Neoplasms in Rats Treated with Pituitary Growth Hormone

III. Reproductive Organs

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This paper deals with the neoplastic and hyperplastic changes in the ovaries, uteri, and mammary glands of rats treated with growth hormone. Abnormalities of these organs occurred in 12 of 15 female rats following the administration of pituitary growth hormone for long periods. The neoplasms occurring in the lungs, lymphatic tissues, and adrenal glands of these rats have been reported in the preceding papers of this series (8, 9).

After reaching the plateau in their growth, female rats of the Long and Evans strain, 237–239 days old, were injected intraperitoneally with pituitary growth hormone daily,1 6 days a week, for a maximum period of 485 days. The initial daily dosage of growth hormone was 0.4 mg.; this was increased at intervals to a maximum of 3.0 mg. A similar group of adult females was injected parenterally with comparable amounts of albumin.

Serial sections of the ovaries were prepared. The uterine horns were fixed, and blocks of tissue from both horns were taken for microscopic examination. The mammary glands were examined both by spreads and by sections. The mammary spreads were prepared by stripping the mammary tissue from the skin and fixing in formol. The spreads were stained with alum carmine and cleared in methyl salicylate.

RESULTS

OVARIES

Control rats.—The ovaries of most of the controls were not remarkable except for certain changes previously reported in rats of the same age. Follicles and corpora lutea in various stages of normal development and regression were pres-

TABLE 1

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* Rats B6508 and BH6092 had macroscopically visible tumors of the pituitary gland.
ent in twelve rats (Table 1). In the remainder of the controls, cystic follicles and very old corpora lutea were present.

As reported previously concerning rats of comparable age, there were pseudo-testicular tubules in all ovaries (3). These structures were formed by pale cells resembling Sertoli cells. In nine rats there were many spherical structures composed of small cells with clear cytoplasm and small round nuclei with scanty coarse chromatin granules (Fig. 1). These cells were apparently derived from the theca interna of involuting follicles. Focal areas of papillary hyperplasia of the germinal epithelium were present in five rats. In one rat there was a small papillary cystadenoma composed of low columnar cells (Fig. 2).

Experimental rats.—The ovaries of the experimental rats were quite different from those of the controls (Table 2). Although follicles and corpora lutea were present in all but two animals, the follicles were not as numerous, and recently formed corpora lutea were only occasionally present. Papillary proliferation of the germinal epithelium occurred in eight rats. Small papillary cystadenomata were present in two rats. Pseudo-testicular tubules were more numerous and often larger than those noted in the controls. Bizarre, small, atypical follicles were present in six rats. Solid tumors of the ovaries were present in two rats.

In the eight rats with papillary proliferation of the capsule, the papillary processes were composed of pseudo-stratified columnar cells with elongated nuclei. These areas were very numerous in both ovaries of one rat (Fig. 3) and in another rat were associated with a cystadenoma (Fig. 4) of the same ovary and multiple tumors of the opposite ovary. The cystadenomata which were present in two rats were small and lined by columnar cells with basally placed hyperchromatic nuclei. The nuclei varied slightly in size and shape. Mitoses were rare.

Pseudo-testicular tubules similar to those observed in the ovaries of the control rats were also present in the ovaries of thirteen of the experimental rats. These tubules were usually larger and more numerous than those in the controls. Areas composed of large interstitial cells with abundant

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**Fig. 1.—**Rat B6508. The germinal epithelium shows papillary hyperplasia. Several atretic follicles composed of abnormally pale thecal cells lie immediately beneath the germinal epithelium. The remainder of the tissue shows very numerous pseudo-testicular tubules and interstitial cells. This degree of abnormality was not encountered in any of the other controls. This animal had an adenoma of the pituitary gland. X125.

**Fig. 2.—**Rat G6460. A small papillary cystadenoma is present. There are four tubular structures, two of which are of the pseudo-testicular type. X125.
pale cytoplasm were present in five of the experimental rats: pseudo-testicular tubules were very numerous in these areas (Fig. 5).

The small atypical follicles were characterized by absence of ova and proliferation of the cells of the granulosa and theca interna. The granulosa cells had hyperchromatic, irregular nuclei. Occasional mitoses were present in both the granulosa and theca interna. The outlines of these follicles were often very irregular (Figs. 6, 7).

In one rat (W6324) the right ovary was grossly involved by neoplastic changes. The left ovary revealed proliferation of the capsule as stated above. The neoplastic right ovary weighed 336 mg., and in this ovary there were three separate lesions was similar to that of a testicular adenoma of the ovary.

A small, solid tumor was present in rat BH6334. This tumor had the pattern of a granulosa-cell tumor. Small atypical follicles were present. The remainder of the ovary was composed of large pale interstitial cells and some pseudo-testicular tubules.

**Uteri**

**Control rats.**—In ten rats the uteri were normal (Table 1). There were a few small endometrial glands. The epithelium was low columnar, and the stromal cells were small. Glandular cystic hyperplasia of the endometrium was present in one animal; endometrial polyps were found in three: and

<table>
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Tumors (Fig. 8). Two of the tumors showed a mixed pattern which suggested a granulosa-cell tumor in some areas and interstitial-cell tumor (luteoma) in other areas. In the areas with the pattern of a granulosa-cell tumor (Fig. 9) the cell types were very similar to those of the atypical involuting follicles described above. Cells resembling those of the granulosa occurred in small compact masses and rosettes; these masses and rosettes were separated by cells resembling those of the theca interna. In areas showing the pattern of a luteoma or interstitial-cell tumor, the cells were moderately large with abundant cytoplasm. In most of these areas the cells were closely packed together; in some areas the cells were quite large and had very pale or clear cytoplasm (Fig. 10). A cyst was present in association with one tumor. The third tumor was composed of numerous large pseudo-testicular tubules with interspersed large pale interstitial cells (Fig. 11). The pattern of this an early adenocarcinoma of the endometrium was present in one rat.

The glandular cystic endometrial hyperplasia was characterized by numerous large and frequently dilated endometrial glands composed of columnar cells. The stroma was edematous, and the cells were slightly enlarged.

The polyps were formed of large endometrial glands. The epithelial cells were columnar in most glands. There was cystic dilatation of some of the glands, and in these the epithelium was flat. The cells of the stroma were normal.

In one rat an adenocarcinoma of the uterus was found microscopically, although there was no grossly visible abnormality of the involved horn. The glands of the tumor were composed of large atypical epithelial cells with abundant cytoplasm and hyperchromatic nuclei. Occasional mitoses were present. The myometrium was not invaded.

**Experimental rats.**—There was hypertrophy of
the myometrium in all rats. The endometrium was normal in eleven rats. Glandular cystic hyperplasia of the endometrium was present in two rats, and in one of these there was also an endometrial polyp. A polyp of the endometrium was present in one additional rat. In one rat (G6534) the entire right uterine horn was greatly enlarged to a diameter of 4.5 cm. by a tumor, and it was adherent to loops of bowel. The neoplastic epithelial cells were large and had large, irregular, hyperchromatic nuclei. Mitoses were numerous. These cells were arranged in atypical glandular structures and small compact groups. The myometrium was extensively infiltrated and replaced by tumor. There were many areas of necrosis with polymorphonuclear leukocytic infiltration and abscess formation (Table 2).

Fig. 3.—Rat G6255. There is marked papillary hyperplasia of the germinal epithelium. The cortex contains many follicles undergoing atresia; the cells of the theca interna are abnormally pale. ×125.

Fig. 4.—Rat W6324. Left ovary. A papillary cystadenoma composed of columnar epithelial cells is present. The tissue at the lower left is composed of small pseudo-testicular tubules. (See Figs. 8-11, right ovary.) ×125.
MAMMARY GLANDS

Control rats.—In twelve rats the mammary tissue was of the virginal pattern; there were a few ducts, occasional alveolar buds, and rare, fully expanded alveoli. In two control rats there was lobulo-alveolar development and solitary small fibro-adenomas; however, both of these rats (B6508 and BH6262) had adenomas of the pituitary gland. One additional control rat had a small fibro-adenoma. These tumors were characterized by proliferating interlobular and intralobular connective tissue which surrounded ducts and a few alveolar buds (Table 1).

Experimental rats.—There was lobulo-alveolar development varying from slight to marked in seven of the rats. The degree and distribution of the lobulo-alveolar development varied from animal to animal. There were areas showing only ducts and alveolar buds interspersed with areas of lobulo-alveolar development. In the rest of the experimental rats the mammary tissue resembled that of the controls and showed only ducts and alveolar buds.

Numerous fibro-adenomata of the mammary tissue were present in seven rats. Most of these tumors were larger than those occurring in the controls. In three rats with fibro-adenomata, the mammary tissue showed only ducts and alveolar buds; in four rats with fibro-adenomata, there was lobulo-alveolar development. In each of two rats (W6324 and G6269), there were eleven fibro-adenomata and fibromata. All the fibro-adenomata were similar and characterized by proliferation of the interlobular and intralobular connective tissue with no significant proliferation of the epithelium. Fibromata of the mammary tissue were present in three rats. The fibrous connective tissue forming these tumors was dense and sparsely cellular. There were no malignant tumors of the mammary tissue (Table 2).

DISCUSSION

The outstanding changes in the ovaries of rats injected with pituitary growth hormone were the presence of atypical small follicles and solid tumors. The absence of ova and the small size of the ovaries indicated that these abnormal follicles had

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Fig. 6.—There are a number of small follicles. Some of these show normal atretic changes. Others show abnormal proliferation of the theca interna and granulosa. X125.

Fig. 7.—Higher magnification of atypical involuting follicle. Note the marked overgrowth of the theca interna and irregular outlines of the proliferating granulosa. There are islands of normal interstitial cells. X355.

Figs. 6 and 7.—Experimental rat G6553. Ovary
undergone involutionary changes, whereas the mitotic activity and alterations in the general pattern of these follicles suggested abnormal stimulation. Our interpretation of these apparently dissimilar findings is that these changes are due to abnormal activity of the anterior pituitary superimposed on involuting follicles. It is considered likely that these atypical small follicles represent an early stage in the development of the solid tumors of the ovaries with the granulosa-cell and interstitial-cell tumor patterns. The occurrence of ovarian tumors in rats following the chronic administration of pituitary growth hormone was also noted in a previous similar experiment (3). Ovarian tumors have not been observed in hypophysectomized rats receiving growth hormone for similar periods (10). It has also been shown that there are morphologic changes in the anterior pituitary following chronic administration of growth hormone to female rats after their weight reached a plateau. There was a decrease in acidophils and an increase in chromophobes; in two rats the histologic picture in the anterior pituitary was of the castration type (5). The above findings suggest that alteration in the function of the anterior pituitary gland following the administration of large amounts of growth hormone is an important factor in the production of ovarian tumors.

Of interest in connection with these ovarian tumors is the work of Biskind and Biskind (2), Li and Gardner (7), and Furth and Sobel (4). These investigators induced ovarian neoplasms by transplanting ovarian tissue into the spleens of castrate rats (2) and mice (4, 7). Biskind and Biskind observed that there was no growth of the intrasplenic transplants of ovarian tissue in rats that were hypophysectomized (1). It has been postulated (11) that this neoplastic transformation is dependent on inactivation by the liver of estrogens from the ovarian transplant in the spleen; this is

3 These hypophysectomized rats were younger and received smaller amounts of growth hormone than the animals in the present report. Because of the greater sensitivity of these rats to growth hormone their rate of growth was comparable to that occurring in normal rats receiving much larger amounts of growth hormone. An additional group of hypophysectomized rats, of the same age as the rats in the present report, is being treated with growth hormone in amounts similar to that used in the present study.

Fig. 8.—Topographic view showing nodular masses of neoplastic tissue which have completely replaced normal ovarian tissue. ×8.

Fig. 9.—Higher magnification of one area showing pattern of granulosa-cell tumor. Note resemblance of the dark area to granulosa and the pale area to the theca interna of the atypical involuting follicle in Figs. 6 and 7. ×355.

activation results in abnormal stimulation of the intrasplenic ovarian tissue by gonadotropins.

No remarkable differences were noted between the uteri of the experimental and control rats.

The mammary tissue in many of the experimental rats showed localized or generalized development; this was in noticeable contrast to the controls. In the latter group, excluding the two rats with spontaneous pituitary neoplasms, the mammary tissue consisted of a few ducts, occasional alveolar buds, and rare alveoli that were fully expanded. There were multiple fibro-adenomata of the mammary glands: these often reached considerable dimensions. A single small fibro-adenoma was present in each of three control rats, and two of these rats had pituitary tumors.

SUMMARY AND CONCLUSIONS

1. The long-term administration of pituitary growth hormone to fifteen normal female rats after their weight had reached a plateau resulted in atypical hyperplasia of small follicles in five rats and in solid tumors of the ovaries in two rats. No similar changes were present in the control rats.

2. The atypical hyperplasia of small follicles is considered an early stage in the development of granulosa-cell and interstitial-cell tumors of the ovary.

3. Fibro-adenomata of the mammary tissue occurred more frequently, were larger, and were often multiple in rats injected with growth hormone.

4. The above changes are regarded as the result of the chronic administration of pituitary growth hormone which causes a marked disturbance in the normal function of the pituitary gland.

REFERENCES


Fig. 10.—This area has the appearance of an interstitial cell tumor. The cells in the lower half have a small to moderate amount of cytoplasm and are arranged compactly whereas in the upper half the cells are larger and form a reticulated pattern. ×355.

Fig. 11.—This was a separate tumor of the same ovary and it was composed entirely of pseudo-testicular tubules and interstitial cells. ×355.
FIGS. 8–11.—Experimental rat W894. Right ovary


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