Several studies have investigated spontaneous pituitary tumors in aged rats. Prolactin-expressing tumors are common and are seen in Sprague-Dawley rats >24 months of age,8,10 old Wistar rats,1 aging Long-Evans rats,6 and Fischer rats.10 Other pituitary tumors in Sprague-Dawley rats are immunoreactive for luteinizing hormone, immunonegative, or growth hormone-prolactin combinations,7 a common finding also in aged Fischer rats.10 Other pleuriphonial tu-

mors have been reported for many strains.1,8,10 We have recently discovered a high incidence of spontaneous pituitary tumor occurrence in Monodelphis domestica, the Brazilian gray short-tailed opossum. Monodelphis domestica is a small, pouchless marsupial that is gaining popularity as a laboratory animal. Pups are born after 14 days of gestation in an extremely immature state, making this opossum an ideal animal model for studying the development of the mammalian nervous system.5,7 We have subsequently characterized these adenomas morphologically and immunohistochemically.

Enlarged hypophyses (pituitary adenomas) were obtained from 50% of Brazilian gray short-tailed opossums >18 months of age. The masses ranged in size from 2 to 7 mm and often compressed the adjacent hypothalamus. Five enlarged hypophyses were placed in Zamboni’s fixative for a minimum of 48 hours and sunk in 30% sucrose. Hypophyses of normal appearance were also collected. Tissue was cut into 20-μm-thick sections on a cryostat and mounted onto poly-L-lysine-coated slides. Immunohistochemical techniques were used to evaluate sequential sections for prolactin (PRL), growth hormone (GH), and adrenocorticotropic hormone (ACTH). Replicate sections were stained with hematoxylin and eosin. The avidin biotin peroxidase technique used has been described previously.2 Rat prolactin, the 11-24 fragment of human ACTH, and human GH (Chemicon) antisera were tested by preabsorbing the antiserum with 1 μg/ml hormone. The percentage of cells stained by a particular antibody in a tissue section was determined by counting the number of stained cells in a sample of ten cells in one high-power field (hpf). This was repeated in five randomly chosen fields for each tissue section. Using this technique on normal tissue, approximately two cells in ten stained for GH or ACTH, and six cells in ten were immunopositive for PRL.

In general, sections of the five pituitary adenomas had similar histopathologic characteristics and were classified according to criteria recently described for aged Wistar rats (Table 1).11 Sections were composed of cells arranged in solid sheets or branching and interconnecting cords supported by a fine vascular stroma. The cords were two to several cell layers wide and often lined on each side by a thin endothelial cell and an empty cleftlike space (Fig. 1). Tumors from opossum Nos. 2–5 also had numerous cystlike spaces (Fig. 1), some of which (10–40% of the cysts) contained red blood cells and low numbers of sloughed cells and necrotic cell debris. The tumor from opossum No. 1 contained only cleftlike spaces that were empty. Neoplastic cells were polygonal to elongate (4–8 μm in diameter) and occasionally pyriform, with moderate amounts of granular eosinophilic cytoplasm.

Key words: Brazilian opossums; immunohistochemistry; marsupial; pituitary; prolactinoma.
Table 1. Morphologic and immunohistochemical characterization of five pituitary tumors from old Brazilian gray short-tailed opossums.

<table>
<thead>
<tr>
<th>Opossum No.</th>
<th>Tumor Size (mm)</th>
<th>Expansion</th>
<th>Histologic Classification</th>
<th>Percent Stained*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 x 2</td>
<td>No</td>
<td>Adenoma</td>
<td>100 2 0</td>
</tr>
<tr>
<td>2</td>
<td>5 x 2</td>
<td>Yes</td>
<td>Adenoma with hemorrhage</td>
<td>96 6 0</td>
</tr>
<tr>
<td>3</td>
<td>5 x 3.5</td>
<td>No</td>
<td>Adenoma with hemorrhage</td>
<td>100 0 2</td>
</tr>
<tr>
<td>4</td>
<td>0.3 x 0.75</td>
<td>No</td>
<td>Nodular hyperplasia</td>
<td>0 100 0</td>
</tr>
<tr>
<td>5</td>
<td>6 x 6</td>
<td>No</td>
<td>Adenoma with hemorrhage</td>
<td>100 2 0</td>
</tr>
</tbody>
</table>

* Percent stained was determined as no. cells stained/10 cells in each high-power field. Five fields were counted in each section. PRL = prolactin; ACTH = adrenocorticotropic hormone; GH = growth hormone.

and a single oval to round nucleus. The nuclei contained small amounts of finely clumped chromatin. Mitoses were rare (** < 1/hpf). The tumor from opossum No. 2 extended beyond the sella turcica into the hypothalamus and ventral brain. Neoparenchyma surrounding this tumor was compressed and contained several dilated axon sheaths. Adenomas from opossum Nos. 4 and 5 were surrounded by a thin (1-3 cell layers wide) connective tissue capsule. All of the hypophysces were enlarged and had a strong avidity for PRL antibody in immunohistochemical tests (Table 1, Fig. 1). Immunoreactivity for GH was negligible in all the adenomas studied. The tumor from opossum No. 3 had one nodular focus of hyperplasia (0.3 x 0.75 mm). This area was well delineated and nonencapsulated, and the cells were well differentiated, with no mitotic figures. The cytoplasm of cells in this focus was stained by the ACTH antibody used in the immunohistochemical procedure and did not stain for PRL (Fig. 2).

Histologically and immunohistochemically, the pituitary adenomas were similar to hemorrhagic adenomas in Wistar rats previously described. Both the Brazilian gray short-tailed opossum and the Wistar rat appear to be predisposed to PRL-positive adenohypophysial adenomas containing blood-filled spaces. In addition, the neoplasms are not invasive but can expand locally and compress adjacent neoparenchyma. The adenoma from opossum No. 1 lacked the cystlike spaces and collections of red blood cells in such spaces. It did, however, stain strongly for PRL and histologically had cell types similar to those present in tumors from the remaining four animals.

The hyperplastic nodule from opossum No. 3 was the only markedly different immunoreactive site. Because the cells were well differentiated and strongly positive for ACTH, we considered that 1) this focus was an area of ACTH-positive adenohypophysis entrapped by the expanding adenoma or 2) the area was an ACTH-immunoreactive adenoma.

Thus, the incidence of spontaneous prolactinomas in the aging Brazilian gray short-tailed opossum appears high. Prolactin-immunoreactive tumors are found in many strains of rat, and prolactinomas characterized by hemorrhagic cysts, a prevalent finding in the old Brazilian gray short-tailed opossum, have been reported for the Wistar rat. Pituitary adenomas in humans constitute as much as 10% of all intracranial tumors diagnosed in living subjects and 10-26% of those tumors diagnosed at autopsy. Over 40% of these intracranial tumors result in an abnormally high secretion of pituitary hormones, most notably PRL. Other pituitary adenomas in humans are predominantly corticotropin, and ACTH-secreting tumors are common in dogs and horses. The Brazilian gray short-tailed opossum, an excellent model for developmental studies, may also provide another model for spontaneous adenoma formation in the aging mammal.
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Fig. 2. Pituitary adenoma; Brazilian gray short-tailed opossum No. 4. Fig. 2a. Well-delineated nodule immunoreactive for ACTH; most surrounding cells are ACTH negative. Fig. 2b. Nodule is not immunoreactive for prolactin, although neoplastic cells surrounding the mass stain intensely. Avidin biotin peroxidase complex method. Bar = 150 μm.

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