Biological Characteristics of Two Transplantable Pituitary Tumors of Syrian Hamsters*

AGNES B. RUSSFIELD, GLADYS FRIEDLER,† AND J. K. FRENKEL

(Children's Cancer Research Foundation and Department of Pathology, Harvard Medical School at the Children's Hospital, Boston, Mass.; and Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, Kansas)

SUMMARY

Three pituitary tumors of the Syrian hamster are described. Two, of anterior lobe origin, arose in females approximately 1 year after radiothyroidectomy. One of these, which exhibited marked cellular atypicality, was lost in the second transplant generation. The other tumor, the cells of which resemble those of normal anterior lobe tissue, has been transplanted for 38 passages. Animals bearing this tumor show splenomegaly, accelerated weight gain due to visceromegaly and increased deposition of fat, and, in females only, gonadal and mammary stimulation and thymic hypertrophy.

A third tumor was found in a castrate male 26 months after an unsuccessful splenic ovarian transplant. This tumor is now in its twelfth passage. No evidence of endocrine stimulation has been observed in animals bearing this tumor, but they exhibit severe splenomegaly. The primary tumor was not studied histologically. However, current passages resemble the primary tumors of pars intermedia which can be induced with estrogens. The propensity of both transplantable tumors for massive metastases which was apparent in early transplant generations has been lost in recent passages.

Spontaneous tumors of the pituitary do not appear to be common in the Syrian hamster. Among thirteen spontaneous tumors occurring in one series of a thousand hamsters of unspecified age, none were of pituitary origin (1). No pituitary tumors were reported among 226 animals autopsied at the end of a normal life span, although the majority of these old hamsters had both benign and malignant neoplasms which were frequently multiple (2). One carcinoma originating in a hamster pituitary has been reported to be in its 60th transplant generation, but information concerning its possible endocrine activity has not been published (16). Although estrogens will induce pituitary tumors in hamsters as they do in some strains of mice and in rats, such tumors in hamsters usually arise from the pars intermedia instead of from the anterior lobe as they characteristically do in other rodents (5, 10, 19). A few estrogen-induced tumors of the anterior lobe have also been reported in hamsters (6). There appear to be no records of successful transplantation of estrogen-induced pituitary tumors in this animal.

This paper describes three pituitary tumors of Syrian hamsters which arose after other types of endocrine manipulation, one of which may secrete a tropic hormone, and two of which have been transplanted through several generations.

MATERIALS AND METHODS

Two of these tumors were obtained from an experiment in which six male and nine female Syrian hamsters were placed on a Remington (13) low iodine diet purchased from the Nutritional Biochemicals Corporation and given only distilled water to drink for a period of 2 months. Each animal then received a subcutaneous injection of 300 µg I131. Following this, the hamsters were maintained on laboratory chow and tap water and observed until they were in a terminal condition.
Another male hamster was castrated and received an ovarian transplant into its spleen. A pituitary tumor was discovered when the animal died 26 months later.

Tumors were perpetuated by mincing tissue into pieces less than 1 mm. in diameter and making subcutaneous trocar implants of 0.1 cc. of tumor into the suprascapular region. From 1957, when the first tumor originated, until mid-1961, laboratory-bred Syrian hamsters were used for transplants. Since then animals have been obtained from the Dennen Animal Industries, Gloucester, Massachusetts.

Hypophysectomized hamsters used for assay of tumor PTHF2 were laboratory-bred and hypophysectomized at the Hormone Assay Laboratory, Chicago, Illinois. Completeness of the operation was determined by reappearance of juvenile fur in these animals and by examination of the sella turcica at the time of autopsy.

When animals died or were sacrificed, complete autopsies were performed. All organs were weighed and examined histologically in sections stained with hematoxylin and eosin.

RESULTS

Primary tumors.—Two radiothyroidectomized females were found to have frank pituitary tumors at 330 and 383 days after administration of I131. Of the remaining seven females used in this experiment, three died and were eaten by their cage mates between 9 and 10½ months after the experiment was started. Three females died with pituitaries of normal size 330, 403, and 515 days after radiothyroidectomy. One female died with an enlarged pituitary at 403 days. In five of the six females which could be examined histologically, including both animals which developed pituitary tumors, no thyroid tissue was grossly visible at autopsy. However, when the tracheas were sectioned, very rare small thyroid follicles were occasionally demonstrable microscopically. No pituitary tumors were found among the six males subjected to radiothyroidectomy.

The first pituitary tumor found (PTHF1) was a soft, globular mass filling the sella turcica and producing compression deformity of the brain. On microscopic examination, the normal architecture of the pituitary had been obscured so that identification of pars anterior, intermedia, and nervosa was no longer possible. In some areas, the tumor consisted of sheets of loosely arranged small cells with fairly uniform, round, central, vesicular nuclei and moderate amounts of finely granular, eosinophilic cytoplasm with irregular cell boundaries. In other areas there were bizarre giant nuclei, pleomorphic nuclei, and occasional multinucleate cells (Fig. 1). This tumor grew when transplanted into intact hamsters but produced no changes in the hosts suggestive of endocrine activity. It was lost in the second transplant generation.

The second pituitary tumor (PTHF2) found in a radiothyroidectomized female measured 7 × 5 × 4 mm, and grossly resembled the first tumor. Microscopically, about one-third of the tissue had undergone hemorrhagic necrosis. The viable portions consisted of sheets of small, fairly uniform cells with rounded or ovoid nuclei containing fine, rather sparse chromatin particles. Cytoplasm was finely granular and eosinophilic. Cell boundaries were so indistinct as to suggest a syncytial arrangement (Fig. 2). Although there was some variation in nuclear size, the extreme pleomorphism characteristic of PTHF1 was not seen, and the tumor cells had a distinct resemblance to the cells of the normal anterior lobe. This tumor was successfully passed into intact male and female hamsters and is now in its 33d transplant generation.

A third pituitary tumor (PTHF3) arose in a male hamster which had been castrated 26 months previously and which had received a splenic ovarian transplant. At the time of death, however, the spleen was of normal size and contained no traces of residual ovarian tissue. The adrenals, also of normal size, contained the cortical nodules characteristic of castrates of this species (9). The pituitary was replaced by a 10 × 5 × 5 mm. tumor mass, all of which was used for transplantation. This tumor is now in its twelfth passage.

Biological characteristics of PTHF3.—Tumor PTHF3, which was found in the radiothyroidectomized female, is readily transplantable, growing in almost 100 per cent of animals inoculated. The latent period has remained at 3 ± 1 weeks throughout the time of observation. Animals will live for 3½—4 months after receiving the tumor, and the tumor itself may attain a weight of 50 gm. The blood supply is rapidly outgrown, so that tumors weighing over 5 gm. usually show central hemorrhagic necrosis. Small tumors are firm, well circumscribed, and present a glistening, semitranslucent, pinkish-gray cut surface. Larger tumors often contain central cystic areas filled with dark red fluid and may exhibit small, irregular, opaque yellow flecks in the solid peripheral portions. Shortly before the death of a tumor-bearing animal, the skin overlying the tumor may ulcerate.

Microscopically, recent transplant generations still resemble the original tumor. They consist of masses of small, reasonably uniform cells with indistinct cytoplasm and rounded, central nuclei, pleomorphic nuclei, and occasional multinucleate cells (Fig. 1). This tumor grew when transplanted into intact hamsters but produced no changes in the hosts suggestive of endocrine activity. It was lost in the second transplant generation.

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which resemble those seen in many human chromophobe adenomas. Mitotic figures are usually abundant (Fig. 3).

In its early passages, metastases from PTHF2 were frequent and often attained a very large size, increasing the weight of the liver to 3 or 4 times its normal value. Metastases were observed in the liver and lungs of nearly all tumor-bearing hamsters, in the spleen in approximately 70 per cent, and in the adrenals, kidneys, or ovaries in approximately 20 per cent. The size of the metastases began to decrease in about the 25th passage, and, although hepatic and pulmonary metastases are still found in the majority of animals which have borne the tumor for 2 months or longer, they are now usually of microscopic dimensions.

In its third passage, PTHF2 was transplanted into three hypophysectomized males and five hypophysectomized females. In both sexes, the transplanted tumor not only grew but restored the atrophic gonads and secondary sex organs to a normal adult weight. No evidence of thyroid stimulation or of growth hormone production was ever found in hypophysectomized animals or in immature, intact litter-mates given implants of the tumor.

In early passages gonadal stimulation was evident in both tumor-bearing males and females. Testicular weights of 5-6 gm. were frequently recorded. With serial passages, however, the gonadotropic potency of the tumor has decreased markedly and is now apparent only in female hamsters. This suggests that the hormone produced has follicle-stimulating properties but that any interstitial cell-stimulating activity has been lost.

When animals given inoculations of PTHF3 in recent transplant generations were compared with controls of the same sex, age, and an initial weight of 90-110 gm., the results shown in Table 1 were obtained. The tumor had no effect on the weight of testes or seminal vesicles but produced a highly significant increase in ovarian and uterine weight. This is illustrated in Figure 4. Histologically, the ovaries contained numerous hemorrhagic corpora lutea. Uteri showed both endometrial and stromal stimulation. The females usually had hyperplastic mammary glands with, however, little evidence of secretory activity.

The weight gain of the animals, exclusive of tumor weight, was accelerated by the presence of PTHF3, especially in the females. This increase in weight seemed to be caused by visceromegaly in part, and in part by excessive deposition of mesenteric fat. No significant increase in thyroid weight was found in either males or females, and there was no histological evidence of unusual thyroid stimulation. Although the average adrenal weight was higher in females bearing PTHF3 than in the controls, this increase appeared to be due to amyloid-like deposits in some of the animals rather than to stimulation of the adrenal cortex. In both males and females PTHF3 produced a highly significant increase in weight of the spleen, which was the site of active extramedullary hematopoiesis. In females, the weight of the thymus was also markedly increased. Average pituitary weight was increased in females, but not to a statistically significant degree.

**Biological characteristics of PTHF3.**—PTHF3, which arose in an old castrate male, is also easily transplantable. Like PTHF2, it has a latent period of approximately 3 weeks and will attain a final weight of 25-30 gm. It appears to be even more susceptible than PTHF2 to spontaneous necrosis and is compatible with a longer life span of the tumor-bearing animal, from 6 to 14 months. The gross characteristics of the two tumors are similar.

Microscopically, PTHF3 is a pleomorphic tumor. Nuclei are much larger than in PTHF2 and vary in size, shape, and chromatin pattern. Cytoplasm varies in amount and may contain strongly eosinophilic granular material. Although cell boundaries are often indistinct, cells tend to assume an angular or spindle shape. The histology of the primary tumor was not studied. However, recent transplant generations in many ways resemble primary estrogen-induced tumors of the pars intermedia (cf. Figs. 5, 6).

Even in its early passages, PTHF3 did not metastasize as widely as PTHF2, nor did the metastases attain as large a size. Maximum percentages observed were 75 per cent hepatic metastases, 50 per cent pulmonary metastases, and 30 per cent splenic metastases. Since about the seventh transplant generation metastases have been decreasing, until they are now both rare in occurrence and microscopic in dimensions.

At no time have phenomena suggestive of hormone production by PTHF3 been observed. No significant differences have been found in weights of pituitary, thyroid, adrenals, gonads, or secondary sex organs in either male or female hamsters bearing PTHF3 as compared with normal controls (see Table 1). Growth rate is not accelerated, and there is no stimulation of mammary glands. No effect of tumor growth on skin or hair pigmentation has been seen. This tumor does, however, produce a striking splenomegaly, which is even more pronounced in females than in males. Microscopically, the enlarged spleens are the site of extramedullary hematopoiesis and occasionally myeloid metaplasia. Proliferation of immature blood cells...
DISCUSSION

The hormonal factors involved in the genesis of the tumor found in the castrate hamster in which splenic ovarian implant was attempted are obscure. Although pituitary tumors have occurred in rats in which this procedure was successful (12), excessive estrogen production by ectopic ovarian tissue seems very unlikely in the present case. Although castration itself may produce short-term pituitary hyperplasia in the Syrian hamster (17), its long-term effects in this species have not been well studied. Whereas the histology of the tumor suggests that it may have been of intermediate lobe origin, the failure to examine the original tumor forbids any definite conclusions. It is not impossible that PTHF3 may have originated spontaneously in a very old hamster without reference to previous endocrine manipulations. The occurrence of pituitary tumors in two of the six radiothyroidectomized female hamsters which could be examined pathologically does suggest, however, that T11 may have played some part in their pathogenesis. The pituitary tumors arising after this treatment in mice characteristically secrete thyrotropic hormone, but some gonadal stimulation has also been observed, especially in females (4). Furthermore, a radiation-induced thyrotropin-secreting pituitary tumor of a mouse has recently been reported which, in its third transplant generation, lost all thyrotropic properties but retained its ability to stimulate the gonads (11).

Both the histology and apparent endocrine activity of PTHF3 support an anterior lobe origin for this tumor. The capacity of the tumor to stimulate the gonads of hypophysectomized hamsters in its early passages suggests an active production of gonadotropic hormone. The origin of the growth-promoting properties of the tumor, which have only been detected in recent passages, is not known. It is possible that the tumor is now secreting growth hormone as well as gonadotropin; how-

<table>
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<tr>
<th>Tumor</th>
<th>Passage</th>
<th>No.</th>
<th>Sex</th>
<th>Wt. gain</th>
<th>Tumor wt.</th>
<th>Pituitary (mg.)</th>
<th>Thyroid (mg.)</th>
<th>Thymus (mg.)</th>
<th>Liver (gm.)</th>
<th>Spleen (mg.)</th>
<th>Adrenals (mg.)</th>
<th>Gonads (gm.)</th>
<th>Sem. ves./uterus (gm.)</th>
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<tr>
<td>PTHF3</td>
<td>Mean S.D.</td>
<td>91 6 M</td>
<td>27.5</td>
<td>8.5</td>
<td>4.7</td>
<td>10.1</td>
<td>75</td>
<td>6.8</td>
<td>110*</td>
<td>37.4</td>
<td>4.36</td>
<td>0.55</td>
<td>0.47</td>
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<td>Controls</td>
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<td>6 M</td>
<td>16.0</td>
<td>9.7</td>
<td>4.9</td>
<td>11.7</td>
<td>88</td>
<td>5.4</td>
<td>92</td>
<td>37.7</td>
<td>4.58</td>
<td>0.35</td>
<td>0.47</td>
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<tr>
<td>PTHF1</td>
<td>Mean S.D.</td>
<td>11 5 M</td>
<td>11.4</td>
<td>6.3</td>
<td>5.2</td>
<td>14.9</td>
<td>67</td>
<td>6.6</td>
<td>890*</td>
<td>32.5</td>
<td>3.89</td>
<td>0.40</td>
<td>0.40</td>
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<tr>
<td>Controls</td>
<td>Mean S.D.</td>
<td>6 M</td>
<td>22.5</td>
<td>12.5</td>
<td>5.8</td>
<td>18.0</td>
<td>68</td>
<td>5.3</td>
<td>91</td>
<td>32.9</td>
<td>2.91</td>
<td>1.24</td>
<td>1.24</td>
</tr>
<tr>
<td>PTHF3</td>
<td>Mean S.D.</td>
<td>30 6 F</td>
<td>39.3†</td>
<td>7.7</td>
<td>8.9</td>
<td>16.6</td>
<td>168*</td>
<td>11.8*</td>
<td>319*</td>
<td>32.7*</td>
<td>187.0*</td>
<td>1.21*</td>
<td>0.314</td>
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<tr>
<td>Controls</td>
<td>Mean S.D.</td>
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<td>14.7</td>
<td>3.1</td>
<td>7.0</td>
<td>15.8</td>
<td>94</td>
<td>6.0</td>
<td>149</td>
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<td>45.5</td>
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<td>0.088</td>
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<tr>
<td>PTHF3</td>
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<td>25.4</td>
<td>8.7</td>
<td>7.5</td>
<td>14.5</td>
<td>74</td>
<td>7.2</td>
<td>555*</td>
<td>31.9</td>
<td>48.6</td>
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<tr>
<td>Controls</td>
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<td>140</td>
<td>18.7</td>
<td>44.0</td>
<td>0.48</td>
<td>0.095</td>
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* Difference between experimental and control, significant at 1 per cent level.
† Difference between experimental and control, significant at 5 per cent level.
however, it is also possible that growth promotion may be a secondary effect due to stimulation of the host's pituitary. If the latter possibility should prove to be correct, this tumor system would offer an interesting parallel to some gonadotropin-secreting tumors of human children which also stimulate the host's pituitary, causing gross hyper trophy and the appearance of "pregnancy cells" with concomitant acceleration of the growth rate (18).

Both the increased growth rate and the excessive deposition of fat in hamsters bearing PTHF2 resemble phenomena observed in parakeets bearing a transplantable chromophobe adenoma, in which, however, minute amounts of growth hormone have been found (14). This parakeet tumor has also been shown to produce marked abnormalities of plasma proteins (15). The splenomegaly associated with extramedullary hematopoiesis produced by both PTHF2 and PTHF1 has been seen in hamsters bearing transplantable tumors of other origins (7, 8) and has been interpreted as an immune reaction to a foreign protein (3). Since the pituitary is an organ normally engaged in active synthesis of protein hormones, it seems possible that further investigation of proteins produced by these tumors in the hamster may be quite as productive as study of their direct endocrine effects.

ACKNOWLEDGMENTS

The assistance of Chris Palacas, Penny Atherton, and Joseph Dias is gratefully acknowledged.

REFERENCES


Fig. 1.—Primary pituitary tumor PTHF1 found in female hamster 380 days after radiothyroidectomy. Note bizarre giant nuclei. Hematoxylin & eosin (H. & E.), X380.

Fig. 2.—Primary pituitary tumor PTHF2 found in female hamster 388 days after radiothyroidectomy. Note small uniform nuclei resembling normal cells of anterior lobe. H. & E., X380.

Fig. 3.—Pituitary tumor PTHF3 in 3rd transplant generation, to show resemblance to original tumor (Fig. 2). H. & E., X380.

Fig. 4.—Uterus, ovaries, and adrenals of female hamster given implant of PTHF2 (30th passage) on right; organs of normal female hamster on left. Note hypertrophy and hyperemia in uterus and ovaries of tumor-bearing animal as compared with control, and similarity of the adrenals in the two hamsters.

Fig. 5.—Pituitary tumor PTHF1 in 11th transplant generation; origin in old castrate male hamster. H. & E. X380.

Fig. 6.—Primary tumor of pars intermedia found in male hamster 74 months following subcutaneous implantation of a 15-mg. pellet of diethylstilbestrol. Note histological resemblance to PTHF3. H. & E. X380.
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