mallory stain, and one by Gomori's (2) aldehyde-fuchsin without prior oxidation by permanganate.

1Abbreviations: PAS, periodic acid-Schiff; AF, aldehyde-fuchsin; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

RESULTS

Types of Adenoma

Adenomata were classified by cytologic characteristics determined by staining reactions. Three groups of adenomata were recognized: thyrotropic, gonadotropic, and chromophobe. Thyrotropic adenomata were composed of cells with PAS-positive, AF-positive granules. These are the characteristics of thyrotropic cell granules. Gonadotropic adenomata were composed of cells with PAS-positive, AF-negative cytoplasmic granules similar to those of normal gonadotrophs. Chromophobe adenomata were composed of cells with no significant amount of granulation. No frankly acidophil tumors were seen.

Gonadotropic adenomata were subdivided into three types. These were:

Gonadotropic Type I. The cytoplasm after PAS staining is coarsely granular or flocculent in appearance, probably from aggregation of submicroscopic granules (Figs. 3, 5, 10). This appearance is typical of normal FSH cells (10).

Gonadotropic Type II. The cytoplasm after PAS staining presents a more uniform appearance probably resulting from a more uniform dispersion of the granules throughout the cytoplasm (Figs. 4, 6, 8, 9). This appearance is typical of normal LH cells (10).

Gonadotropic Type III. In these adenomata, the PAS reaction is confined to a small area adjacent to the nucleus. From comparison with the other types of gonadotropic tumor in which the PAS reaction in the Golgi region is often much stronger than elsewhere, it is considered that the PAS-positive material in these cells is located in the Golgi region. The nature of the specimens did not permit the demonstration of the Golgi apparatus for which a special fixation is necessary. The cells of these adenomata were...
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did not have any specific character which could be used to relate them to either FSH cells or LH cells.

**Description of Adenomata**

**Thyrotropic Adenomata.** The cells of these adenomata were irregular in shape with multangular outlines. The depth of staining by AF varied greatly from cell to cell. There was a tendency towards darker staining at the cell periphery giving a characteristic hard outline to the cell. These appearances are characteristic of normal thyrotrophs. Very large cells with large nuclei were sometimes present. Mitoses were occasionally seen, usually in large pale cells.

The thyrotropic adenomata were composed of a mass of cells without organized structure. Basement membranes stained by PAS were notably absent and blood vessels were few or absent. The adenomata were always small in this series, less than 0.5 mm in diameter and without any capsule or signs of compression of surrounding tissue. They were found in the interior of the pars anterior lobe, most frequently in the caudal half of the gland.

**Gonadotropic Adenomata.** The cells of this group of adenomata were more uniform in size than those of thyrotrophic adenomata and more regular in shape, being typically round or oval in outline. The size of the cells tended to be uniform within any one tumor but the average size varied among the tumors. The degree of granulation and hence depth of staining by PAS was typically uniform throughout the tumor. Within the cells the tendency was for deepest staining to be present in the interior of the Golgi region and in Type III tumors, only the interior of the Golgi region was PAS-positive. Negative images of the Golgi body were commonly seen in Type I and II adenomata (Figs. 5, 6).

Gonadotropic adenomata uniformly showed a regular organization into acini. These were composed of solid masses of cells without central lumen, round in cross-section and completely enclosed in strongly PAS-positive basement membranes (Figs. 3, 4). This organization is not normally found in the pars anterior of the rat although common enough in other species. The prominence of the basement membranes and the complete enclosure by them of spheroid cell masses is so constant a feature of these adenomata and so distinctive as to be diagnostic of this group. In the thyrotropic and chromophobe tumors, PAS-positive basement membranes are limited to the immediate vicinity of blood vessels and are therefore less developed than in the normal pars anterior. In the normal pars anterior, basement membranes radiate from the vicinity of blood vessels and subdivide the tissue into prismatic strands without enclosing groups of cells into isolated masses. Gonadotropic tumors are well vascularized with vessels of normal appearance.

Gonadotropic adenomata were always small, less than 0.8 mm in maximum diameter. In the larger series of animals, reported elsewhere, an occasional large adenoma of the gonadotropic Type III has been found occupying most of one side of the pars anterior (3).

There is a difference in the distribution of Type I gonadotropic and Type II gonadotropic tumors (Figs. 1, 2). Type I tumors were found almost exclusively in the most anterior part of the pars anterior on either side of the stalk in a region where the portal vessels enter the pars anterior. This is a region rich in FSH cells (Figs. 3, 5). Type II adenomata were found further back, often in close proximity to the pars intermedia (Fig. 7) but also quite frequently in a more lateral position. These were therefore somewhat anterior to the site of maximum frequency of thyrotropic adenomata.

Very often adenomata of Types I and II were found in the same side of a pituitary gland. In these cases the Type I tumor was always anterior to the Type II tumor (Figs. 1, 11). Frequently, the two adenomata coalesced at their maximum diameters and formed a collision tumor in which Type I cells were anterior and Type II cells posterior (Fig. 12). The appearances could only be explained by the fusion of two growths arising independently. There was no suggestion of a single growth differentiating into Type I and Type II cells in different areas.

Capsule formation was not observed but some signs of displacement and compression of surrounding tissue were observed. Type III adenomata were infrequent in this series. They occurred in the same region as the Type II adenomata.

**Chromophobe adenomata.** These were composed of rounded cells of uniform appearance with relatively scanty cytoplasm which did not stain with any conventional stain. The nuclei were uniform and similar in size to those of normal anterior lobe cells. Mitoses were more frequent in basophil adenomata but were not numerous.

There were no basement membranes or connective tissue in chromophobe adenomata except in the immediate vicinity of blood vessels. The tissue either formed a uniform mass of cells with widely dilated blood vessels or was arranged in wide columns which enclosed sinuses, sometimes blood-filled, sometimes empty. In areas where blood-filled sinuses were present, there were usually macrophages containing large inclusions which were brown in unstained sections. These inclusions were strongly PAS-positive in PAS-stained sections. Their presence was considered to indicate that hemorrhages and cell necrosis were occurring in the tumor. Chromophobe tumors were usually larger than the other varieties, sometimes occupying half the anterior lobe. The surrounding tissue showed signs of compression, but there was no capsule formation.

**Incidence of Tumors**

Frequency of occurrence of the various types of adenoma are shown in Table 1. The significance of difference in incidence was tested by the chi square test.

**Thyrotropic Adenomata.** The incidence of this type of adenoma was the same in male and female rats. Treatment with radioactive iodine increased the incidence of this type of tumor in both sexes. The increase is not statistically significant for either sex alone but is significant ($P < 0.02$) for both sexes combined. Thyroid irradiation by X-rays did not increase the incidence of this type of adenoma in male rats. The difference between male rats irradiated by X-rays and those receiving radioactive iodine is highly significant ($P < 0.01$).

**Gonadotropic Adenomata.** The high incidence of gonadotropic adenoma in male rats was an unexpected finding. Of 144 male rat pituitaries examined, there were only 39 (26%) in which a gonadotropic adenoma of one type or other was not found. This is in striking contrast with the female rats in which only two adenomata of this class were found in 78 animals.
The difference between the sexes is in the high incidence of Type I and Type II which are seen only very occasionally in female glands in this and other series (5) using the same strain of rat. During the examination of the sections, an impression was gained that there was a pronounced tendency for both Type I and Type II tumors to occur in association. By the chi square test this association is highly significant \( (P < 0.01) \). Irradiation either by \( ^{131} \text{I} \) or by X-rays did not affect the incidence of this class of tumor.

**Chromophobe Adenomata.** These were quite common. There was no difference between the two sexes either as controls or after radiiodine treatment. Radioiodine treatment but not X-ray irradiation increased the incidence of chromophobe adenomata. The difference for both sexes combined is highly significant \( (P < 0.01) \).

**DISCUSSION**

**Classification of Adenomata.** It will be seen that the thyrotropic adenomata are composed of angular cells without basement membranes. On the other hand, those of the gonadotropic class are composed of rounded cells which are organized into follicular or acinar structures delineated by basement membranes. These characteristics are correlated with the staining responses of the cytoplasmic granules and substantiate the fundamental character of the classification by granule characteristics.

Impairment of thyroid efficiency as a result of internal irradiation of the thyroid by \( ^{131} \text{I} \) increased the incidence of thyrotropic adenomata without altering the incidence of gonadotropic adenomata. On the other hand, there was a marked sex difference in the incidence of gonadotropic adenomata but not in that of thyrotropic adenomata. These results show that the classification of the adenoma by the staining reactions of the granules is of functional significance.

Chromophobe adenomata do not in general show indications of functional properties or specific origin. Sometimes these spontaneously arising adenomas are associated with milk secretion in the mammary glands and acidophil granules may be present especially in the cells at the periphery of the growth. Although none of the animals in this series showed these characteristics, it is probable that some at least of these adenomata originate from lactogenic-hormone-secreting cells. Estrogen administration which stimulates prolactin secretion in rats causes a high incidence of chromophobe tumors. In this series administration of \( ^{131} \text{I} \) caused a significant increase in the incidence of chromophobe adenomata. The mechanism of this effect is obscure. Subtotal thyroidectomy was found to decrease the incidence of chromophobe tumors in this strain of rat as reported elsewhere (3). It is possible that direct radiation effects on the pituitary gland or on tissues other than thyroid are involved.

In these experiments, direct irradiation of thyroid lobes by X-rays did not increase the incidence of thyrotropic adenomata. In another investigation, using female rats (4), an increased incidence of such adenomata was recorded but this was not statistically significant. Presumably, unilateral irradiation does not decrease thyroid efficiency sufficiently to cause a hormonal imbalance of a degree which would be revealed in studies of tumor incidence.

Some of the basophil adenomata observed in these studies are composed of cells which, to microscopic examination, are not distinguishable from normal basophils. Each adenoma consists of a clone of cells produced by a focal hyperplasia. Since normal adenohypophysial cells show only a very limited ability to multiply as evidenced by the lack of regeneration of adenohypophysial tissue from fragments left in situ after hypophysectomy, the growth of these clones is considered to be itself an expression of abnormality. For this reason, we call these clones tumoral. In another investigation, using female rats (4), an increased incidence of such adenoma was recorded but this was not statistically significant. Presumably, unilateral irradiation does not decrease thyroid efficiency sufficiently to cause a hormonal imbalance of a degree which would be revealed in studies of tumor incidence.

Type I gonadotropic adenomata could, however, be regarded as focal hyperplasias of FSH basophils and Type II gonadotropic adenomata as focal hyperplasias of LH basophils. The existence of these two distinctive types and the correlation of their distribution in the gland with that of the two normal gonadotropic

**TABLE 1**

**Pituitary Adenomas in Rats Treated with \( ^{131} \text{I} \) Injections or X-irradiations of the Thyroid Gland**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Sex</th>
<th>Treatment</th>
<th>No. of pituitaries examined</th>
<th>Pituitaries with adenoma</th>
<th>No. of pituitaries of each type of adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gonadotropic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>Controls</td>
<td>31</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>2a</td>
<td>M</td>
<td>( 25 \mu \text{c} {^{131\text{I}}} )</td>
<td>48</td>
<td>44</td>
<td>24</td>
</tr>
<tr>
<td>2b</td>
<td>M</td>
<td>( 4 \times 10 \mu \text{c} {^{131\text{I}}} )</td>
<td>65</td>
<td>56</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>X-irradiation, ( 500-1000 ) R to both lobes of thyroid; ( 1000 ) R to one lobe of thyroid</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Controls</td>
<td>30</td>
<td>13</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>( 3 \times 10 \mu \text{c} {^{131\text{I}}} )</td>
<td>48</td>
<td>30</td>
<td>63</td>
</tr>
</tbody>
</table>
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cell types would show that these adenomata derive from one or other of the two gonadotropic cell types. The basophil adenomata are always small, and their presence cannot be ascertained before sectioning. This precludes a demonstration of the hormonal content of the two distinctive gonadotropic types of adenoma by bioassay. They would provide favorable material for studies using fluorescent antibody technics. The high incidence of gonadotropic adenomata, especially of Types I and II in male rats of the Long-Evans strain, was unexpected but is not a peculiarity of this strain. In our own material of Wistar rats, 18-24 months old, gonadotropic cell adenomata were found in 8 pituitaries of 23 male rats, while 112 female rats showed no such adenomata. The sex difference in the incidence of gonadotropic adenomata is therefore not a peculiarity of the Long-Evans strain.

The sex difference may be a consequence of the continuous activity of FSH and LH cells in the male in contrast to the cyclic activity of these cells in the mature female. After gonadectomy, when there is continuous secretory activity in the gonadotrophs, both sexes show an equal high incidence of gonadotropic adenomata (5).

ACKNOWLEDGMENTS

I am indebted to Dr. H. D. Purves for revision and rewriting of this paper.

REFERENCES


Figs. 1-12. Stained with periodic acid-Schiff and hematoxylin (Lillie).

Fig. 1. Double adenoma in anterior pituitary. The darker stained adenoma (P) at the periphery contains coarsely granulated Type I cells, the lighter one in the center (C) finely granulated cells of Type II. Pars intermedia visible above. Radioiodine treatment. 2.5 μ. X 60.

Fig. 2. Area between the two adenomas of Fig. 1, showing some mixing of both cell types, but mainly finely granulated (Type II) ones. 2.5 μ. X 150

Fig. 3. Coarsely granulated cell of peripheral adenoma of Fig. 1. 2.5 μ. X 400.

Fig. 4. Finely granulated cells of central adenoma of Fig. 1. 2.5 μ. X 400.

Fig. 5. Higher magnification of Fig. 3. 2.5 μ. X 900.

Fig. 6. Higher magnification of Fig. 4. 2.5 μ. X 900.

Fig. 7. Double adenoma in control pituitary (C, C). Both adenomata contain finely granulated cells. One lies in the “sex zone.” Parts of the hypophysial cleft and of the pars intermedia are seen at extreme right. 5.0 μ. X 90.

Fig. 8. Type II cells of one of the adenomas of Fig. 7. 5.0 μ. X 400.

Fig. 9. High magnification of the other adenoma of Fig. 7. Finely granulated cells, showing the coarse granules, typical for old cells of Type II. 2.5 μ. X 900.

Fig. 10. Coarsely granulated Type I cells from the periphery of another rat's pituitary (degranulating?). 2.5 μ. X 900.

Fig. 11. Double adenoma of rat pituitary after X-irradiation of thyroid gland. P is a Type I adenoma; C a Type II adenoma. 5.0 μ. X 90.

Fig. 12. Coalescent double adenoma. In earlier sections, two independent adenomas each with one gonadotroph cell type only could be seen. 5.0 μ. X 400.
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