Dependent and Autonomous Mammotropin Pituitary Tumors in Rats; Their Somatotropic Features*

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The discovery by several investigators (6, 26, 39) that large quantities of estrogen will induce pituitary tumors has been amply confirmed, but there has been little agreement as to the character of these tumors. Lacour (see [5]) noted that they were organophilic, and Zeckwer (38) suggested they were mammotropin. These conclusions have been confirmed by studies on the histogenesis of the tumors and by hormone assays (4, 27). Dunning et al. (7) observed that pituitary tumors induced by estrogen could be grafted on estrogen-treated but not on normal rats; this would indicate that such tumors are conditioned neoplasms. Gardner (14), however, found no regression of primary estrogen-induced tumors in mice after estrogen withdrawal.

The present report describes studies on the transplantation of pituitary tumors in rats of the strain of origin, and of the hormonal secretions of these tumors as indicated by changes in organs of tumor-bearing hosts. All first-generation grafts were dependent tumors, but some of them became autonomous upon successive passages. Similarities between changes caused by mammotropin tumors induced in mice by ionizing radiations (34, 35) and those in rats by estrogens led us to conclude that they are essentially identical in character. The observations made suggest that the mechanism leading to their induction is essentially the same.

MATERIALS AND METHODS

Animals.—Experiments were performed on Fischer rats inbred in our laboratories and derived from litters obtained through the courtesy of Dr. Wilhelmina Dunning, who found this strain especially sensitive to pituitary tumor induction by estrogens (7).

Estrogen treatment.—For primary tumor induction and for conditioning of transplanted tumor hosts, fused estrogen pellets (diethyl stilbestrol, 25 per cent; cholesterol, 75 per cent) were implanted subcutaneously in the back of the neck.

Transplantations.—Tumor grafts were made as described for mice (14).

Autopsies.—Autopsies were performed on all animals that died or were killed. The term “well matched controls” indicates animals of the same age, sex, and treatment other than tumor graft. They were killed and autopsied, together with tumor-bearing rats. Tissues were fixed and prepared for microscopic examination as previously described (18).

Nomenclature and abbreviations.—These were explained in the preceding paper (18): Mt = mammotropin; MTH = mammotropin hormone; MtT = tumor composed of mammotropin cells. Similarly, At = adrenotropin, Tt = thyrrotropin, etc. Mammotropin, abbreviated MTH, will be used synonymously with mammotropin except in citations when other also presumably synonymous terms preferred by the authors (prolactin or luteotrophin) will be used.

RESULTS

Induction and transplantation.—Primary tumor induction was highly successful; a single pellet containing 7–8 mg. of stilbestrol was sufficient to induce tumors in most rats. Six such tumors from animals killed after 170–482 days of stilbestrol treatment were transplanted. Few of the primary grafts grew in normal hosts; all grew in hosts which received a stilbestrol pellet of about the same potency as that given to the rat in which the tumor was induced (Table 1). The latency period of grafted tumors as ascertained by palpation (158–567 days) was nearly as long as that required for their induction. Rats with first-generation tumor grafts had either primary tumors or much enlarged pituitaries at autopsy. The periods indicated in Table 1 merely express the periods of observation of animals with such tumors, since most animals were either sacrificed for experimental purposes or died of intercurrent disease.

Strain 4 was studied most extensively and will be discussed in detail. Grafts were first palpable 188 days after the injection of primary tumor fragments—the shortest latency period noted for a primary graft. Subpassages of Strain 4 were highly successful in estrogen-treated animals (Table 2). In passage Ic there was a marked shortening of the
**TABLE 1**

TRANSPLANTATION DATA OF SIX ESTROGEN-INDUCED PITUITARY TUMORS IN RATS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Tumor size (mm.)</th>
<th>Induction period (days)</th>
<th>Passage</th>
<th>No. +</th>
<th>Observation period (days)</th>
<th>Av. Range</th>
<th>Sex</th>
<th>No. inj.</th>
<th>Observation period (days)</th>
<th>Av. Range</th>
<th>Sex</th>
<th>No. inj.</th>
<th>Observation period (days)</th>
<th>Av. Range</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>5</td>
<td>170</td>
<td>Original</td>
<td>0/4</td>
<td>F</td>
<td>358 (329-408)</td>
<td>2/3</td>
<td>F</td>
<td>326 (287-354)</td>
<td>348 (331-364)</td>
<td>364</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In</td>
<td>1/2*</td>
<td>F</td>
<td>364 (346-394)</td>
<td>2/3</td>
<td>F†</td>
<td>253 (206-320)</td>
<td>275 (252-380)</td>
<td>398</td>
<td></td>
<td></td>
<td></td>
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<td>2</td>
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<td>5</td>
<td>179</td>
<td>Original</td>
<td>0/3</td>
<td>F</td>
<td>390 (324-447)</td>
<td>3/4</td>
<td>F</td>
<td>260 (239-339)</td>
<td>285 (346-366)</td>
<td>447</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>In</td>
<td>2/2*</td>
<td>F</td>
<td>400</td>
<td>1/1</td>
<td>F</td>
<td>319 (318-319)</td>
<td>319 (318-319)</td>
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<tr>
<td>3</td>
<td>F</td>
<td>6×8</td>
<td>398</td>
<td>Original</td>
<td>0/4</td>
<td>F</td>
<td>332</td>
<td>1/1</td>
<td>F</td>
<td>222</td>
<td>111</td>
<td>172</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>In</td>
<td>0/2</td>
<td>F</td>
<td>346 (314-378)</td>
<td>1/2</td>
<td>F</td>
<td>118</td>
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<td>319</td>
<td>Original</td>
<td>0/4</td>
<td>F</td>
<td>331 (506-335)</td>
<td>5/5</td>
<td>F</td>
<td>168</td>
<td>255 (145-335)</td>
<td>398</td>
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<tr>
<td>5</td>
<td>M</td>
<td>5×10</td>
<td>417</td>
<td>Original</td>
<td>2/2*</td>
<td>F</td>
<td>450</td>
<td>1/1</td>
<td>F</td>
<td>380</td>
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<tr>
<td>6</td>
<td>M</td>
<td>8</td>
<td>432</td>
<td>Original</td>
<td>7/8</td>
<td>M†</td>
<td>308 (223-367)</td>
<td>7/8</td>
<td>M†</td>
<td>483 (366-460)</td>
<td>483 (366-460)</td>
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</table>

* The tumors were minute, discovered at autopsy.

† Subpasses are detailed in Table 2.

† Gonadectomised.

**TABLE 2**

TRANSPLANTATION DATA OF ESTROGEN-INDUCED PITUITARY TUMOR STRAIN 4

<table>
<thead>
<tr>
<th>Donor</th>
<th>Tumor size (mm. av.)</th>
<th>Latency (days)</th>
<th>Passage</th>
<th>Sex</th>
<th>No. +</th>
<th>Av. Range</th>
<th>Observation period (days)</th>
<th>Range</th>
<th>Sex</th>
<th>No. inj.</th>
<th>Observation period (days)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>F</td>
<td>10</td>
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<td>0/4</td>
<td>F</td>
<td>435</td>
<td>321 (506-585)</td>
<td>5/5</td>
<td>F</td>
<td>158</td>
<td>253 (145-335)</td>
<td>5/5</td>
</tr>
<tr>
<td>La</td>
<td>F</td>
<td>10</td>
<td>138</td>
<td>2/4†</td>
<td>F</td>
<td>326</td>
<td>282 (229-320)</td>
<td>7/5</td>
<td>F</td>
<td>136</td>
<td>282 (97-407)</td>
<td>5/5</td>
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<tr>
<td>Ib</td>
<td>F</td>
<td>15</td>
<td>138</td>
<td>0/3</td>
<td>M</td>
<td>328</td>
<td>257 (227-317)</td>
<td>8/5</td>
<td>M</td>
<td>157</td>
<td>165 (165-171)</td>
<td>8/5</td>
</tr>
<tr>
<td>Ic</td>
<td>F</td>
<td>20</td>
<td>138</td>
<td>4/4†</td>
<td>F</td>
<td>328</td>
<td>257 (227-317)</td>
<td>8/5</td>
<td>M</td>
<td>157</td>
<td>165 (165-171)</td>
<td>8/5</td>
</tr>
<tr>
<td>IIa, b</td>
<td>M</td>
<td>30</td>
<td>97</td>
<td>1/1</td>
<td>M</td>
<td>46</td>
<td>260 (111-159)</td>
<td>4/4</td>
<td>M</td>
<td>77</td>
<td>129 (110-146)</td>
<td>4/4</td>
</tr>
</tbody>
</table>

* Of tumor-bearing period.

† The tumors were minute, discovered at autopsy.

† Gonadectomised.
latency period (77 days), and in this passage the tumor began to grow in normal female hosts. Passage IIa (made from passage Ib) also contained autonomous cells. An autonomous variant thus arose in the course of the first subtransfer generation.

**Morphologic characteristics of tumors.**—The grafted tumors remained localized at the site of injection but were not encapsulated. They were traversed by connective tissue septa which divided them into nodules of irregular size and shape. Although invasion of adjacent tissue was the rule, no gross metastases have been discovered, not even in the regional lymph nodes. The transplanted dependent tumors resembled the primary tumors, which have been well described elsewhere (37).

The tumor cells were of relatively uniform size and appeared in sections either as sheets (Fig. 3) or detached, the latter resembling plasma cells (Figs. 1, 2). Most cells were nongranular, but many had small to coarse acidophilic granules in varying numbers. Many cells, both granular and nongranular, contained large perinuclear negative Golgi images, as previously noted in primary tumors induced by radiation (38). Tumor cells were often in cords and occasionally lined up along blood sinuses with nuclei oriented toward the base. Necrosis and hemorrhages were common and often extensive.

Sections thus far examined from the autonomous variant (Figs. 4–6) show distinct differences from the dependent strains. The nuclei were, in general, larger, and many were greatly enlarged with large intranuclear inclusions (Fig. 6). Many were hyperchromatic, and there was great variability as to their size and shape. Mitoses were more common than in dependent MtT.

**Mammary gland.**—Both dependent and autonomous tumors were associated with extensive hyperplasia of mammary ducts and alveoli distended with milklike secretion (Figs. 7–12). In some cases, cysts measuring 2–3 cm. in diameter developed (Figs. 7, 8, 10, 12). When such cysts were punctured, a milky fluid was released under considerable pressure. There were scattered areas in some mammary glands in which secretion was minimal, and the cells were arranged in solid nodules (Fig. 15) or in adenomatoid ducts (Figs. 14, 15). The cells were enlarged, hyperchromatic, and contained few or no secretory globules. Mitotic figures were common.

A well developed mammary tumor was seen in an estrogen-treated rat which had a conditioned MtT of Strain 1. The animal was killed at 16 months of age 1 year after grafting and was found to have a firm MtT weighing 69 gm. Microscopic examination showed a gradation from secretory cells, through solid sheets of none secreting cells with many mitotic figures, to sheets composed of cells with anaplastic features (Fig. 16). Scattered hyperplasic areas were noted in several glands. The relative importance of estrogen and MtH in the genesis of such tumors is being currently investigated.

Studies of nineteen animals suggest that the extent of cystic ductal dilation is directly related to the size of the tumor: (a) in six animals bearing dependent MtT of 2–10 mm. average diameter, mammary stimulation was predominantly alveolar, ductal hyperplasia was slight, and cysts were few and small; (b) in nine animals with dependent MtT of 10–20 mm. diameter, the mammary glands of three were composed mainly of milk cysts of 5 or more mm. in diameter, the glands of the other six resembled those of the previous group; (c) all four animals bearing dependent MtT of 25–35 mm. diameter had mammary glands greatly enlarged with milk cysts.

**Ovaries.**—The ovaries of intact dependent MtT hosts displayed a varied picture. In most animals, they were small-normal or markedly atrophic and yellowish. Most had small follicles, and, often, there was a diffuse interstitial luteinization. Occasionally, large follicles were present. Discrete normal corpora lutea were found in only one of the eleven female hosts thus far examined microscopically. The ovaries of rats with autonomous MtT had either predominantly corpora lutea, or many developing follicles with interstitial luteinization, or both.

**Adrenals.**—In both conditioned and autonomous MtT-bearing rats the adrenals were often enlarged and congested (Table 3; Figs. 7–9). The cortical cells of such glands were swollen and packed with sudanophilic fat droplets (Fig. 18). Swelling and vacuolization were most marked in the reticular and fasciculate zones and diminished peripherally. In several rats with dependent MtT the entire cortex was involved. In extreme cases, superimposed hemorrhage caused distortion of the normal cortical structure (Fig. 19).

**Liver.**—Stilbestrol-treated rats with grafted dependent MtT displayed liver changes similar to those of mice with grafted radiation-induced MtT. The organ was usually much enlarged (Table 3; Figs. 7–9), had a mottled brown-red surface, and was congested. Enlarged parenchymal cells with hyperchromatic, large nuclei and occasional mitotic figures formed irregular foci throughout the liver. Adjacent normal cells were often compressed, and the normal lobular architecture was distorted (Fig. 22). In some regions there were indications of degeneration (Fig. 23) with sudanophilic droplets in the cytoplasm. Pyknotic cells were present in variable, usually small, numbers. Liver
enlargement also occurred with autonomous MtT, but preliminary observations indicate that foci of enlarged cells were less common with these than with dependent tumors (Fig. 24). Cytoplasmic vacuoles were present and one animal showed extensive fatty degeneration (Fig. 25).

Spleen.—The spleens were uniformly larger than normal and bore large erythrocytopenic and myelopoietic foci throughout the pulp.

Kidneys.—Most dependent MtT hosts had moderately to greatly enlarged kidneys (Table 3) with red-brown, mottled surfaces (Figs. 7—9). The spaces of the Bowman's capsules, proximal convoluted and collecting tubules contained hyaline casts (Fig. 20). Degeneration and necrosis of the tubular epithelium were common; regeneration of some tubules was indicated by hypercellularity, similar correlation with tumor size was not evident in females.

**DISCUSSION**

**General features of MtT.**—The present studies confirm the acidophilic and mammotropic character of estrogen-induced pituitary tumors noted earlier by others (4, 5), point to somatotropic effects of MtH also noted earlier (2), and confirm the dependent character of these cells (7). However, these findings were made independently in pursuit of discoveries of induction of pituitary tumors by ionizing irradiation (34, 32) and of the thyrotropic character and conditionality of pituitary tumors induced by radiothyroidectomy (10—12). Most of these observations were contrary to our expectations. Pituitaries are known to be radio-resistant;

**TABLE 3**

| EFFECT OF MAMMOTROPIC TUMORS AND OF DIETHYlstilbestrol ON ORGAN WEIGHS |
|--------------------------|---------------------------|-------------------|
| ANIMALS                  | Body weight (gm.)         | Liver (gm.)       |
|                          |                           | (4—6.6)          |
|                          |                           | (2.5—9.1)        |
|                          |                           | (0.15—0.5)       |
|                          |                           | (0.7—1.1)        |
|                          |                           | (14—10)          |
|                          |                           | (1.5—10)         |
|                          |                           | (3.5—1.4)        |
|                          |                           | (0.2—0.4)        |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |
|                          |                           | (0.005—0.011)    |

The figures give mean values; the ranges are in parentheses.

* One animal had a small primary pituitary tumor, probably MtT; the testes of all were markedly atrophic.

† Four had small primary pituitary tumors.

with a few mitotic figures. Autonomous MtT in normal rats caused similar but less extensive changes.

**Somatotropic effects.**—A small number of rats were weighed in order that the increase in weights, which was obvious to autopsy of most tumor-bearing rats, could be expressed in approximate figures. Table 3 shows the magnitude of increases in body weights and in liver, kidney, and adrenal weights. Diethylstilbestrol alone caused loss of body weight and enlargement of the pituitary due to proliferation of Mt. In the first group there was some correlation between tumor size, tumor-bearing period, and weight changes in organs (not detailed in Table 3). The liver weighed 4.3 per cent of body weight in an animal with a 5-mm. tumor, and 10.5 and 14.1 per cent in two animals with 40-mm. tumors. The corresponding kidney weights were 0.3, 1.6, and 2.3 per cent, respectively; the adrenal weights 0.02, 0.04, and 0.1 per cent, respectively. A

estrogens were expected to induce anti-estrogenic, that is, “ICSH”-secreting, gonadotropic pituitary tumors which would stimulate androgen production; and radiation atrophy of the ovaries was expected to cause development of either follicle-stimulating or luteinizing pituitary tumors.

One observation, though suggested by physiological events, has not been evident in research of others, namely, the dependence of proliferation of mammotropes on estrogens. This conclusion was reached independently by the senior author and by the Wisconsin group, of which a junior author was a member (4, 27). Lyons et al. (5, 29) have indicated that MtH has a direct action on the mammary gland, and the term “mammotropes” was suggested by their work. Some workers look upon pituitary hormones as influencing only secretion of the mammary gland (hence, the name “prolactin”) and relate growth of the gland to gonadal hormones; others believe that the pituitary hor-
mone stimulating mammary growth is different from prolactin (32).

Dependence of mammotropes on estrogens for growth should have been expected. During infancy the mammary gland is undeveloped and is "awakened" at puberty with maturation of the ovaries. Pregnancy is associated with hypersecretion of estrogens (and of other gonadal hormones), enlargement of the pituitary, and development of the mammary gland. Increase of acidophiles in the pituitary during lactation has been reported (5), but these acidophiles have usually been related to growth hormone. However, the observations of Bates, Riddle et al. indicate production of splanchnomegaly with prolactin (2).

Normal hosts, i.e., hosts that are not conditioned (in this case, animals without estrogen pellets), are excellent natural selectors of autonomous Mt variants, which are cancer cells by all definitions. It is difficult to conceive of the origin of an autonomous tumor cell other than by assumption of a mutation-like change. Contrariwise, it is unnecessary to postulate that the tumor cells of dependent (conditioned) neoplasms are altered cells, even though such tumors may be fatal.

Mammary gland stimulation.—The role of prolactin in mammary gland development and lactogenesis has been recently reviewed by Cowie and Folley (5). It is generally agreed that MtH is necessary for milk secretion, while estrogens alone stimulate ductal growth (see 5). According to Lyons et al. (23), MtH also stimulates mammary gland growth directly. Small intraductal milk cysts have been noted in rats which had been treated with estrogens for several days (4, 5), but estrogens stimulate the mammotropes. Meites and Turner found by bioassays an increased amount of prolactin in pituitaries of animals of various species treated with estrogenic hormones (see [5]). In the assays of Meyer and Clifton (27) MtH was the only hormone present in approximately normal concentration in primary estrogen-induced MtT. The quantity of MtH produced and released in rats bearing grafted dependent MtT is expected to be greater than in rats with primary MtT because of the larger size of the former. The evidence presented suggests that MtH causes both proliferative and secretory changes in the mammary gland, even in gonadectomized rats. Turner et al. found that full growth of the mammary gland in hypophysectomized animals can be attained by the administration of estrogen and the lactogenic hormone without other hormones (33). This is in agreement with our unpublished experiments with MtT, Strain 4. Our observations explain the findings (see 17) that estrogens can induce lactation in goats and guinea pigs without "luteotrophin" injections: estrogens stimulate proliferation and secretion by "luteotropes" (Mt) of the hosts. This procedure is being exploited commercially, and it will be interesting to watch domesticated animals so treated over prolonged periods for development of MtT. The specificity of mammary gland stimulation by MtT has been discussed in the preceding article (13).

Rats of the Fischer strain are not susceptible to mammary tumors (7, 8). Only one such tumor was seen in animals of the present series, but most animals were sacrificed too early for mammary tumors to appear. Furthermore, under the experimental conditions used here, secretory changes outweighed and possibly checked proliferative changes. The two are generally believed to be independently regulated, and a search for the possible role MtH may play in the development of mammary tumors calls for special studies.

The chief influence of the pituitary on the development of mammary cancer is believed to depend on its power to stimulate the ovaries to secrete estrogens (3). The suggestion that pituitary "mammogens" play some part in the development of MtT was made by Turner (32), but supporting evidence has been lacking. Our experiments, however, do indicate that MtH stimulates the mammary gland directly; in these experiments there was no histologic evidence of increased production of estrogens. Physiologic doses of estrogens are, however, required to maintain the Mt. Viewed in the light of these findings and of recent success with hypophysectomy for mammary cancer (28), some old experiments became of great current interest. Loeb et al. found (20—22) that repeated transplantations of histocompatible pituitaries led to an increased incidence of mammary gland tumors, but not in absence of the ovaries. Tumor induction was attributed in part to enhanced supply of estrogens, but it is uncertain whether the surviving grafts in ovariectomized animals contained Mt. The extensive literature which followed this discovery was reviewed by Burrows and Horning (3). Korteweg and Thomas (18) found that hypophysectomy prevents the development of mammary cancer. The crucial experiment would be to attempt the induction of mammary cancer in hypophysectomized animals either with estrogens and supporting maintenance doses of pituitary hormones other than MtH, or with MtH in the absence of, or with only small maintenance doses of, estrogens.

The breast of the normal human male can be induced to lactate by administration of the lactogenic hormone ("luteotrophin") following estro-
gen administration; women, even in the 7th decade, secreted milk when stimulated with "luteotrophin" (Huggins and Dao, 17).

MtT may be a unique tool for studies of both the rate and mechanism of MtH production and secretion and the relation of MtH to normal and abnormal mammary growth.

Role of lutein cells.—The terminal stages of maturation of follicles, ovulation, and the development of corpora lutea of rats are thought to be under the control of luteinizing hormone, while the maintenance of and secretion by formed lutein body is believed to be controlled by MtT (see [5]).

The early effect of estrogen treatment is an increased output of luteinizing hormone and formation of corpora lutea. However, when estrogens are given in large amounts over longer periods, gonadotropins are suppressed (see [5]). The frequent absence of corpora lutea in the ovaries of MtT hosts is noteworthy. Assays of primary estrogen-induced MtT failed to disclose the presence of either luteinizing or follicle-stimulating hormones (27). If MtH stimulates secretion of ovarian hormones, the latter remain to be identified.

Interstitial luteinization, common in animals bearing MtT, has also been observed in mice bearing primary TtT. It is noteworthy, therefore, that many mice with primary TtT also displayed mammary gland stimulation of ductal type (9). Hyperplasia of the ovarian stroma was found to be disproportionately common in women with breast cancers; excessive estrogenic and pituitary hormones were considered to be significant factors in breast cancer development (31). The specificity and hormonal relationship of ovarian changes to mammary gland stimulation is not clear.

Morphology of dependent and autonomous tumors.—Histologic examination of dependent and autonomous TtT disclosed marked differences: the former resembled a culture of normal cells, whereas the latter possessed the textbook features of a malignant tumor (9). In view of this, the histologic pattern of dependent and autonomous MtT was closely followed soon after onset of autonomy. That the sequence of events here is the same as with TtT is shown by the illustrations (Figs. 1–6). Cells of dependent growths are fairly uniform in size, shape, and chromatophilia. The nuclei are relatively small. Development of autonomy is accompanied by the appearance of cells possessing larger nuclei, giant cells, and greater variations in size of both cells and their nuclei. Mitotic figures are often numerous with dependent tumors, but this merely signifies that cells are proliferating. The character and number of chromosomes and histochemical differences between cells of these two basically different types of tumors remain to be studied.

Such differences as indicated above are expected to be clear-cut only if the character of tumors chosen for comparison has been verified by transplantation assays: dependent tumors should show good growth in conditioned hosts and none in histocompatible normal hosts. Autonomous tumors should be obtained from normal hosts, which apparently act as selectors of this variant cell type. Autonomous tumor cells will also grow in conditioned hosts, and so a cell population in the latter may be composed of both types of cells.

The above findings and considerations may help to explain the response of certain human neoplasms to hormonal therapy. Remissions are almost invariably followed by relapses. It seems to us that the tumors most responsive to hormones are composed of predominantly dependent, with few autonomous, cells. Hormones control the dependent cells, hence the remission. Hormones do not inhibit the autonomous cells which proliferate, causing the relapse. Huggins and Dao (16) found that "it was usually possible to correlate histologic appearance in a useful manner with response to the operation employed" (adrenalectomy and oophorectomy in the treatment of carcinoma of the breast). The now classical Papanicolaou test of malignancy is that of morphologic identification of autonomous tumor cells. The experience gained in the study of experimental dependent and autonomous tumors calls for more extensive and precise correlation between morphologic and biologic behavior of tumors in both man and experimental animals.

Adrenal change.—The mechanism and meaning of marked enlargement of the adrenals in rats bearing grafted MtT are puzzling. A similar hypertrophy of the adrenal cortex has been described in mice bearing autonomous TtT (9) and in mice that had been given amphenone (15). Since such changes also occurred in normal rats with autonomous MtT devoid of uterine stimulation, they are not the result of estrogen excess. Marked adrenal enlargement is usually considered to be caused by hypersecretion of MtH, with resulting hypersecretion of AH. Thymic involution, the most sensitive index of hypersecretion of glucocorticoids, was not a constant finding in MtT or TtT hosts that were sacrificed. We believe, therefore, that the adrenal enlargement is not due to overactivity but to fatty degeneration. Hertz et al., assaying adrenals of mice treated with amphenone, arrived at the same conclusion (15).

Vacuolization of cytoplasm of cells of the fasciculate and reticular zones, believed to be due to
fatty degeneration, has been described in rats that had been treated with methyltestosterone (29). In addition, “colloid” granules giving glycoprotein reactions have been noted in adrenal of such animals (30). Massive doses of AtH caused “tubule” formation in the adrenal cortex with hemorrhagic necrosis in extreme cases (36). In our animals receiving excessive quantities of steroid hormones or AtH endogenously (from AtT or steroid-secreting tumors, respectively) such changes were not noted. Secreting Leydig and lutein-cell tumors caused adrenal atrophy, while AtT caused enlargement of the adrenals with enlargement of cells of the cortex, but with no degenerative changes. The difference in results may be explained by the supposition that high hormone levels in the blood of some steroid and pituitary tumor-bearing animals are attained gradually, while in animals treated with large quantities of exogenous hormones very high hormone levels are attained suddenly. Stimulation under the former conditions is gradual and sustained; in the latter the stimulation is sudden, striking, and transitory.

The pathogenesis of the adrenal lesions in MtT hosts has not yet been worked out, but the possibility that they may be caused by steroid hormones or AtH deserves further inquiry.

Somatotropic features of MtT.—The consistent occurrence of somatotropic changes with MtH activity in both female mice and rats with all mammotropic tumor strains studied leads us to conclude that MtH itself has somatotropic properties. If the tumors were composed of a mixture of two cell types (Mt and St), one would expect the St, which is independent of estrogen, to grow selectively in ovariectomized or hypophysectomized animals. Mammotropic changes are markedly dependent on treatment with estrogen in mice; somatotropic changes are not. In male mice bearing autonomous MtT, mammary gland stimulation was usually absent, but somatotropic effects were evident. Grafts of autonomous MtT on castrated male mice with ovarian grafts caused marked mammary gland stimulation. Bates, Riddle et al. (2) demonstrated splanchonemal in hypophysectomized pigeons that were treated with “prolactin,” and the somatotropin-like action on the tibial plate has recently been reported by Geschwind and Li (see [5]), with highly purified MtH.

The greatest degree of mammotropic and somatotropic change in the present studies was found in female rats bearing estrogen-dependent tumors. Estrogen treatment alone did not stimulate the liver under the conditions of these experiments.

The proliferative mechanism of liver is stimulated by numerous agents (Paget, see [24]) including tumors (Mider, see [24]). Increased weight of liver was reported by Annau et al. (1). This increase is very slight in comparison with that caused by the pituitary tumors mentioned. When, in the course of passages, the specific hormonal secretions of these tumors diminishes, so goes their ability to cause enlargement of viscera. Furthermore, distinct enlargement of organs other than the liver is not caused by common tumors.

The complexity of growth hormonal action is indicated by many reports at a recent symposium (23). Our studies suggest that at least three pituitary hormones have general growth promoting effects: StH, MtH, and TtH. The nature of these hormones, their effects on different metabolic pathways, and the character of the somatotropic changes caused in different organs remain to be studied.

Kidney changes.—Although these have not yet been specifically investigated and the mechanism of their development is not yet clear, the following observations may be of interest. The most conspicuous changes occurred in estrogen-treated rats with dependent MtT and lesser changes in normal rats with autonomous MtT. In the hamster, estrogens are known to cause profound kidney damage, culminating in tumor formation (19, 25). This is believed to be due to failure of the hamster liver to inactivate estrogens as do livers of rats and mice. In our rats pretumorous proliferative changes were not detected in the kidneys. The nephrotic change occurred late in the course of the tumor-bearing period and was associated with marked albuminuria. In mice with autonomous MtT not treated with estrogens, similar nephrotic damage was noted frequently when the tumors were large (13). The usual absence of nephrotic damage in mice bearing transplanted pituitary tumors other than MtT type suggests a relation to excessive quantities of MtH. It is noteworthy that renal cortical necrosis is a rare, but albuminuria a frequent, complication of late pregnancy.

Concept of MtT induction.—The similarity in morphologic and physiologic characteristics of radiation-induced MtT in mice and estrogen-induced MtT in rats strongly suggests their essential identity and a common basic induction mechanism.

Radiation at first depresses the ovaries, but regeneration follows frequently, with hyperestrogenization. Little is known about pituitary tumors occurring in castrated mice, but, in these, adrenal hyperplasia with compensatory secretion of estrogens is the rule (Dickie and Woolley, see [18]). The
endocrine imbalance in these two situations frequently results in the development of hormone-secreting tumors: ovarian in irradiated mice, and adrenocortical in castrate mice. Thus, it can be postulated that, in all three experimental situations discussed, sustained hyperestrogenization is the common denominator for induction of pituitary tumors and that these tumors are of the same character.

SUMMARY

Pituitary tumors induced in the rat by sustained hyperestrogenization are dependent neoplasms in that, when transplanted, they will grow only in estrogen-treated hosts. Upon subpassage, these dependent tumors give rise to autonomous variants.

These tumors are markedly mammotropic and somatotropic. Evidence is presented to suggest that this is not due to an admixture of two types of cells or to two different hormones, but is an inherent characteristic of one hormone secreted by mammatropes.

Nephrosis, as yet unexplained, is a common secondary change in animals bearing such tumors. Hyperestrogenization is the common mechanism by which three procedures (irradiation, castration, and administration of estrogens) will induce mammatropic pituitary tumors.

ACKNOWLEDGMENTS

The excellent technical assistance of Mrs. Lena Zompetti is acknowledged.

All photographs were taken by Mr. John Carabites.

REFERENCES

For articles cited but not listed here, see the bibliography of Cowie and Folley (6).


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Note: All sections were stained with hematoxylin and eosin. Dependent mammotrophic tumors were carried in diethylstilbestrol-treated females or castrate males, autonomous tumors in castrate males or intact females.

Fig. 1.—Dependent pituitary tumor (Strain 4); tumor cells detached, fairly uniform in size and shape; cytoplasm moderate to abundant; nucleus eccentrically located. X×630.
Fig. 2.—Same tumor as Figure 1. X×1150.
Fig. 3.—Dependent pituitary tumor (Strain 5); tumor cells fairly uniform in size and shape; cytoplasmic boundary indistinct; two mitotic figures. X×630.
Fig. 4.—First-generation autonomous pituitary tumor (Strain 4); cells vary greatly in size; several mononuclear giant cells. X×630.
Fig. 5.—Same tumor as in Figure 4. X×1150.
Fig. 6.—Same tumor as in Figure 4 showing part of a nodular area composed of bizarre giant cells. The bulk of the tumor was composed of smaller cells as shown in Figure 4. Magnification same as that of Figure 4. X×630.
Fig. 7.—Female 8-month-old rat with large dependent tumor (Strain 4) in right thigh. Great enlargement of mammary glands with milk cysts; hypertrophy of liver, spleen, kidneys, and adrenals.

Fig. 8.—Castrated, estrogen-treated male rat, 5 months of age, with large dependent tumor (Strain 4) in right thigh. Great enlargement of the mammary gland with milk cysts and hypertrophy of liver, spleen, kidneys, and adrenals. Compare the size of organs with those of the control (Figure 9).

Fig. 9.—Castrated estrogen-treated male control, 6 months of age. Same magnification as in Figure 8.
Fig. 10.—Mammary gland of rat bearing a very large dependent tumor (Strain 4); cystic dilatation of ducts with secretion. $\times 44$.

Fig. 11.—Mammary gland of rat bearing a small dependent tumor (Strain 4); predominantly alveolar stimulation with milk secretion. $\times 44$.

Fig. 12.—Mammary gland of a female rat bearing a large autonomous tumor (Strain 4); ducto-alveolar hyperplasia with slight secretion. $\times 44$.

Fig. 13.—Mammary gland of a rat bearing a medium-sized dependent tumor (Strain 5); solid areas of hyperplasia of the epithelium with no secretion. $\times 280$. 
Fig. 14.—Mammary gland of a female rat bearing a large autonomous tumor (Strain 4); adenomatoid hyperplasia. ×44.

Fig. 15.—Same mammary gland as in Figure 14; higher magnification of adenomatoid hyperplasia. ×280.

Fig. 16.—Mammary gland of rat bearing a very large dependent tumor (Strain 5) with intraductal papilloma (possibly carcinoma in situ). ×630.
Fig. 17.—Adrenal cortex of a 13-month-old untreated female rat. ×150.

Fig. 18.—Greatly enlarged adrenal of a 7-month-old male rat bearing a medium-sized dependent tumor (Strain 4); hypertrophy and fatty vacuolization of cells of the fasciculate and reticular zones. ×150.

Fig. 19.—Early necrosis with hemorrhage in adrenal of a 9-month-old female rat bearing a large dependent tumor (Strain 4). ×150.

Fig. 20.—Kidney of a 6-month-old male rat with very large dependent tumor (Strain 4); hyalin deposits distend glomerular spaces and many tubules; necrosis of some tubules, cellular and albuminous casts in others; fibrosis of interstitium. ×150.
FIG. 21.—Liver of a normal 5-month-old male rat. ×150.

Fig. 22.—Liver of a 5-month-old male rat with large dependent tumor (Strain 4); compression of normal cells by foci of hypertrophied cells some of which show large and hyperchromatic nuclei. ×150.

Fig. 23.—Liver of a 6-month-old male rat with large dependent tumor (Strain 4); foci of hypertrophied cells some of which have intensely vacuolated cytoplasms ("foam cells") and others have very large hyperchromatic nuclei; leukemoid blood picture. ×150.

Fig. 24.—Microscopic appearance of a greatly enlarged liver of a 5-month-old female rat with an autonomous tumor of medium size (Strain 4). Compare with Figure 21. ×150.

Fig. 25.—Intense fatty degeneration with great enlargement of the liver of a 7-month-old female rat bearing a large autonomous tumor (Strain 4). ×150.
Dependent and Autonomous Mammotropic Pituitary Tumors in Rats; Their Somatotropic Features

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Cancer Res 1956;16:608-616.