Spontaneous Basophilic Tumors of the Pituitary Glands in Gonadectomized Mice*†

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The presence of spontaneous pituitary tumors and lesions following the appearance of adrenal cortical carcinomas presents a new line of evidence for the close relationship of adrenal cortex and pituitary dysfunctions in experimental animals and of these dysfunctions to tumor formation and their possible inherited tendencies. In man, there are not sufficient data to say conclusively whether the adrenal cortex or anterior pituitary abnormalities are the primary causal agents in the development of various syndromes, such as Cushing's syndrome, (Cushing's disease) and adrenal virilism. In fact the clinical evidence of these syndromes is so confusing that it is difficult to try to ascribe the causal agent to any one endocrine gland and/or its secretions. Kepler (22) recently has given a critical review of Cushing's syndrome and its association with Crooke's changes of the basophile cells of the anterior pituitary, i.e. hyalinization of the basophile cells. He made several postulates about the mechanism that might be involved, to be discussed later, but did not definitely say that either the anterior pituitary or the adrenal cortex was the primary cause. Hyperadrenocorticism, however, usually plays an essential part in this syndrome. In the experimental animals under discussion in the present report it is definite that the adrenal cortical carcinomas produced both masculinizing and feminizing effects. 

A review of the literature indicates that spontaneous hypophyseal abnormalities in mice are extremely rare. Slay found only one abnormality in 11,000 mice examined (38). Tumors of the mouse hypophysis interpreted as chromophobe adenomas have been produced following estrogenic treatment (18). Burrows described such a tumor in a male mouse following estrogenic treatment (5). Gardner and Strong have shown that the pituitary response to estrogen treatment was strain limited and that the strain C57 Black mice were more susceptible than other strains (16). Pituitary tumors of the above type were observed following implantation of pellets containing an estrogenic substance, diethylstilbestrol, in strain ce mice in this laboratory (45).

Castration changes in the pituitaries of rats have been described by many workers, Addison (1), Biggart (2), Guyer (20), and Severinghaus (30). They have noted that there is an increase in the number of basophiles and that these basophiles become vacuolated to such an extent that they are called "signet ring" cells (80). These changes, however, have not been found to any extent in the mouse hypophysis following gonadectomy (19).

The effects of early gonadectomy in mice of various strains have been intensively studied by Woolley, Fekete, and Little (14, 41, 42, 48). It was observed that the strains used reacted differently to gonadectomy. Experiments were carried out with several inbred strains, namely, JAX, C57 Black, C57 Brown, dba, ce A and CSH. Their results will be summarized briefly here. In strains C57 Black, C57 Brown and A the accessory reproductive organs remained small, the submaxillary gland unstimulated, that is, small and dark; no obvious changes in the hypophyses or extensive changes in the adrenals were noted (46). However, the dba and ce mice reacted quite differently. The dba gonadectomized animals developed nodular hyperplasia of the adrenal cortex; this modified cortex seemed to be supplying hormonal stimulation to the organs ordinarily influenced by the hormones of the gonads themselves. The accessory reproductive organs in gonadactomized female mice were stimulated and, as histologically determined, they were feminized (14). A few mammary tumors also appeared in this experimental group. The pituitaries were examined and some abnormalities noted but changes were not studied in detail at that time. The ce mice not only developed nodular hyperplasia of the adrenal cortex but, later, adrenal cortical carcinomas, the latter first appearing in the hyperplastic areas. These carcinomas produced both masculinizing and feminizing hormones as evidenced by the histological picture of the submaxillary glands and the accessory reproductive organs in both gonadectomized sexes (42, 43, 12). Changes

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† Animal tissues were collected previous to the fire of October 23, 1947 and most of the tissues were saved. Some detailed notes and slides have been missing since that date.
in the hypophyses were observed in a few mice but not studied intensively.

After differences in the reactions of the various strains to early gonadectomy became known, it was decided to study reactions after gonadectomy on animals of the F1 generation of reciprocal crosses of some of these strains. As the animals were examined it became evident that frequent hypophysial abnormalities were occurring several months after the appearance of adrenal tumors in some of the crosses. Concurrent with this, there was very extensive and unusual mammary gland development.

The other data from these crosses will be reported separately and only the pituitary abnormalities and the relationships of the mammary glands to these abnormalities will be discussed in the present report.

**MATERIALS AND METHODS**

Approximately 800 F1 mice from reciprocal crosses between dba and C57 Black, dba and ce, ce and C57 Black, and A and C3H strains were gonadectomized from 1 to 3 days after birth. The mice were then returned to the nest and allowed to mature and age without any further treatment. Virgin females and unmated males from these crosses were used for control animals. All animals, experimental and control, were maintained on a diet of Purina fox chow and water ad libitum, and at a temperature of approximately 70° F.

Animals from both the experimental and control groups were autopsied at monthly intervals from 15 days and 1 month up to 24 months of age and beyond. Gross and histologic observations were made on the endocrine glands and accessory reproductive organs for comparative studies. The present report is on the macroscopic and histologic findings of the pituitary and mammary glands from these animals. Numerous abnormalities were encountered in the hypophyses of some of the experimental animals but not in the control animals. The pituitaries of the control animals and of the experimental animals that did not show gross abnormalities were fixed in 10 per cent formalin in normal saline, embedded in paraffin, and sectioned at 8 micra. Several longitudinal sections were cut from each pituitary and stained with Mayer's haematoxylin and aqueous eosin.

For the study of the abnormal pituitaries a good differential staining method was necessary. Trials with a number of fixatives in conjunction with various staining methods showed that the mouse pituitary was difficult to stain differentially, as compared to the rat or the hamster. A method was finally developed using either a modified Zenker-formol solution or a modified Bouin solution for fixation. The sections were stained with a combination of Mallory's and McFarlane's triple staining methods (11). Abnormal glands were cut in cross section and serially sectioned at 4 micra.

A few normal pituitaries beyond 12 months of age were fixed and stained in the same way as the abnormal pituitaries for purposes of comparison.

The mammary glands were left on the skins of the animals and were fixed in modified Tellyesnicky's fluid. These glands were then studied with the aid of the dissecting microscope and some representative glands from various animals were removed and sectioned. Most of these tissues were stained with haematoxylin and eosin.

**GROSS OBSERVATIONS ON THE PITUITARIES**

All gross observations on the pituitaries and mammary glands were made with the aid of a dissecting microscope (7 X ocular and 10 X objective).

The mouse hypophysis lies on the sphenoid bone and is covered by a tough membrane, the intrasellar dura (Fig. 1). At autopsy the anterior lobe is usually light pink in color and the intermediate and posterior lobes have an opaque color, the pars nervosa being slightly whiter than the pars intermedia. Normal hypophyses in mice measure about 2.16 mm. laterally X 1.00 mm. anterioposteriorly. In older mice the glands are slightly smaller, 1.90 mm. X 0.9 mm. (measurement on microscopic sections).

In some of the gonadectomized F1 mice, the pituitaries were enlarged and nodular. For instance one abnormal gland measured 3.7 X 2.6 mm. Two kinds of nodular areas were noted in these glands. The first type was a well defined opaque nodule or lesion at the periphery of the anterior lobe that did not alter the normal contour of the gland (Fig. 2). The second type was a protruding hemorrhagic nodule (Fig. 3). These nodules could be found at either or both tips of the anterior lobe and sometimes were so extensive that they pushed the intermediate and posterior lobes aside but did not seem to invade them. Characteristically they gave the impression of penetrating deeply into the anterior lobe.

These nodular areas occurred after 14 months of

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**Fig. 1.—Diagrammatic sketches of the mouse pituitary.**

A. dorsal view of whole normal gland as seen at autopsy. Pars intermedia is lined and pars nervosa is stippled. B. longitudinal section of the gland, the residual lumen is between the pars anterior and the pars intermedia. C. cross section of gland, the third ventricle, d the dura, e the subdural space, am the arachnoid membrane, and b the brain.
age, sometimes both kinds in one gland, sometimes only one. Table 1 shows the data on the eight F1 crosses that were made. It shows that there are strain variations and emphasizes the fact that the pituitary tumors occur at 14 months of age or later. In all cases the individuals that showed hypophyseal tumors had well developed adrenal tumors. The table does not show whether each adrenal possessed a tumor, but includes all the animals that had one or more adrenal tumors.

The alveolar development that was found along with the hypophyseal changes was very pronounced; the alveoli were large and sometimes the ducts were concealed by the alveolar growth. This growth was similar in gonadectomized animals, both females and males. The only difference was that all five pairs of mammae were present in the gonadectomized females, while in the castrated males some of the glands were absent, probably because the rudiments for some of the glands were never formed.

MAMMARY GLANDS

Concurrent with the gross hypophyseal changes, there was very extensive alveolar development of the mammary glands. It has been reported previously that following the appearance of adrenal cortical carcinomas in gonadectomized mice of the ce strain there was some mammary development, such as growth and extension of ducts, the appearance of end buds (42, 43) and some alveolar development. Where this latter occurred, it was correlated with abnormal hypophyseal changes (46).

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MICROSCOPIC OBSERVATIONS ON THE PITUITARIES

The Opaque Nodules.—The opaque nodules that occurred in the hypophyses of the experimental animals were not encapsulated. They were composed of densely arranged cells.

The cells were mainly of two types: (a) small cells, closely packed in disorderly arrangement and, (b) very large cells arranged in small acinar clusters separated from one another by delicate reticular fibers. Both types of cells could be found in one opaque nodule, or there might be only one type in an opaque nodule.

The small cells had light staining cytoplasmic basophilic granules and remnants of a Golgi apparatus comparable in form to the negative image of the Golgi apparatus in the normal mature basophile (i.e., a doughnut shaped negative image associated with basophiles). The nuclei of these cells were slightly different from those found in normal anterior lobe cells. The chromatin was found only in a light ring at the periphery and there was a large yellow-staining nucleolus as well as several very small fuchsinophile nucleoli. While a few fuchsinophile nucleoli are present in normal nuclei, no yellow nucleoli were observed in the nuclei of the cells of normal glands. Some of these

TABLE 1

FINDINGS FOR GONADECTOMIZED MALES AND FEMALES OF EIGHT F1 CROSSES AT TWO AGE GROUPS

<table>
<thead>
<tr>
<th>Cross</th>
<th>Sex</th>
<th>No. of mice</th>
<th>6 to 15 Months</th>
<th>14 Months and beyond</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adrenal tumors</td>
<td>Pituitary tumors</td>
</tr>
<tr>
<td>dba 9 × C57 6</td>
<td>♀ ♀</td>
<td>8</td>
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<td>0</td>
</tr>
<tr>
<td>C57 9 × dba ♀</td>
<td>♀ ♀</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dba 9 × ce ♀</td>
<td>♀ ♀</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ce 9 × dba ♀</td>
<td>♀ ♀</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ce 9 × C57 ♀</td>
<td>♀ ♀</td>
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<td>0</td>
</tr>
<tr>
<td>C57 9 × ce ♀</td>
<td>♀ ♀</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A ♀ × CSH ♀</td>
<td>♀ ♀</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CSH ♀ × A ♀</td>
<td>♀ ♀</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The scale in which all cell sketches are reproduced is 2 cm. = 0.01 mm.

Fig. 4.—Large transitional basophiles and a degranulating basophile. There is degranulation of the transitional cells and several nuclei are lobular. From 18 months old gonadectomized male (ce 9 X dba♂) F1 hybrid WK 450. Mag. ×450.
cells were partially degranulated, only a little cytoplasm being found adjacent to the nucleus and at the periphery of the cell.

The cytoplasmic elements of the large cells were always basophilic and the cytoplasmic elements appeared either finely granular or foamy. The negative image of the Golgi apparatus that was observable by the staining methods used was comparable to that found in normal ripe basophiles, doughnut shaped. Some of these cells had large polymorphic and/or lobular nuclei and some cells were multinucleate. The nucleoli were very large, filling almost the entire nucleus. They stained pale blue and appeared to be vesicular.

Occasionally among the above described small and large cells, very small cells were found with a clear bluish gray cytoplasm and little or no granulation. In the nuclei of the cells, the nucleoli were large and fuchsinophile staining.

There were many variations in the amount of granulation of the cells that comprised the opaque nodules. There were no mitotic figures observed in any of these cells. These cells have been designated transitional basophiles and the opaque nodules shall be called focal basophile adenomas.

The acidophiles were absent in the opaque areas while those that were found adjacent to the opaque nodules were very densely arranged and were somewhat larger than average acidophiles in normal glands. Normal acidophiles ranged from 9.92 micra to 10.41 micra while the acidophiles adjacent to the nodules were from 10.17 micra to 13.81 micra in diameter. Other than the size difference the acidophiles seemed normal. The staining reactions of the a granules seemed normal and no abnormal nuclei were observed. The negative image of the Golgi apparatus which forms a cap over the nucleus in acidophiles also appeared normal.

The Hemorrhagic Nodules.—The nodules, which appeared to be hemorrhagic grossly, were also non-capsulated. They frequently contained hemorrhagic cysts and were surrounded by scattered clusters and strands of abnormal basophilic cells. In some nodules free blood cells were found amid strands of abnormal basophiles. In some areas the basophiles showed evidence of loss of cohesion. These basophiles were coarsely granular and the negative image of the Golgi apparatus was hypertrophied, and filled almost the entire cytoplasmic area. In glands with large hemorrhagic areas and basophiles, usually no other cell types were present.

Other Abnormalities.—There were several other abnormalities that are worthy of mention and that do not fall into the types given above.

Two types of colloid cysts were noted in the hypophyses examined, both occurring in the anterior lobe. The first and most common type was lined by thin epithelial cells. The colloid was usually basophilic, only occasionally being acidophilic. These cysts were usually very small and occurred in clusters or singly.

The second type of colloid cyst was lined by cuboidal ciliated cells (Fig. 9). Some flat non-ciliated cells were found occasionally. The appearance of these non-ciliated cells might be due to a gradual transformation from non-ciliated to ciliated cell type. The colloid in these cysts was always basophilic.

From the study of the glands it is evident that cysts are common in the anterior lobe of the pituitary. In the present series they were found most often in control males and gonadectomized females as is shown in Table 2. These are by no means absolute figures since none of the glands of the control animals and only some of the glands of the experimental animals were serially sectioned, therefore many cysts may have been missed.

Another abnormality was found in several pituitaries of gonadectomized and control animals. Groups of two to six cells were surrounded by normal anterior lobe cells. These encircled cells had very large nuclei and the cytoplasm was either very light staining or seemed to be absent. It was thought that they might be degenerating basophiles, but later they were diagnosed as phagocytes.

In a castrate male F1 (db9 X C57 Black), 21 months of age six giant cells were found.2

In WK 706, a 26 months old castrate male F1 (ce9 X dba), the pituitary contained a yellow nodule. Microscopic analysis showed that this nodule was composed of cells containing yellow lipochrome pigment. Close to this area cholesterol clefts separated by flattened epithelial cells were found. This area of tissue joined both lobes but did not invade either lobe to any extent. Such cholesteatomas have been described and do occur occasionally in the hypophysis.3 In the adjacent anterior lobe amyloid degeneration occurred in the blood vessel walls.

The only abnormality that occurred in the control series, other than the hyaline cysts, was a chromophobe adenoma that was found in a 30

1 Diagnoses were made by Miss Elizabeth Fekete of this laboratory.
2 There seems to be no simple explanation as to why these should have been present in the pituitary. Diagnoses by Miss Elizabeth Fekete of this Laboratory.
3 Cholesteatomas are formed by epithelial cells that were left behind at the time of the closure of the neural crest and grow slowly throughout life to form these benign tumors (10).
FIG. 5.—Group of acidophiles, chromophobes and a basophile from a normal section of an abnormal gland. From hypophysis of a 17 months old gonadectomized male (dba♀ × cc♂) $F_1$ hybrid WK 431. Mag. ×439.

FIG. 6.—Hemorrhagic cysts lined by abnormal basophiles in the pituitary of a 23 months old gonadectomized male (ce♀ × dba♂) $F_1$ hybrid WK 615. (Photomicrograph made from lantern slide; original negative lost, magnification unknown since laboratory fire.)

FIG. 7.—Hypertrophied basophiles; note enlarged negative image of the Golgi apparatus and large nucleoli. No other cell types present in this section. From 17 months old gonadectomized male (dba♀ × ce♂) $F_1$ hybrid WK 431. Mag. ×439.
Fig. 8.—Region of abnormal basophiles showing loss of cohesion of these cells. From WK 431. Mag. X439.

Fig. 9.—Cyst lined by ciliated epithelium at the periphery of the anterior lobe of the pituitary of an 18 months old gonadectomized female (ce♀ × dba♂) F1 hybrid WK 448. Mag. X132.

Fig. 10.—Section of abnormal pituitary showing enlarged sinuses. Section is composed of transitional basophiles and hypertrophied basophiles. From WK 448. Mag. X93.

Fig. 11.—Mammary gland of a 20 months old gonadectomized male (dba♀ × ce♂) F1 hybrid WK 516. Note secretion globules in the alveolar cells and ducts distended with secretion. Mag. X132.
months old control female F₁ (C57 Black X dba♂).¹

MICROSCOPIC OBSERVATION OF THE MAMMARY GLANDS

The mammary glands of most of the experimental animals with hypophyseal tumors were examined histologically and showed alveolar cells and alveoli filled with secretion and the ducts distended with secretion (Figs. 11 and 12). In some of the glands there were areas of hyperplastic nodules (so-called precancerous lesions) and in four castrate males and a few gonadectomized females, mammary gland carcinomas were found. The mothers of these F₁ animals were dba strain mice. These mice had the milk factor from strain dba, and therefore with proper endocrine stimulation the mammary tumors might be expected, at least in intact animals. In other experimental animals where the milk factor was absent, the glands were well developed, but hyperplastic areas (so-called precancerous lesions) and mammary tumors never occurred.

In studying the mammary glands of castrate animals where no pituitary abnormalities occurred, there was no alveolar development observed. The amount of growth up to the stage of alveolar formation of these glands depended on the extent of abnormality occurring in the adrenals.

DISCUSSION

The results show that these abnormal pituitaries present several significant facts. The first and perhaps most important, is that in all cases the adrenal cortical tumors preceded the appearance of the gross hypophyseal tumors. Second, two types of nodules or tumors were present and both types were composed of basophiles, and third, the pituitary alterations were correlated with physiological changes in the animals that were manifested particularly in the mammary glands.

Very few spontaneous hypophysal abnormalities have been reported previously in mice. Most of the abnormalities that have been reported were found to be disturbances of chromophobe cells and were observed following treatment with estrogens (5, 6, 16). Many spontaneous chromophobe cell adenomas have been found and studied in rats of advanced age (37, 40), and following treatment with various hormones (36, 38, 39).

For purposes of differentiating the present series of tumors of the anterior pituitary from chromophobe adenomas that have been reported, it might be said first of all that we are dealing with a different endocrine background than that used in the study of chromophobe adenomas. Here the hormonal stimulation arises from adrenal cortical hormones in castrate animals. Chromophobe adenomas have been observed both in intact old mice and rats and following estrogenic treatment in

TABLE 2

<table>
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<tr>
<th>SEX</th>
<th>NUMBER OF ANIMALS</th>
<th>CILIATED CYSTS</th>
<th>NON-CILIATED CYSTS</th>
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<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>ϕ</td>
<td>186</td>
<td>14</td>
<td>7.52</td>
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</tr>
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<td>ϕ♂</td>
<td>211</td>
<td>14</td>
<td>6.64</td>
<td>43</td>
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<tr>
<td>Total</td>
<td>424</td>
<td>21</td>
<td>4.95</td>
<td>57</td>
</tr>
</tbody>
</table>

*There is little difference in the percentage occurrence between the gonadectomized and control groups, but there is a statistically significant sex difference in the occurrence of non-ciliated cysts in the control F₁ hybrid mice.

Yet both species. Chromophobe adenomas produce a condition stimulating hypopituitarism when they occur following estrogen treatment, while in this series, from the physiological condition of the animal as expressed in the accessory reproductive organs, a state of hyperfunction exists. Part of the hormonal stimulation is directly attributable to the hormones secreted by the adrenal tumors but the extreme alveolar development of the mammary glands seems to depend upon the presence of the abnormal basophilic cells in the pituitary.

Reasons for believing that these various types of cells found in the abnormal areas of the pituitaries were basophiles are manifold. Their staining reaction with aniline blue showed that they had a great affinity for that dye. The negative image of the Golgi apparatus, which is quite different in normal acidophiles and basophiles, was always like that found in mature basophiles even though at times it was greatly hypertrophied. This hypertrophy indicates a heightened activity of the cell (15). There was obviously great physiological activity from the intense alveolar development of the mammary glands. Severinghaus states that with chromophobe adenomata there is no physiological alteration in the animal except that caused by de-
struction of the anterior lobe and invasion of adjacent tissues. Furthermore, basophile cell disturbances are almost invariably associated with changes in the adrenal cortex (30).

Another factor in favor of classifying the present series of pituitary abnormalities as basophile is the size of the tumors. Where chromophobe adenomas occur they are described as large, filling the entire base of the skull and exerting pressure on the brain (16, 40, et al.). However, the tumors described here and those basophile adenomas reported elsewhere (17) have never attained this great size. They are discrete nodules not of sufficient volume to exert great stress on surrounding tissues. They have been observed to interrupt the normal contour of the intermedia and very occasionally of the posterior lobe.

The nodules found in the pituitaries of these mice were non-capsulated, but no invasion of other lobes or surrounding tissues was observed. Mitoses were rare, but evidences of amitotic division were prominent, a condition thought to be non-existent in a normal hypophysis. By calling this condition amitotic division the authors are merely calling attention to the fact that the multinucleate cells were dividing in this manner or mitoses were incomplete and the cytoplasm did not divide. Another supposition is possible, and that is that mitosis was occurring too rapidly to permit the cytoplasm to divide. Selye has formed this opinion from studying the pituitaries of rats after hormone treatment (28).

The large transitional basophiles were in orderly acinar groups, while the small transitional cells and the abnormal basophiles were in dense clusters. These small cells may have, as Severinghaus states, passed a phase of hypersecretory activity and have become exhausted. The nodules formed by large transitional basophiles and small transitional cells follow closely Ewing's description of diffuse hyperplasia in the pituitary in man.

"On sections the growth may be diffuse . . . or focal adenomas may appear as opaque spots. . . ." "The arrangement of cells is orderly and mitoses are rare. While the gland usually remains solid, small cysts filled with colloid may develop and hemorrhages may occur" (12).

The relations of cell types and various hormones have been established. Acidophiles are associated with growth-promoting factors; these cells are said to be absent in dwarf mice (33) and there is an overabundance of them in acromegals, in man (17). Pregnancy affects both acidophiles and basophiles (30). Basophiles are modified by thyroidectomy (31), states of thyroxine deficiency (18), and thyroid hyperplasia and adenomata (19). Also, evidence has been secured that basophiles secrete the adrenocorticotrophic hormone (25). No where as yet in the present series have been found morphological differences correlated with the presence of these pituitary tumors other than the unusual development of the mammary glands.

From the literature very little reference was found, however, to mammary gland growth allied with pituitary disfunction. It has usually been modified by steroid hormone injection. It is known that mammary development follows treatment with some steroid hormone preparations, and in certain strains chromophobe adenomas occur. It is reasonable to assume that the development of the mammary glands is due to the interaction of the hor-

**Fig. 12.** Mammary gland of 18 months old gonadectomized female (dba $\delta \times$ ce$\sigma$) $F_1$ hybrid WK 61. The alveolar spaces and the ducts are distended with secretion. Mag. X132.

**Fig. 13.** Mammary gland adenoma (at right) and carcinoma (at left) from a 16-1/2 months old gonadectomized male (dba $\delta \times$ ce$\sigma$) $F_1$ hybrid WK 62. In the adenomatous area there is some secretion in the alveoli. Mag. X93.
mone with various pituitary factors, or the chromophobe adenomas are transitional basophile types and hence would secrete the factors necessary for the mammary gland growth or at least part of its growth. Evidence against the latter is the difference in tumor size, and in spontaneous chromophobe adenomas no physiological action on the accessory reproductive system has been recorded. That this is true is shown by our observations that with the presence of the hypophysial nodules, the excessive mammary gland development was the constant feature. The mammary gland development depends in part on the release of gonadotropic factors (34), a reason for believing that the substances associated with the abnormal basophiles are gonadotropin-like.

In parabiotic experiments, where a castrated rat was joined to an intact rat, it has been observed that there is an increase in the number of basophiles in the pituitaries of both animals, interpreted as storage of gonadotropic hormones (27, 35).

Hypertrophy of the Golgi bodies, and degranulation occur; these latter indicate an increase in secretion and its release (53). Thus it might be assumed with pituitary basophilism, that: (1) the storage and release of gonadotropins is greatly increased. A greater production of gonadotropins might affect the mammary glands in these experimental mice particularly since the gonads are not present and the hormones from the adrenals may either not regulate the gonadotropic action of the pituitary as the gonads do, or (2) the adrenal cortical secretions may be slightly different from those of a normal gonad, or (3) the pituitary secretions may act directly.

It is not the purpose of the authors to discuss the hormonal aspects involved in the present series at this time, since the substances elicited from the adrenal cortical tumors have not yet been identified, but to say briefly that the substance or substances elicited from the pituitary and adrenal glands seem to be of a gonadotropic and gonadal nature respectively. The effects produced on the mammary glands and accessory reproductive organs are similar to the effects produced by the hormones from the ovaries or combinations of ovaries and testes when they are present. It is well known that there are close relationships between the hypophysis and gonads and the hypophysial and adrenals. Recently much evidence has been reported on the interrelation of the pituitary and the mammary glands through the action of marnmagenic hormones that affect the duct extension and lobule alveolar growth (23, 26). Gardner has stated that the hypophysis and the mammary glands are so closely related that the mammary gland development cannot be discussed unless the role of the pituitary is taken into consideration (16).

The presence of mammary tumors in certain of these animals with anterior pituitary tumors and also in the parental strain dba would seem to establish for gonadectomized animals the factors necessary for mammary tumor production. It is known that in intact animals the proper genetic constitution (such as in strains dba or C3H), hormonal influences and Mammary Tumor Inciter (M.T.I.) are necessities for spontaneous mammary tumor appearance generation after generation (9). Now in these animals under discussion, it is possible to postulate that the genetic constitution and

### TABLE 3

**HYPOTHESIS TO SHOW THAT MAMMARY TUMOR PRODUCTION IN GONADECTOMIZED MALE AND FEMALE MICE IS DEPENDENT UPON FOUR *FACTORS***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genetic constitution</th>
<th>Adrenal tumors</th>
<th>Pituitary tumors</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(Hormonal influence)</td>
<td>M.T.I.†</td>
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</tr>
<tr>
<td>dba</td>
<td>+</td>
<td>+</td>
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* The genetic constitution of the animal, the hormonal influences which are supplied by the adrenal tumors, the presence of mammary tumor inciter, and the composition of basophilic pituitary tumors.
† Mammary tumor inciter.
been known to occur as a part of this and other syndromes. McLetchie (24) has stated that the castrate condition and Cushing's syndrome have much in common as in the latter there is frequently gonadal atrophy. Selye reports that it is very probable that in these cases tumorous hypophyses are capable of producing an excess of such pituitary hormones as adrenotropic, mammotropic, lactogenic and perhaps other factors (29). McLetchie declares further that:

"... it is the rule rather than an exception to find adreno-cortical hyperplasia associated with basophil adenoma and again in some cases of adreno-cortical carcinoma, basophilia, that is the relative increase in basophil cells, is present... hypersecretory processes of the basophile cells and the adrenal cortex are complimentary, the one producing the other" (24).

In the adreno-genital syndrome, and in intersexuality, it has been thought that the pituitary becomes abnormal first and then the adrenal changes follow (4). In Addison's disease however, the adrenals become abnormal and the hypophysial changes follow (8). In this experimental series the gross adrenal changes are primary. In some crosses adrenal cortical tumors occur as early as 8 months of age, while the hypophysial changes are not grossly observed before 14 to 18 months in the dba X ce crosses and later in other crosses. It is possible to postulate that the pituitary may be thrown into unbalance primarily by castration, exert its effect on the adrenals in the absence of the gonads, and the adrenals in turn then become abnormal. The abnormal adrenals then secrete excess or unusual hormones which react on the pituitaries and they, then, become abnormal.

It seems pertinent to further compare the syndrome in mice with Cushing's syndrome in man. This may well be an animal counterpart of Cushing's syndrome. The latter syndrome is a baffling problem both as to cause and therapy (9). Kepler (21) in an analysis of the problem has made several assumptions that might be applied to the mice. He states that the pituitary may become overactive primarily and cause the development of adrenal hyperplasia or neoplasia and then the abnormal adrenal cortices function excessively. If the abnormal basophilic cells are the primary cause of the adrenal disturbance, unless they produced only the adrenotropic agent and no other tropins, some manifestations should remain following removal of the adrenal tumors. Now, rather than suppose that the basophiles are secreting only one hormone, let us assume they are responsible for, and producing, at least one other tropin, one that affects mammary development since this seems to be the case in these animals. Woolley has transplanted an adrenal tumor from a ce mouse with a basophilic pituitary tumor and hyperplastic mammary glands. The transplanted adrenal tumor did not cause overdevelopment of the mammary glands in the new hosts, ce gonadectomized mice (44). It may be assumed that the pituitary tumor either directly or indirectly, rather than the adrenal tumor itself was responsible for the mammary gland growth. Here then, in mice at least is a factor that seems to be missing or that has taken another form of expression in man. The present situation then would be analogous to eosinophilic tumors in man (22) where there are other factors influenced by the anterior lobe cells. This would further advance the possibility that the pituitary is the primary site of the disturbances rather than the adrenals.

Castration in rats and mice causes increased gonadotropic potency. One might assume that the gonadotropins are transformed into a substance either identical with adrenocorticotrophic hormone (ACTH) or similar to ACTH that can act on the adrenals. Other workers have given serious consideration to the idea that since one cell type seems to produce so many hormones, the cell types may in effect secrete one or two basic precursors (17) that act in various ways according to the demands made upon it by other glands.

It is felt however, that the investigation of these tumors is by no means complete. Since there is a gross macroscopic change and histological change, perhaps there are identifiable biochemical changes also. Too, the developmental stages need to be studied more completely.

No cell types have been identified as exact counterparts of Crooke's change, the hyalinization of the basophile cells. It might be speculated that the large and often multinucleate abnormal cells in these mice are similar to the hyaline basophiles or Crooke's cells, but the hyalinization in the basophiles of the pituitaries of the mice is less acute, if present at all. These abnormal basophile cells in the mice compare more closely with the hyper- trophic amphophiles described by Mellgren and others (25). Mellgren believes that these cells, the amphophiles, are later stages in the degranulation and hyalinization of the basophile cells. This might be the case in the mouse pituitaries since these anterior pituitary abnormalities seem to be in well-advanced stages of alteration at the time of autopsy. Collection of the early stages was not possible in this series because the experiments were well advanced at the time the pituitary abnormali-

4 Cushing's syndrome indicates only those cases in which an adrenal tumor is present.
ties were first noted macroscopically. It is hoped that more information on analogies and developmental processes will be learned by a study of pituitaries of younger gonadectomized animals.

From the preceding discussion, it is very evident that while the abnormalities of the pituitaries are a fact, their function, i.e. the function of the cells composing the abnormalities, with respect to the secretions they may produce is very speculative. In classifying the abnormalities as basophile disturbances, it is realized that this designation may be controversial. However, on the basis of effects on the mammary glands of the animals and the discussion of pituitary adrenal relationships, this classification is, at present, justified.

SUMMARY

Mice of the \( F_1 \) generation reciprocal crosses of JAX strains C57 Black, dba, ce, A and C3H were gonadectomized at 1 to 3 days of age. Virgin females and unmated males were used as control animals. At autopsy, from 14 to 26 months in the experimental groups, pituitary abnormalities occurred. Adrenal cortical carcinomata were also present. The highest incidence of hypophyseal abnormalities occurred in reciprocal hybrids of strains dba and ce. Histological analysis of the hypophyses showed that they were hyperplastic and some were adenomatous. These changes were considered to involve the basophile chiefly and it was decided that the abnormalities were basophilia (possibly adenomas) and focal adenomas. The most striking change, concurrent with the hypophyseal tumors, was the extensive differentiation and over-development of the mammary glands. This change became a criterion for predicting the presence or absence of the hypophyseal changes.

The possibilities as to hormonal activity of the hyperplastic and adenomatous areas of the pituitary were discussed and it was thought that in the combination of pituitary-adrenal disfunction, substances were secreted similar to the gonadotropic and gonadal hormones that reacted most differentially on the mammary glands. The pituitary tumors were compared to Cushing's syndrome in man. The pituitary response, i.e. the appearance of the tumors was, to some extent, a strain-limited factor, as is the pituitary response to hormonal injection.

It was postulated that although the gross pituitary changes were secondary to the changes observed in the adrenal, the fundamental change might have occurred first in the pituitary, reacted on the adrenals, and secretions emanating from the adrenals further reacted on the hypophysis so that gross changes occurred eventually after the adrenals were tumorous.

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CORRECTION

The title of the last column of Table I in the paper by Bass and Feigelson, Cancer Research, 8: 507, October 1948, should read “Dry weight mean” instead of “Wet weight mean.”

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Spontaneous Basophilic Tumors of the Pituitary Glands in Gonadectomized Mice

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