Amphophil Tumors of the Hypophysis
Induced in Mice by $^{131}$I

AGNES S. BURT,† BENJAMIN H. LANDING,‡ AND SHELDON C. SOMMERS§

(Departments of Pathology, Massachusetts General Hospital, Children’s Medical Center, and Harvard Medical School; the Cancer Research Institute, New England Deaconess Hospital, and the Children’s Cancer Research Foundation, Boston, Mass.)

It is often implied that chromophobe adenomas of the hypophysis are without significant endocrine function (11). However, some hypophysial tumors experimentally produced in mice by destroying their thyroids with $^{131}$I have been described as functioning chromophobe adenomas (9, 10).

A review of the literature on the effects of thyroid deficiency in laboratory animals reveals wide discrepancies in the hypophysial changes reported. Most workers agree that the hypophysis becomes hyperplastic and increases in weight and that there is a marked reduction in the relative percentage of acidophils. However, the hyperplastic cells are regarded as “basophils” by some workers and as “chromophobes” by others. Some of these reports are summarized in Table 1, which also shows that a wide variety of fixatives and stains has been used in arriving at the conclusions given.

In a recent report of a case of human thyroid aplasia (8), it was suggested that the hyperplastic weakly Schiff positive cells (“sparingly granulated basophils”), which made up a large part of the hypophysis of the patient, might be analogous to cells of the “chromophobe adenomas” which have been induced in mice by destroying the thyroid with radioactive iodine. In view of the different physiological roles commonly ascribed to “basophils” and “chromophobes” in the hypophysis, an investigation of the effects of fixation and staining on the histology of the adenomas which can be induced in mice by $^{131}$I seemed desirable.

MATERIALS AND METHODS

A total of 125 C57 mice, both males and females between 6 and 10 weeks of age, were treated with one dose of 300 $\mu$C $^{131}$I in 0.5 ml of aqueous solution injected subcutaneously. They were maintained on Purina Laboratory Chow, which contains 0.005 per cent potassium iodide according to the supplier’s analysis. The animals were observed frequently for abnormal behavior and were sacrificed at intervals from 185 to 441 days following the administration of radioactive iodine.

Complete autopsies were performed and tissues prepared for microscopic examination. Some pituitary tumors were used for transplantation experiments. A total of 38 hypophyses from C57 mice, 24 male and fourteen female, were used in the histological studies reported here. These hypophyses were fixed variously in 10 per cent neutral formalin, acetone, Zenker formol, Zenker acetic, Bouin’s fluid, formol-sublimate, or were frozen-dried. Sections fixed by these methods were stained with hematoxylin and eosin, Mallory’s aniline blue technic, the periodic acid Schiff reaction with an Orange G counterstain (27), aldehyde fuchsin (14), and phosphotungstic acid hematoxylin.

RESULTS

Gross pathologic changes.—Gross enlargement of the hypophysis was evident in some animals sacrificed 249 days after the injection of $^{131}$I. A frank hypophysial tumor was discovered at 284 days. The macroscopic appearance of the hypophysis varied, however. A few animals sacrificed over a year after administration of radioactive iodine had no gross tumors. The size of those tumors found ranged from 85 to 205 mg. The normal hypophysial weight in this strain is approximately 2 mg.
Histological changes.—All hypophyses examined showed an obvious reduction in the relative percentage of acidophils present. However, even in those glands which had been transformed into frank tumors it was possible to find very rare acidophilic cells scattered throughout the hyperplastic areas (Fig. 1). Sections of the neck region of most of these animals showed that the thyroid had been entirely replaced by scar tissue. In about one-third of the mice some regenerated thyroid cells were present. Adjacent vessels had hyaline thickening of the type characteristic of radiation reaction.

The earliest glands examined contained two types of cells which could be stained as “basophils” by the various technics used. One of these was a small, densely granulated cell which did not appear to increase in numbers as the tumors developed. Instead, rare cells of this character were found scattered throughout the hyperplastic cells comprising the adenomas (Fig. 2). Morphologically, this type of cell resembled the “gonadotrophic basophil” of the rat (30) and the “normal basophil” (25) or “maximal mucoid cell” (28) of man (Fig. 3).

The second type of “basophil” cell was usually much larger and had an indistinct, often irregular cell outline. The nucleus was very large, ovoid, vesicular, and often contained one or more prominent nucleoli. After proper fixation, faint but unmistakable granules could be seen scattered through the abundant cytoplasm (Fig. 4). Even in the earliest glands examined, this type of cell was greatly increased in numbers as compared with a normal mouse hypophysis. As tumors developed, they were seen to consist almost entirely of this type of cell. Mitotic figures were abundant (Fig. 6). Morphologically, this hyperplastic cell

### TABLE 1

**Histological Changes in the Adenohypophysis Following Experimental Production of Thyroid Deficiency in Animals**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Animal</th>
<th>Surgical thyroidectomy</th>
<th>Fixation*</th>
<th>Stain*</th>
<th>Histological change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43 ca rabbits</td>
<td>Surgical thyroidectomy</td>
<td>Regaud's, ZF</td>
<td>MAB, acid fuchsin Me green</td>
<td>Hyperplasia and hypertrophy of chromophobes</td>
</tr>
<tr>
<td>55</td>
<td>43 ca Long-Evans rats</td>
<td>Surgical thyroidectomy</td>
<td>Champy's, osmic acid</td>
<td>MAB</td>
<td>Increased basophils</td>
</tr>
<tr>
<td>56</td>
<td>43 ca, 4 white rats</td>
<td>Surgical thyroidectomy</td>
<td>Helly's</td>
<td>MAB</td>
<td>Increased cells with blue cytoplasm and pale granules</td>
</tr>
<tr>
<td>59</td>
<td>43 ca, 4 Wistar rats</td>
<td>Surgical thyroidectomy</td>
<td>Formalin</td>
<td>MAB</td>
<td>Increased basophils</td>
</tr>
<tr>
<td>19</td>
<td>43 ca Sprague-Dawley rats</td>
<td>Surgical thyroidectomy</td>
<td>Susa, Bouin's</td>
<td>AF</td>
<td>Increased AF-basophils</td>
</tr>
<tr>
<td>31</td>
<td>Rats</td>
<td>Surgical thyroidectomy</td>
<td>Formalin</td>
<td>PAS</td>
<td>“Thyrotropic basophils” in normal rats are PAS+, AF+. After thyroidectomy are AF— and weakly PAS+</td>
</tr>
<tr>
<td>23</td>
<td>Rats</td>
<td>Sulfon-guanidine</td>
<td>ZF</td>
<td>MAB</td>
<td>Increased in vacuolated, hyalinized basophils</td>
</tr>
<tr>
<td>7, 8</td>
<td>43 ca, 4 CSH mice</td>
<td>Thiourea, thiouracil</td>
<td>Champy's, osmic acid</td>
<td>none</td>
<td>Adenomatous hyperplasia of β-cells, changes in Golgi network</td>
</tr>
<tr>
<td>34</td>
<td>Wistar rats†</td>
<td>PABA, Selenium dioxide, 2-aminothiazole</td>
<td>Formalin†</td>
<td>H &amp; E</td>
<td>Increased chromophobes or degranulated chromophils; chromophobe adenomas</td>
</tr>
<tr>
<td>30</td>
<td>Rats</td>
<td>Methyl thiouracil, Surgical thyroidectomy</td>
<td>Formalin</td>
<td>PAS</td>
<td>Increased weakly PAS+ basophils</td>
</tr>
<tr>
<td>38</td>
<td>43 ca A strain mice</td>
<td>Propyl thiouracil</td>
<td>Helly's†</td>
<td>MAB†</td>
<td>Chromophobe adenomas</td>
</tr>
<tr>
<td>15, 16</td>
<td>C57, A, I, CSH mice†</td>
<td>Helly's†</td>
<td>MAB†</td>
<td>Chromophobe adenomas</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>43 ca Swiss mice</td>
<td>Surgical thyroidectomy</td>
<td>Formalin</td>
<td>MAB</td>
<td>Hypertrophy and hyperplasia of basophils</td>
</tr>
<tr>
<td>18</td>
<td>43 ca mongrel dogs</td>
<td>Surgical thyroidectomy</td>
<td>Formalin</td>
<td>PAS</td>
<td>Hypertrophy and hyperplasia of, cells which are aniline blue+AF—, PAS—</td>
</tr>
<tr>
<td>10</td>
<td>43 ca CSH mice</td>
<td>Surgical thyroidectomy</td>
<td>Formalin</td>
<td>MAB</td>
<td>Chromophobe adenomas</td>
</tr>
</tbody>
</table>

*AF = aldehyde fuchsin
Formalin = formol-sublimate
H & E = Hematoxylin and eosin
MAB = Mallory-aniline blue
PAS = Periodic Acid Schiff
ZF = Zenker Formal
†Personal communication from the authors.
closely resembled the “thyrotrophic basophil” of the rat (30) and the “sparsely granulated basophil” (25) or the “intermediate mucoid cell” (28) of man (Fig. 5).

As hyperplasia increased, these cells occasionally became vacuolated and lost most or all of their granules. The nuclei sometimes increased in size, often to such dimensions that they suggested polyploidy. The nucleoli also became enlarged, often nearly filling the nucleus (Fig. 7). Cells which have undergone this change have been described as “thyroidectomy cells” in animals and bear a striking resemblance to the “hypertrophic amphophils” reported in human adrenal hyperactivity (25), in women with carcinoma of the breast (9), and in human thyroid aplasia (3) (Fig. 8).

The hyperplastic cells which made up the adenomas could be made to take on staining characteristics of either “basophils” or “acidophils,” depending on the fixation used. With Zenker formol, Bouin’s, or formol-sublimate they were Schiff positive. However, the Schiff reaction was weak or negative after acetone or Zenker acetic fixation or after freeze-drying. After pure 10 per cent formalin the Schiff reaction was very weak, but it was strongly positive if electrolytes such as HCl or HgCl₂ were added to the fixing solution. These cells were also strongly positive to aldehyde fuchsin after Zenker formol or formol fixation; the reaction was somewhat weaker after acetone, Zenker acetic, or formol-sublimate; and it was negative after freeze-drying. The tumor cells stained shades of lavender with hematoxylin and eosin.

The adenomatous cells gave a mixed staining reaction with Mallory technic, usually containing both blue and red cytoplasmic granules. Blue granules predominated after Zenker formol and formol-sublimate fixation, red granules after Bouin’s fluid, Zenker acetic, acetone, or freeze-drying. After Zenker acetic, Bouin’s, formol-sublimate, or freeze-drying they could also be stained with phosphotungstic acid hematoxylin, although less intensely than the true acidophils. Weak or negative Schiff reactions obtained after improper fixation, and the mixed character of the staining with Mallory technic or hematoxylin and eosin could easily lead to the identification of these tumors as “chromophobe” by workers to whom “chromophil” means a densely granulated, brightly staining cell.

### TABLE 2

<table>
<thead>
<tr>
<th>Fixative</th>
<th>PAS</th>
<th>AF</th>
<th>PTAH</th>
<th>MAB</th>
<th>H &amp; E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen-dried</td>
<td>+</td>
<td>0</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zenker acetic</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Formol</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bouin’s</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Formol sub.</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zenker form.</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Abbreviations:**

- Bas = densely granulated basophil.
- SGB = “sparsely granulated basophil” or “amphophil.”
- Ac = acidophil.
- PTAH = phosphotungstic acid hematoxylin.

Other abbreviations as in Table 1.

True chromophobes (using this term to denote cells in which no granules could be demonstrated by any of our technics and in which the nuclei were small or of moderate size) were rare in all but the earliest glands.

**Effects of fixation on staining reactions.**—The results of the experiments on the effects of fixation on the staining of the chromophil granules are summarized in Table 2. In general, the densely granulated basophils were both Schiff- and aldehyde fuchsin-positive with all fixatives. The weakest reactions were obtained after acetone fixation and with freeze-drying. These cells stained various shades of blue with the Mallory technic and purple with hematoxylin and eosin. They did not stain with phosphotungstic acid hematoxylin after any of the fixatives tried.

The acidophils, in contrast, were Schiff- and aldehyde fuchsin-negative. They stained bright red with Mallory technic, orange with Orange G, and pink with eosin. They took phosphotungstic acid hematoxylin (PTAH), except after acetone fixation. The greatest affinity for PTAH was noted after Zenker acetic or formol-sublimate fixation or after freeze-drying.

DISCUSSION

The possible functional significance of these hypophysal changes will be discussed in a later

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*Note: The table and text are formatted to match the original layout of the document, including page numbers and section titles.*
paper in which other tissues, including endocrine organs, of these mice will be described. However, the close morphological resemblance between the adenomatosus cells in these animals and the hyperplastic cells in the human case of thyroid aplasia, as shown in Figures 4-8, seems worthy of note. It suggests that the hypophyseal “sparsely granulated basophils” and “hypertrophic amphophils” described by Mellgren (25) as characteristically increased in some types of human adrenal hyperactivity may actually have much broader endocrinological implications.

The physico-chemical reactions which take place in tissues exposed to the conventional histological fixatives are exceedingly complex, so that the interpretation of these results in precise chemical terms is not possible at present. The Schiff positivity of the basophilic cells has been interpreted as a test for glycoprotein (27, 28), although there is evidence that the Schiff reagent will react with a variety of other substances which either contain aldehyde groups or from which aldehydes can be liberated by mild oxidation (21). It is perhaps significant that the Schiff reaction was most strongly positive after those fixatives which are themselves oxidizing agents or which require treatment of sections with Lugol’s solution. The exception to this was the weak reaction after Zenker acetic, which seemed to promote the staining of the acidophil elements at the expense of the basophils.

Aldehyde fuchsin, as has been shown previously (30, 33), is a somewhat less selective stain than the Schiff reagent, and was sometimes positive when the Schiff reaction was not. It has been suggested that the basis of this difference is that aldehyde fuchsin will react with strong sulfur acids as well as with aldehyde groups, whereas the Schiff reagent will not (33). However, since some orthochromatic tissues which are Schiff-negative are aldehyde fuchsin-positive, this conclusion is as yet in doubt (20).

Phosphotungstic acid hematoxylin appears to stain strongly basic protein groups in the nuclear chromatin and in the acidophil granules. It seems noteworthy that after those fixatives which produced the strongest affinity for PTAH in the acidophils, the cells of the induced hypophyseal adenomas, which were closely related to basophils by other staining methods, could be stained like acidophils by PTAH.

This amphiphilic character of the adenoma cells was perhaps best shown by their variable reaction to Mallory aniline blue. In this technic, the aniline blue seems to stain glycoprotein or aldehyde-containing substances, while the fuchsin stains basic proteins (32). Our experiments showed that the adenoma cells could be stained so as to have predominantly red, blue, or mixed granulation, depending upon the fixative used. Other workers (24, 32) have found that Mallory stained cells will vary in color with the same fixative, depending on pH of the staining solution and the method of differentiation used. This fact would seem to account for many of the discrepancies reported by individuals investigating the effects of thyroid deficiency on the adenohypophysis by means of the Mallory technic.

This does not, of course, explain the negative aldehyde fuchsin reaction reported in the hyperplastic cells of the rat hypophysis following thyroidectomy (31) or the negative Schiff and aldehyde fuchsin staining in dogs which had received radioactive iodine (19). Here, however, the functional state of the cells must be taken into consideration. In rats studied soon after thyroidectomy, the cytoplasmic granules may be so fine and sparse as to be easily missed on microscopic study, whereas later large granules may accumulate (30). In our mice, no granules of any description could be demonstrated microscopically in some of the hypertrophic, vacuolated thyroidectomy cells, although the existence of intermediate forms showed them to be derived from granulated cells which could be stained with the Schiff reagent, aldehyde fuchsin, and aniline blue.

As recently pointed out by Pearse (38), the terminology used for hypophyseal cell types is very confused. The findings reported here would suggest that the clear distinction traditionally made between hypophyseal “acidophils” and “basophils” has been exaggerated. The hypophysial adenoma cells developing after radiothyroidectomy form an intermediate category, having predominantly “basophilic” characteristics but possessing some of the staining properties of “acidophils” also. Since the word “chromophobe” carries with it a connotation of physiological inactivity which does not seem to be warranted in these tumors, it would appear desirable to classify them in some other fashion. Among the variety of terms available for the radiothyroidectomy-induced hypophyseal tumors, use of the designation “amphophil adenoma” would seem at present to be most nearly accurate.

The vesicular nuclei and the large nucleoli found in these amphophilic cells are said to be characteristic of active protein synthesis (4). The sparse cytoplasmic granulation suggests rapid discharge rather than storage of protein material. “Chromophobe adenomas” have been found to occur more frequently in patients dying of tumors.
than in those dying of other causes (5). They have been reported in rabbits with spontaneous tumors of the mammary glands (17) and endometrium (18), in mice with estrogen-induced mammary tumors (6), and in rats in which multiple tumors had been induced by growth hormone (22). Hypertrophic amphiphils appear to be increased in women with carcinoma of the breast, although hypophyseal adenomas may or may not be present (9). It seems possible that the sparsely granulated amphiphilic family of cells, through the production of some types of trophic hormone, may play a more important role in the endocrine imbalances of tumor formation than has been generally recognized.

SUMMARY

1. Histological studies of the hypophyses of 88 C57 mice suggest that the hypophyseal adenomas induced by large doses of I\(^{131}\) are derived from cells which are morphologically similar to the "thyrotrrophic basophils" of rats and to the "sparsely granulated basophils" or "intermediate mucoid cells" of man. The "thyroidectomy cells" found in the adenomas bear a strong resemblance to the "hypertrophic amphiphils" previously reported in some types of human adrenal hyperactivity and in women with carcinoma of the breast.

2. By use of suitable fixation and staining methods, the adenoma cells can be shown to resemble not only the hypophyseal "basophils" with affinities for Schiff reagent, aldehyde fuchsin, and aniline blue, but also the "acidophils" with affinities for phosphotungstic acid hematoxylin and eosin. If stained with Mallory aniline blue under proper conditions or with hematoxylin and eosin, they sometimes resemble "chromophobes." It is suggested that these tumors be called "amphiphil adenomas."

3. On purely morphological grounds, the amphiphils appear to be actively secreting cells. It is suggested that the adenomas frequently reported in the hypophyses of tumor-bearing animals and men may have some functional significance.

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