Observations on the Morphology and Histochemistry of the Mouse Pituitary Implanted in the Anterior Eye Chamber

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Previous investigations from this laboratory have indicated that the transplanted mouse pituitary is capable of maintaining thyroideal metabolism of I\(^{131}\) in the host but is incapable of maintaining the size of any of the target endocrine glands, including the thyroid, above that of the hypophysectomized state (1). It was hoped that cytologic and histochemical study of such functionally limited heterotopic glands might reveal some information concerning the morphologic basis of their peculiar physiologic activity. The present study makes use of the periodic acid-Schiff technique, which demonstrates histochemically the glycoprotein of the basophils; a selective trichrome stain; and a toluidine blue-ribonuclease method for identifying ribonucleic acid. The mice used were of the same genetic constitution as those in the previous functional studies and the implants were prepared in like manner. Whole mouse pituitaries were implanted, and observations made of all 4 parts of the gland. The implants were taken for examination at intervals beginning 1 day after implantation and extending to 14 months so as to document the sequence of changes taking place in the implanted tissues.

Morphologic studies of transplanted pituitaries in rats (2-8), guinea pigs (9), and mice (10) have been reported. However, the present study combined the utilization of mice and observation of all lobes of the gland with the differential and histochemical techniques employed, and in addition surveyed the implants at early and frequent intervals following transplantation.

Methods

All animals used in the study were F\(_1\) derivatives of BALB/c \(\times\) C3H mice. Whole pituitary glands were dissected from newborn mice of either sex and implanted, 1 per host, into the anterior eye chamber of 42 normal

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2-month-old female mice by a technique previously described (11). Host mice were maintained for intervals ranging from 1 day to 14 months after implantation. Animals were killed at the times following implantation listed in table 1. At each interval 2 to 7 mice were killed with ether, and the graft-bearing eye was removed and fixed in buffered mercuric chloride-formol (12). For comparison, pituitaries were also secured from normal mice of both sexes at various intervals from birth to 3 months. Serial paraffin sections, 3 and 5 μ, were prepared of each implant in situ.

The majority of sections were stained by the periodic acid-Schiff method (PAS), which demonstrates the sites of glycoprotein, or by a modified Mallory triple stain (13), which is selective for demonstrating pituitary cell types. Control PAS slides were exposed to saliva for 15 minutes at room temperature prior to staining. Staining for ribonucleic acid was performed with toluidine blue in acetate buffer at pH 4.5 or 5.0. Before staining, 1 of a pair of adjacent slides was incubated 1 to 1½ hours at 37° C. with 100 μg. of ribonuclease in 0.5 ml. distilled water. During this period the adjacent control sections were allowed to stand in distilled water at the same temperature. As a certain amount of extraction of the stain takes place during the dehydration in alcohol, care was taken to carry each slide simultaneously with its control through all parts of the procedure.

The volumetric measurements of the pituitary implants, given in table 2, were calculated by means of planimetry of serial sections.

Observations

General Morphology of Anterior Lobes of Implanted Pituitaries

One to 8 days after implantation.—All the 12 transplanted pituitaries examined at 1 to 8 days following implantation showed varying but sometimes marked degrees of degeneration and necrosis. One of the 8-day implants showed intact cells and little necrosis. The sinusoids of the anterior lobe were often distended and engorged with blood. Cellular and amorphous debris were trapped in residual lumina of Rathke's pouch and in scattered necrotic areas within the anterior lobe. In both anterior and intermediate lobes nuclei were frequently predominantly pyknotic or hyperchromophilic. The cytoplasm of the epithelial cells tended to be indistinct, cell boundaries undelineated, and the tissue appeared somewhat edematous. In the anterior lobes of most of the implants, centrally placed necrotic tissue and extravasated erythrocytes could be seen surrounded by an irregular cortex of relatively healthy cells (fig. 1). Within the debris, chromophils in which no stainable nuclear material remained could readily be identified, but the cytoplasm was intact and could be selectively or specifically stained (fig. 2).

One day after implantation, small blood vessels appeared to enter the implant from the iris at points of contact. However, judging from the extravasated blood in the tissue and the dilatation of the anterior-lobe sinusoids, which were packed with erythrocytes, revascularization was
not well established until approximately the 8th day. It must be pointed out that in all but 1 of the implants of 1 to 8 days, varying amounts of intact, healthy epithelial tissue could be seen (fig. 3), but their intact state could not be correlated with the length of time after implantation. One of the 3-day implants looked completely necrotic throughout the serial sections, and no attachment to the iris could be found.

The tissue degeneration in the initial stages after implantation was apparently not due to mechanical injury to the pituitaries by the dissection procedure prior to implantation. Three pituitaries from newborn animals were dissected as for implantation, allowed to stand in Tyrode's fluid 20 to 60 minutes, then fixed and prepared for histologic examination. Except for a thin marginal rim, 2 or 3 cells thick, showing histologic signs of damage, these pituitaries were not detectably different from 4 other pituitaries of newborn mice that were fixed in situ before dissection and histologic preparation.

Eleven days or more after implantation.—At 11 or more days, the epithelial tissue was intact, cell boundaries were distinct, and the vascular pattern was more or less normal with the exception of 1 unusually small 15-day implant. The only indication, in the remainder of the series, of the degeneration of tissue that occurred during the first week was the presence of rather large cavities within the epithelial tissues containing variable amounts of blood, and, in the 11- to 15-day groups, some debris as well. The debris was apparently absorbed or phagocytosed progressively with time and was not seen after 15 days.

Between the day of implantation and day 15 the implanted tissue grew (table 2) in spite of the necrosis that occurred in the early period. Mitoses were frequently seen in all implanted anterior lobes after the 8th day but were quite infrequent before this time.

Beginning at 36 days, small vacuoles were seen scattered in the “chromophobic” anterior-lobe tissue but it was difficult to determine whether this vacuolation was intracellular or intercellular. However the 14-month implants had numerous large “chromophobes” which clearly contained large nonstaining vacuoles.

Anterior-Lobe Cytology

Two of the 3 staining methods used in this study stain differentially the 3 main classes of pituitary cells, traditionally called “basophils,” “acidophils,” and “chromophobes.” “Basophils” are stained by the recolorized leucofuchsin of the PAS procedure and by aniline blue in the Mallory technique. Either of these stains will allow differentiation among “basophils” of “thyrotrophs” and “gonadotrophs” on a morphologic and distributional basis (14) in certain species, including the mouse. “Acidophils” are stained by the orange G counterstain of the PAS method, and by acid fuchsin in the Mallory method. “Chromophobes,” as their name implies, are not stained by these nor by any of the traditional pituitary stains. However, some of the “chromophobes” are stained by basic dyes, e.g., toluidine blue, by virtue of their ribonucleic
<table>
<thead>
<tr>
<th>Days after implantation</th>
<th>No. of mice</th>
<th>Neural lobe</th>
<th>Intermediate lobe</th>
<th>Anterior lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Present but variably intact or edematous (degenerate).</td>
<td>Generally not completely intact; variable amount of necrosis.</td>
<td>Much necrosis, blood-engorged sinusoids, edema, nuclear pyknosis.</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>Present but atrophied to small remnant.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>&quot;</td>
<td>Intact; small PAS-positive cells, some with negative Golgi image. Mitosis present. Volume of lobe decidedly increased.</td>
<td>Almost uniformly intact, predominantly vesicular nuclei, compact sinusoid arrangement.</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>No recognizable remnant in any case.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>&quot;</td>
<td>Cell size increased, staining somewhat increased, more frequent Golgi images.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>3</td>
<td>&quot;</td>
<td>Cell size definitely greater, Golgi images larger and more frequent, less mitosis, light and dark cells.</td>
<td>Some vacuolation of cytoplasm.</td>
</tr>
<tr>
<td>51</td>
<td>3</td>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4½ mos.</td>
<td>2</td>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 mos.</td>
<td>7</td>
<td>&quot;</td>
<td></td>
<td>Considerable numbers of vacuolated cells, some scattered pyknotic nuclei.</td>
</tr>
</tbody>
</table>
### Anterior lobe—Continued

<table>
<thead>
<tr>
<th>Acidophils</th>
<th>Basophils</th>
<th>Chromophobes</th>
<th>Cytoplasmic basophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good numbers of acidophils in intact portions. Also many dead acidophils lacking stainable nuclei in interior necrotic areas.</td>
<td>Same comments as for acido- phils.</td>
<td>Nothing unusual regarding chromophobes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good numbers present.</td>
<td>No definite baso- phils seen.</td>
<td>Some large phobes, no Golgi images.</td>
<td>Present but not obviously greater than in normal 11-day pituitary.</td>
</tr>
<tr>
<td>Variable numbers, probably less frequent than above.</td>
<td>A few pale PAS + cells, vesicu- lar nuclei.</td>
<td>Some Golgi images in phobes; vesicular nuclei; nucleoli.</td>
<td>Much greater than in normal 20-day-old pituitary. Heavy staining in cytoplasm ca. Golgi zone.</td>
</tr>
<tr>
<td></td>
<td>A few in one im- plant clear and pyknotic.</td>
<td>Increasing nos. of Golgi images.</td>
<td>Increasing cytoplas- mic basophilia.</td>
</tr>
<tr>
<td>Markedly reduced numbers, with high proportion of pyknotic nuclei and shrunken cyto- plasm.</td>
<td>Possibly a few in one implant.</td>
<td>(Less mitosis) Chromophobes begin to line up along sinuso- ids as &quot;palisades&quot; with nuclei in basal position.</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>None seen</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Even more and larger Golgi, palisade forma- tion through- out.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased size of cells and nucle- oli.</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2.—Implants in normal hosts and normal pituitaries

<table>
<thead>
<tr>
<th>Time after implantation (days)</th>
<th>Implants in normal hosts</th>
<th>Normal pituitaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.0449</td>
<td>0.0209</td>
</tr>
<tr>
<td>25</td>
<td>0.1321</td>
<td>0.0288</td>
</tr>
<tr>
<td>36</td>
<td>0.1476</td>
<td>0.0454</td>
</tr>
<tr>
<td>51</td>
<td>0.2480</td>
<td>0.1345</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age and sex (days)</th>
<th>Normal pituitaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ?</td>
<td>0.0382</td>
</tr>
<tr>
<td>1 ?</td>
<td>0.0390</td>
</tr>
<tr>
<td>3 M</td>
<td>0.0451</td>
</tr>
<tr>
<td>3 F</td>
<td>0.0749</td>
</tr>
<tr>
<td>8 M</td>
<td>0.0760</td>
</tr>
<tr>
<td>26 F</td>
<td>0.1612</td>
</tr>
<tr>
<td>33 M</td>
<td>0.3630</td>
</tr>
</tbody>
</table>

acid content. Thus, in respect to basic dyes, these cells are not literally “chromophobes.” For simplicity, the traditional terminology will be employed in this paper but the terms will be enclosed in quotation marks for reasons that will be indicated in the Discussion.

“Chromophils” were far less numerous in all the implants than in normal pituitaries. Among the implants the highest proportions of “chromophils” were seen in intact areas during the first week. After the first week, cells with specifically stainable granules became progressively more scarce. This loss of stainability was in contrast to the marked increase in chromophils which took place in the normal mouse pituitaries following the day of birth. Figure 22 illustrates a typical area from the lateral portion of a 54-day-old mouse pituitary. In contrast to the predominantly “chromophobic” anterior lobes of 51-day implants (fig. 23), both “basophils” and “acidophils” are numerous and large.

The nuclei of “acidophils” in 1- to 8-day implants were either vesicular or pyknotic, reflecting the state of relative intactness or degeneration of parts of the gland. In the 11- to 25-day implants well-granulated “acidophils” were present in all cases (fig. 21), though fewer than normal, and almost all had clear, vesicular nuclei with conspicuous nucleoli. In the 36-day, 51-day, and 4½-month implants, however, “acidophils” were extremely scarce and their nuclei were usually pyknotic. In these, the cytoplasm was generally reduced in size and often compressed in shape.

“Basophils,” like “acidophils,” could be recognized in some of the intact areas of anterior-lobe tissue of implants during the first week. Their nuclei were likewise either pyknotic or vesicular. Their cytoplasm stained faintly with aniline blue or with PAS, and was generally more voluminous than that of acidophils. In most of the remainder of the...
series, cells having the tinctorial characteristics of “basophils” could not be differentiated even upon serial examination of sections. In 6 out of the 16 implants of 11 to 51 days, a very few cells could be discerned which stained faintly with aniline blue or PAS, and these were generally rather large cells as compared with “acidophils.”

“Chromophobes” obviously constituted the major portion of anterior-lobe tissue of transplants older than 11 days. Beginning at 15 days, it was apparent that some of the “chromophobes,” which were distinguished by their inability to take up the standard pituitary stains, differed morphologically from the classical “chromophobes” of normal pituitaries. Many of the nonstaining cells were conspicuously larger than is usual for “chromophobes,” and, furthermore, negative Golgi images could clearly be seen in the cytoplasm. The proportion of total cells constituted by the large Golgi-containing “chromophobes” increased markedly with time, so that in the 51-day and 4½-month groups they were in large majority. These cells almost always had vesicular nuclei, and quite commonly had large, brilliantly stained nucleoli.

Two morphologically distinct types of “chromophobes” could be recognized, and these had a different distribution in the implanted anterior lobes. One type, small in number, was localized in those portions of anterior-lobe tissue which were adjacent to what would normally be the lateral and anterior portions of intermediate lobe and to the pars tuberalis (fig. 6). This area was characterized by Purves and Griesbach (14) as the “sex zone” where gonadotrophic “basophils” are concentrated. The cells in this area in the implants tended to be relatively large in comparison to the other anterior-lobe cells, and to have distinct, though non-staining, granules in the cytoplasm. The presence and character of the cells in this area were rendered more conspicuous by their formation into acini demarcated by abundant connective tissue and sinusoids—a similar circumstance to that found in this region in the intact mouse pituitary gland. PAS-positive or aniline blue-positive cells, though infrequently seen, were most commonly found in this region.

The second type of “chromophobe,” much more plentiful than the one described above, was distributed uniformly throughout the remainder of the anterior-lobe tissue (figs. 4, 5, and 6). The cytoplasm, unlike that of the first cell type described, was nongranular, and was smooth and homogeneous in appearance. Negative Golgi images were more readily distinguished in these cells. These more numerous “chromophobes” were arranged in a conspicuous “palisade” formation along the sinusoids (fig. 10). The “palisade” appearance was produced by the alignment of cells of uniform size and shape, with basilar nuclei and supranuclear Golgi images. Some evidence of palisade formation was first seen in the 20-day implants, but by 51 days these dominated the appearance of the anterior-lobe tissue.

Most of the “chromophobes” of the implants showed conspicuous cytoplasmic basophilia with toluidine blue, excepting those of the “sex zone” which were virtually unstained (fig. 7). This staining of the an-
terior-lobe cells and of a smaller proportion of intermediate-lobe cells was apparently due to ribonucleic acid, as all the cytoplasmic basophilia was destroyed by the ribonuclease pretreatment. The homogeneous mass of cytoplasmic nucleoprotein was characteristically localized peripherally in the cell producing a ring-shaped configuration enclosing the unstained Golgi area (fig. 9).

Pituitaries implanted for 11 and 20 days were compared as to degree of RNA staining with normal glands from 11- and 20-day-old mice. Figures 13 to 16 demonstrate the results and show that the normal glands from both age groups displayed very little staining, mostly of a diffuse character, but some perinuclear masses were present. In the 11-day implant there was an indication of the possible presence of more cytoplasmic basophilic material than in its normal counterpart, whereas, in the 20-day implant the amount of RNA had obviously increased and was localized in the typical fashion described above. Studies of later implants bore out the indication that the cytoplasmic RNA content may increase with time after implantation and that it occurs in larger concentration than in normal pituitaries of corresponding age.

In the normal intact pituitaries, RNA-containing cells (presumably "acidophils") were found primarily in the lateral areas of the gland while the "sex zone," and "basophils" generally, were negative. Within individual cells, RNA was most frequently seen as an amorphous mass of deeply staining material closely adherent to the nuclear wall, while occasionally it was present as a thin rim at the extreme periphery of the cell. Figures 11 and 12 illustrate the staining pattern for nucleoprotein compared with that of the PAS-positive material in the "sex zone" from a 2½-month-old female mouse.

**Neural Lobe**

The neural lobe could be recognized at 1, 3, and 5 days following implantation in either intact or degenerate form (figs. 1, 3, and 21). None of the subsequent implants contained any recognizable neural lobe.

**Intermediate Lobe**

In the first week after implantation, mitoses were rarely seen in the intermediate lobe. Numerous pyknotic nuclei were frequently seen and cells were generally very small and they stained poorly. However, by 11 days a definite increase in cell size, cytoplasmic staining, and frequency of mitosis was observed. In addition, negative Golgi images were clearly definable in the cytoplasm and the nuclei had generally become large and vesicular with prominent nucleoli. In the stages after 11 days, the cell size, the frequency and size of the Golgi images, and the size of nucleoli increased further, as did the apparent over-all size of the pars intermedia. At the same time, the staining intensity of the intermediate-lobe cells was only slightly increased. In the 36-day group and in all subsequent groups, some of the cells stained well with PAS or aniline blue, while others stained scarcely at all (fig. 18). A size difference in
the tinctorially distinct cells was striking: the nonstaining cells were larger and more frequently showed a large Golgi image.

The relatively greater increase in total volume of the intermediate lobe as compared with that of the anterior lobe is reflected in the ratios of calculated total anterior-lobe volume to intermediate-lobe volume for implants and for normal pituitaries (table 2).

Intermediate-lobe cells had far less histochemically demonstrable cytoplasmic basophilic material (RNA) (fig. 8). Many cells, in fact, did not stain at all, in contrast to the positive reaction of most anterior-lobe cells.

**Pars Tuberalis**

Cells of the pars tuberalis were seen in implants throughout the series (fig. 19). They were readily identified by their content of strongly PAS-positive, saliva-labile material, presumably glycogen (15). The glycogen in the epithelial layer of the cornea (16) surrounding the implants was also revealed by this method, and thereby provided additional control for the salivary digestion. Neither the corneal epithelium nor the pars tuberalis displayed metachromasia with toluidine-blue staining, while the mucopolysaccharide-containing substantia propria was strongly metachromatic. These results are consistent with the view that the material stained by PAS in the pars tuberalis is glycogen.

**Fourteen Months after Implantation of Pituitaries**

The 7 implants that had been allowed to remain in the eye 14 months were all very enlarged, and it was apparent from gross examination that they were considerably larger than normal adult mouse pituitaries. They consisted predominantly of anterior-lobe tissue in which no “chromophils” could be seen (fig. 24). The “chromophobes” of these implants were very enlarged, displayed greatly hypertrophied Golgi images, and were occasionally vacuolated. There was some evidence of palisade formation of cells along sinusoids as in the implants described above. Some nucleoli of tremendous size were seen which filled most of their respective nuclei.

In contrast to all the earlier implants, the intermediate lobe after 14 months comprised only a small fraction of the total implant. The cytology of the intermediate lobes, however, was similar to that in the several previous groups. In addition there were no longer any large empty “pockets” in the implants, these presumably having been filled up by growth of the tissue. Thus, between 4½ and 14 months, the intermediate lobe remained apparently unchanged, whereas the anterior lobe showed marked hyperactivity, hypertrophy, and hyperplasia.

**Discussion**

A large portion of some of the first-week implants was obviously necrotic tissue, and it is possible that functional effects of such “grafts”
might be due to absorption of hormones from dead cells rather than true secretion. It may also be reiterated that in the early implants the tissue was not uniform in condition throughout serial sections of the implant; the more intact tissues tended to be adjacent to the iris, the source of revascularization. These findings strongly indicate that, for a true assessment of the morphologic state of an implant, serial sections are required.

The degranulation of anterior-lobe cells after transplantation, observed in the present study, has been reported by previous observers (2,3,5-7,10). Degranulation has also been noted following stalk section (17,18) and hypothalamic lesions (19). Because of the impaired function of the anterior pituitary when severed from its connections with the hypothalamus, the scarcity of specific granulation has been interpreted as the cytologic manifestation of loss of activity (20). However, transplanted anterior lobe has been reported to be capable of maintaining a number of its usual functions, i.e., stimulation of thyroïdal iodine metabolism (1), release of corticotrophin in response to systemic stress (6,7), partial adrenal maintenance (5), and luteotrophin secretion (21,22). Since such implants are composed almost entirely of "chromophobes," it may be postulated that the "chromophobes" are, in fact, the elements responsible for some or all of the functional activity. Accordingly, at least some of the "chromophobes" may be poorly granulated "chromophils" whose rate of secretion exceeds that of the rate of synthesis. Supporting this concept are the findings in this study, first, of a rich cytoplasmic RNA content of the "chromophobes" of the implanted pituitaries, implying a high level of protein synthesis (23), and second, of the simultaneously occurring morphologic features (enlarged Golgi images and nucleoli and increased cell size) generally considered indicative of heightened secretory activity. Of course, as these cytochemical and cytologic features by themselves do not necessarily imply the elaboration and secretion of biologically active pituitary hormones, the synthetic activity of these cells could conceivably be, at least in part, of a nonhormonal nature.

That so-called "chromophobes" may be among the active secretory elements in pituitaries functioning in situ has been postulated previously on the basis of observations of estrogen-treated animals (24). In such pituitaries, enlarged cells showing cytologic evidence of activity were seen (enlarged Golgi and nucleoli and numerous mitochondria). Thus, as Severinghaus (25) has pointed out, before assigning a cell to the category of quiescent "stem-cell chromophobes," it seems necessary to observe other cytologic features in addition to its tinctorial properties.

The occurrence of increased cytoplasmic RNA in normal pituitaries has been observed following estrogen injections (24,26). Desclin (26) observed that the cytoplasmic basophilic masses were practically never seen in normal rat "basophils" or "chromophobes," and rarely in eosinophils, but they were abundant during pseudopregnancy, pregnancy, lactation, or estrone treatment in "chromophobes" and "acidophils." He also noted that while aniline blue stained the regular "basophils,"
both before and after ribonuclease treatment, this dye was poorly re-
active on the cells that stained with basic dyes. Wolfe (24) observed that during estrogen treatment, when both “acidophils” and “basophils”
degrnulate, the degranulating “acidophils” and the cytologically active
“chromophobes” were characterized by intense cytoplasmic basophilia. Wolfe distinguished between “active” and “inactive” “chromophobes” on the basis of these cytologic criteria. Desclin and Gregoire (2) studied the morphology of pituitaries transplanted in the kidney for 15 days and found that estrogen injection resulted, as in their normal pituitaries, in greatly increased numbers of cells staining with basic dyes. These cells were characterized by Golgi areas of larger dimension displacing the basophilic cytoplasm peripherally in the cell; thus they resembled the RNA-rich “chromophobes” of Wolfe’s estrogen-stimulated pituitaries and those implanted in mice for 20 days and longer in the present study. Although the morphologic resemblance between the large “chromophobes” of the different preparations does not necessarily imply a functional similarity, the appearance of these cells in response to estrogen in both normal and implanted glands suggests that, in these circumstances, these cells are a morphologic expression of the functional response to estrogen. Furthermore, the findings that grafted pituitaries are capable of luteotrophin secretion with (21) and without (22) estrogen treatment are consistent with the idea that the large, RNA-rich “chromophobes,” presumably degranulated “acidophils,” are involved in luteotrophin production in the implants and in pituitaries in situ in the case of es-
trinized or lactating animals.

In view of the fact that the implanted rat pituitary was found capable of responding to stress (6,7), it is interesting to note that Finerty, Hess, and Binhammer (28) found increased cytoplasmic RNA in “acidophils” and “chromophobes” of normal rat pituitaries 1 hour and more after stress.

Thyrotrophin is known to be produced by transplanted mouse pitu-
itories (1). The PAS procedure is believed to stain thyrotrophin or its precursor in normal pituitaries, but since the anterior lobes of the implants were virtually negative to PAS after the first week, no information as to a possible localization of thyrotrophin production was forthcoming.

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4 The traditional terminology for the pituitary cells has been criticized in the past as being inappropriate and unreasonable (21). Both “acidophils” and “basophils” are stained with acid dyes in the standard trichrome procedures; and “acidophils” have been stained with basic fuchsin. Further, “basophils” stain poorly, if at all, with any of the basic dyes, while both “acidophils” and “chromophobes” may exhibit a high degree of cytoplasmic basophilia. Because of the literal inaccuracy of these terms they have been enclosed in quotation marks throughout this paper. It should be recorded here that a recent publication (27) has presented evidence that throughout most of the pH range of the staining solutions, an acid dye, fast green, stained “acidophils” more intensely than “basophils,” and a basic dye, methylene blue, stained “basophils” more intensely than “acidophils.” The authors concluded on the basis of their study that the nomenclature acidophil, basophil, and chromoboe is informative and should be retained. However, the data were derived from the use of only 1 acid dye and 1 basic dye; at pH values between 6 and 7 the staining curves crossed so that the acid dye stained “basophils” more strongly than “acidophils” with increasing pH; and between pH 6 and 7 each dye stained either “acidophils” or “basophils” with equal intensity. Further, the authors point out that the acid dye aniline blue stains “baso-
phils” more intensely than “acidophils” at each pH. Further work may show that unqualified retention of the old terminology is indeed reasonable. At present it might be preferable to remember that the names of the cells refer to distinct morphologic entities irrespective of tinctorial implications.

5 Martins (9) also reported this effect in intra-ocular transplants using the methyl green-pyronine stain.
Because of the marked hyperplasia seen in the intermediate lobe following transplantation of the pituitary or following the induction of hypothalamic lesions, it was considered possible that the intermediate lobe might be the source of a thyrotrophic hormone which particularly stimulated the metabolism of radioiodine by the thyroid. Previous studies had indicated that thyroidal $^{131}I$ metabolism was maintained to a much greater extent in both the above experimental circumstances than was thyroidal growth (1). Accordingly a series of mice was implanted with anterior lobe alone or with intermediate plus posterior lobe and a very small amount of anterior lobe. Functional studies indicated that all the thyrotrophic activity of transplanted pituitaries was accounted for by the anterior lobe alone (unpublished data).

Similarly, assays performed by Dr. R. W. Bates on isolated anterior, intermediate, or posterior lobe of both the rabbit and the rat showed that only anterior-lobe tissue caused thyroidal iodine depletion in the chick.

A preliminary experiment revealed no apparent morphologic differences between implants in animals which were: (a) untreated, (b) hypophysectomized, (c) hypophysectomized and castrated, (d) hypophysectomized and fed propylthiouracil.

The atrophy of the neural lobe in this series confirms the findings of many other investigations involving separation of the neural lobe from the brain. Atrophy of the neural lobe has been observed following transplantation (5,8), stalk section (17,18), and hypothalamic lesions (29,30). That it has not been reported more frequently in implants is probably due to the fact that in most studies only anterior lobe has been implanted.

The intermediate-lobe hypertrophy in the ocular implants of the present study may be compared to the findings of Etkin in the frog (31). Transplantation of the epithelial portions of the pituitary, either as a primordium or as the differentiated gland, resulted in hypertrophy and hyperplasia of the intermediate lobe, associated with excessive pigmentation of the host. Similar results were also produced by infundibular lesions. This suggested a restraining influence of the infundibulum on intermediate-lobe growth and activity. As far as we are aware, no previous reports have been made of intermediate-lobe hypertrophy in transplanted mammalian pituitaries. However, Brooks (17) found in the stalk-sectioned rabbit that, in comparison with anterior and posterior lobe, the intermediate lobe was much larger than normal and the cells were larger and stained more deeply. He described a granular formation in the cytoplasm, which may be the negative Golgi image and macula densa, and notes that these granular aggregates were never seen in normal glands. Barrnett and Greep (18) found that the intermediate lobe was usually enlarged after stalk section in the rat. Intermediate-lobe hypertrophy was reported in the rat, after hypothalamic lesions, by Bogdanove and Halmi (30) and has also been seen in this laboratory. Thus there are 4 types of preparations now reported in which interference with the brain-pituitary connections is followed by hypertrophy of the interme-
mediate lobe. It may be of interest that intermediate-lobe hypertrophy is also seen in intact animals following certain hormone injections. Estrogen produced this effect very markedly in the hamster (32,33) and to a lesser extent in the rat (34). Corticotrophin caused hypertrophy and cytologic changes in intermediate-lobe cells in kittens (35).

Previous reports on the maintenance of pars tuberalis cells after transplantation have apparently not been made. It is possible that these cells are not generally observed due to their inconspicuous appearance when ordinary histologic methods are employed. The preservation of glycogen and its demonstration with PAS staining (15) made possible the identification of the pars tuberalis in these implanted pituitaries. Since the function of the pars tuberalis is not known, the significance of its persistence in implants cannot be surmised.

**Summary**

1) Pituitaries of newborn mice were implanted in the anterior eye chambers of normal adult female mice of the same derivative strain (BALB/c × C3H), and the resulting viable transplants taken for cytologic study at varying intervals from 1 day to 14 months.

2) It was found that during the first week following implantation (1–8 days), the pituitaries were in varying stages of degeneration or necrosis, with marked stasis of blood and engorgement of sinusoids. Large areas of dead tissue could frequently be seen in one interior cavity.

3) Anterior-lobe tissue grew well subsequent to the first week after implantation to the eye, though probably not so well as the intermediate lobe. The stainability of anterior-lobe cells was much less than that of the normal gland; "acidophils" appeared less frequently with time until they were very rare by the 51st day, while just a few "basophils" were discernible in a minority of the specimens in the 15- to 36-day groups.

4) The "chromophobes" of implanted anterior lobes were morphologically dissimilar to those present in normal pituitaries, being much larger and having cytologic manifestations of activity. At the later time intervals these "chromophobes" formed striking "palisade" patterns and comprised a larger proportion of the cell population.

5) The pars tuberalis with its content of glycogen survived well in the implants of this series. Mitoses were not observed in this lobe.

6) The intermediate lobe, after the regression of the first week, exhibited considerable capacity for growth, attaining a size comparable to that of normal in situ pituitaries of a corresponding age. Mitoses were numerous, and the cells stained well with PAS or aniline blue, while negative Golgi images were prominent in the ample cytoplasm.

7) The neural lobe could be seen in implants of 1 to 8 days; in some of these it had a relatively intact appearance, and at other times it was markedly degenerated. At 11 days, only atrophic remnants were identified, but by 15 days and in the remainder of the series the presence of the neural lobe or remnants thereof could not be ascertained.
References


PLATE 37

FIGURE 1.—Pituitary taken from the anterior eye chamber 5 days after implantation, containing a large mass of debris, D, in the residual lumen. The neural lobe, NL, has lost most of its nuclei and stainable droplets; the intermediate lobe is only a thin layer. Pars tuberalis, PT, is strongly positive to PAS. Anterior lobe, AL, peripheral to the debris, is edematous and somewhat disorganized, and nuclei are vesicular. PAS and hematoxylin. × 150

FIGURE 2.—Higher magnification of a portion of the debris in the same implant as in figure 1, illustrating "dead" "chromophils" in which the nuclei no longer stain. Erythrocytes are also in the field. Modified Mallory. × 1250
FIGURE 3.—Another 5-day implant in which the 3 lobes appear intact. PAS and hematoxylin. $\times 150$

FIGURE 4.—Anterior lobe of a 51-day implant illustrating “chromophobes” of large size, the nuclei of which are oriented toward sinusoid; large Golgi images, prominent nucleoli. Modified Mallory. $\times 1700$

FIGURE 5.—Anterior lobe of another 51-day implant, similar features as in figure 4. PAS, hematoxylin, and orange G. $\times 1700$
PLATE 39

FIGURE 6. Same 51-day implant as in figure 5. A blood vessel separates intermediate and anterior lobes. Just to the right of it is the area of anterior lobe containing many of the larger, more granular type of "chromophobes" surrounded by abundant connective tissue, and farther right are the predominating type of "chromophobes." PAS, hematoxylin, and orange G. × 360

FIGURE 7.—Another section from same implant as in figure 6. Demonstration of RNA in the cytoplasm. "Chromophobes" immediately to the right of the blood vessel, BV, did not take the stain, while those farther right stained well. Toluidine blue. × 360
Figure 8. Another area from same section as in figure 7; intermediate lobe in upper half of photograph, anterior lobe in lower half. Note differences in size of cells and pattern of RNA staining between IL cells and AL "chromophobes." Toluidine blue. × 690

Figure 9. Higher magnification of field from same section illustrated in figures 7 and 8, showing typical RNA staining of cytoplasm and nonstaining Golgi zone of "chromophobes." × 1700
Plate 41

Figure 10.—Anterior lobe of 51-day implant, “palisade” formation of nuclei of “chromophobes” along sinusoids, nonstaining cytoplasm with visible Golgi images oriented away from sinusoids. PAS, hematoxylin, and orange G. × 390

Figure 11.—In situ pituitary of female mouse 2½ months old (host to a 15-day implant). Most rostral and ventral area of anterior lobe, where greatest concentration of “basophils” is seen. “Basophils” appear gray in the photograph. The dark cells above the arrow are acidophils, A. PAS, hematoxylin, and orange G. × 280

Figure 12.—Another section from the same area of the pituitary shown in figure 11 for comparison of localization of PAS and RNA staining. The large “basophils” are very slightly stained or unstained, while considerable staining reaction is present in the “acidophil” area above. Note the intense perinuclear staining reaction in some of the cells. Toluidine blue. × 280
Plate 42

All sections stained for RNA at the same time. In each case only nuclear staining was possible in adjacent sections pretreated with ribonuclease. Toluidine blue. × 690

Figure 13.—In situ anterior lobe of an 11-day-old normal male mouse. Only some diffuse staining of cytoplasm, except for a cell (arrow) in the approximate center with a dark perinuclear mass.

Figure 14.—Anterior lobe of 11-day implant, not obviously different from a normal implant.

Figure 15.—In situ anterior lobe of a normal 20-day-old female mouse.

Figure 16.—Twenty-day implant in which there are numerous large cells with peripheral masses of RNA typical of the older implants. In the center of the photograph a group of such cells is arranged in a circle bounded by connective tissue of a sinusoid.
Figure 17. Intermediate lobe of a normal 54-day-old male mouse. Note homogeneous staining of cytoplasm, small Golgi (arrows). PAS, hematoxylin, and orange G. × 1700

Figure 18.—Intermediate lobe of a 51-day implant. Some cells much darker and smaller than most of the cells, very large Golgi images (arrows) in the large, paler cells. PAS, hematoxylin, and orange G. × 1700

Figure 19.—Fifteen-day implant with strongly PAS-positive pars tuberalis. Blood vessels passing through connective tissue from iris to implant. PAS and hematoxylin. × 210

Figure 20. Section adjacent to that in figure 19, pars tuberalis is PAS-negative. (The nuclear staining is stronger in this section than in fig. 19.) Saliva, PAS, and hematoxylin. × 210
FIGURE 21.—Eleven-day implant composed of healthy epithelial tissues. Large pouch containing some blood. “Chromophils” in piece of AL projecting into pouch. Mitoses in IL. PAS, hematoxylin, and orange G. $\times 176$

FIGURE 22.—Anterior pituitary of a 54-day-old normal male mouse. “Acidophils” are yellow; PAS-positive cells, pink. Golgi images in cytoplasm of both types of “chromophils”. PAS, hematoxylin, and orange G. $\times 1700$

FIGURE 23.—Anterior lobe of a 51-day implant. Comparison with fig. 22 illustrates the difference in staining properties between the implant and a normal male mouse pituitary of comparable age. Note cytoplasmic detail, particularly of “palisade” cells for comparison with RNA staining (figs. 8 and 9). PAS, hematoxylin, and orange G. $\times 735$

FIGURE 24.—Anterior lobe of an implant after 14 months in the eye. Note increased cell size, vacuoles, large nucleoli. Some extraordinarily large nucleoli not easily distinguished in the photograph, as they stain poorly. PAS, hematoxylin, and orange G. $\times 735$