Toxicologic Pathology ISSN:0192-6233
Volume 20, Number 1, 1992

Pathogenesis of Blood-Filled Cavities in Estrogen-Induced Anterior Pituitary Tumors in Male Sprague-Dawley Rats*

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Abstract

The formation of blood-filled cavities in developing tumors of the anterior pituitary of estrogen-treated male Sprague-Dawley rats was studied in a serial sacrifice experiment. Two treated and 2 control rats were killed at each of 15 time points ranging from 7–272 days after sc implantation of an estradiol-17β pellet. The pituitaries were examined using light and electron microscopy. Changes at 7–9 days after implantation included epithelial cell swelling and trabecular arrangement. At 11–13 days, epithelial cells were further enlarged. Arrangement of epithelial cells in islands and endothelial degeneration were first seen at this interval. Also, any sinusoids were distended, whereas some were compressed by swollen epithelial cells. At 16–81 days, scattered necrotic and immature epithelial cells were present, and cell size decreased. Endothelial degeneration and both distended as well as compressed sinusoids were more prominent at this time. Loss of basement membrane was first seen during this interval. At 114–133 days, small hemorrhagic areas partially lined by epithelium were first seen; sinusoidal compression, endothelial necrosis, and loss of basement membrane were more frequent, but there was less sinusoidal distention. Between 150 and 272 days, epithelial cells were increasingly pleomorphic and arranged in nodules, and there was an increase in number and size of the hemorrhagic areas. Sinusoidal compression, endothelial necrosis, and loss of basement membrane were abundant, whereas sinusoidal distention had almost disappeared at this interval. Local compression of sinusoids and perhaps compression of pituitary surface veins due to epithelial cell swelling, were thought to play a primary role in the development of ischemic endothelial damage leading to loss of endothelial lining and basement membrane, and eventually to the formation of blood-filled spaces partially lined by epithelial cells.

Keywords. Light microscopy; electron microscopy; hemorrhagic tumors; epithelial cell swelling; endothelial cells; compression; ischemia; basement membrane

Introduction

Pituitary tumors are common in aged rats and humans (1, 2, 8, 12, 13, 16). Spontaneous rat pituitary tumors often contain abnormal vascularization (5, 6), and in particular, blood-filled cavities. Therefore, classification as hemorrhagic tumors has been proposed (1, 8, 16). Similar vascular abnormalities occur frequently in human pituitary adenomas (4, 6, 7, 14). Ischemia due to compression of pituitary stalk blood vessels by the tumor leading to hypoxic damage of endothelium has been suggested as a mechanism by which these vascular changes in human tumors develop (7, 14). The pathogenesis of hemorrhagic areas in spontaneous rat pituitary tumors is not known.

Estrogen-induced pituitary tumors in rats have been widely used as a model for spontaneous human and rodent pituitary neoplasms (3, 10, 18, 19). In a previous study, the first histopathological changes were seen at day 9 after estrogen implantation when acidophilic cells became hypertrophic. At day 16, cord-like structures of hypertrophic cells lined by endothelial cells and distended sinusoids were common features. At day 98, the sinusoidal lining showed

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interruptions causing intraparenchymal hemorrhages that contained some necrotic cells. From day 186 and onwards, the pituitaries became tumorous and contained areas that could be divided into different morphological areas (20).

However, the development of pituitary vascular lesions in estrogen-treated rats has not been systematically studied. The purpose of this study was to establish sequential changes in the rat pituitary morphology following exposure to estrogen in an attempt to determine the histopathogenesis of blood-filled cavities in pituitary tumors. To this end, an experiment was conducted with serial sacrifices between 7 and 272 days after the start of estrogen treatment, and changes in pituitary morphology were examined at the light and electron microscopic levels.

Materials and Methods

Under ether anesthesia 30 male Sprague-Dawley (Hsd/CPB:SD) rats (Harlan CPB, Zeist, The Netherlands), 4–5 weeks of age, were each sc between the scapulae implanted with a single pellet containing 25 mg estradiol-17β (Organon, Oss, The Netherlands). There was an equal number of control rats, which were sham operated in an identical manner but without the deposition of a pellet.

All 60 rats were kept in stainless wire-mesh cages (2 or 3 animals per cage; controls separated from experimental animals) in a well ventilated room maintained at 20°C with relative humidity 40–70% and 12 hr of lighting per day. Rats were fed the Institute's powdered, natural ingredient diet for rats. The animals had free access to food and tap water.

Two treated and 2 control rats were sacrificed by decapitation while under ether anesthesia at each of the following time points: 7, 9, 11, 13, 16, 25, 35, 81, 114, 133, 150, 168, 217, 241, and 272 days after the estradiol pellet implantation. All animals were treated under the protocol and housing arrangement approved by the Institutional Committee for the Humane Use of Animals.

At necropsy, the pituitaries were carefully dissected and fixed in a 2.5% glutaraldehyde solution buffered with 0.1 M sodium cacodylate (pH 7.4), and post-fixed in a 1% osmium tetroxide solution in the same buffer. After rinsing, the pituitaries were dehydrated in a graded series of acetone, and embedded in a glycid ether 100/Araldite mixture. One μm-thick sections were stained with toluidine blue, and ultrathin sections with uranyl magnesium acetate and lead citrate. The periodic acid-silver methamine staining method for carbohydrates (9) was used to demonstrate basement membrane material. The ultrathin sections were examined using a Phillips EM401LS at 60 kV.

Results

The presence or absence, in comparison with controls, of alterations in the epithelial and perivascular compartments of the anterior pituitary, and a semi-quantitative assessment of their severity were recorded for each time point as determined by light and electron microscopy. The time points were grouped when no differences in alterations were observed between time points. The results of this assessment are summarized in Table I, and the changes, in comparison with those of control tissue (Fig. 1), are briefly described below by time period.

Days 7 and 9 Post-Implantation (Fig. 2)

The earliest change observed in epithelial cells was an increase in the size of many cells containing increased amounts of rough endoplasmic reticulum (RER), Golgi structures, and electron dense secretory vacuoles. The epithelial cells were arranged in more or less distinct trabeculae of a few cells thick lined by sinusoids, rather than more diffusely as seen in controls (Fig. 1).

The earliest vascular change was a slight distention of the pericapillary interstitial space containing collagen that appeared somewhat dispersed.

Days 11 and 13 Post-Implantation (Fig. 3)

Some epithelial cells were now arranged in islands or foci, often surrounding a sinusoid, rather than in trabeculae. The RER proliferation and enlargement of epithelial cells were more marked than at 7–9 days, as was their arrangement in trabeculae.

Many sinusoids were now slightly distended, whereas a few displayed some compression of the lumen by enlarged epithelial cells (Fig. 3). Endothelial cells showing plasma membrane blebbing were now also regularly seen, and there were a few endothelial cells with prominent degenerative changes, particularly vacuolization, or frank necrosis. In addition, distention of pericapillary spaces and dispersion of collagen were more prominent than at days 7–9.

Days 16, 25, 35, and 81 Post-Implantation (Fig. 4)

The epithelial cells appeared to contain less RER than at 11–13 days. These cells were also increasingly arranged in islands from 16–81 days post-implantation, whereas trabecular arrangement was decreasing in frequency. Occasional scattered necrotic and degenerated epithelial cells were observed from 16 days post-implantation onwards; the latter were characterized by cell swelling, RER degranulation, and swelling of endoplasmic reticulum and some mitochondria. The frequency of occurrence of these necrotic/degenerated cells did not change much be-

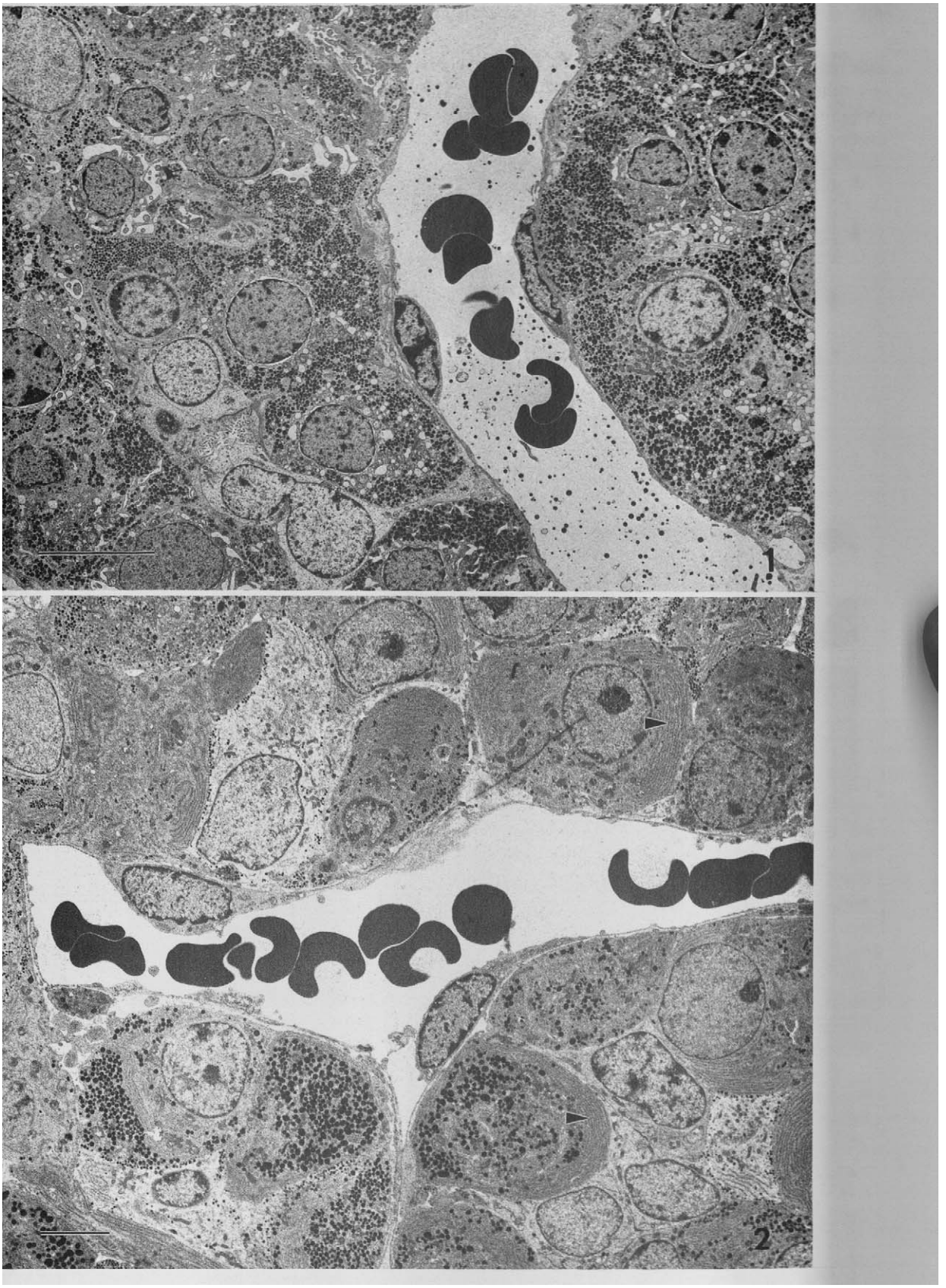

FIG. 1.—Control pituitary; sinusoids are lined by epithelial cells on a single basement membrane. Bar = 10 μm.

FIG. 2.—Pituitary after 7 days of estrogen treatment. Endothelial cells are increased in size and contain increased amount of rough endoplasmic reticulum (RER) (arrowheads). Epithelial cells are arranged in more or less distinct trabeculae (not shown). Bar = 5 μm.

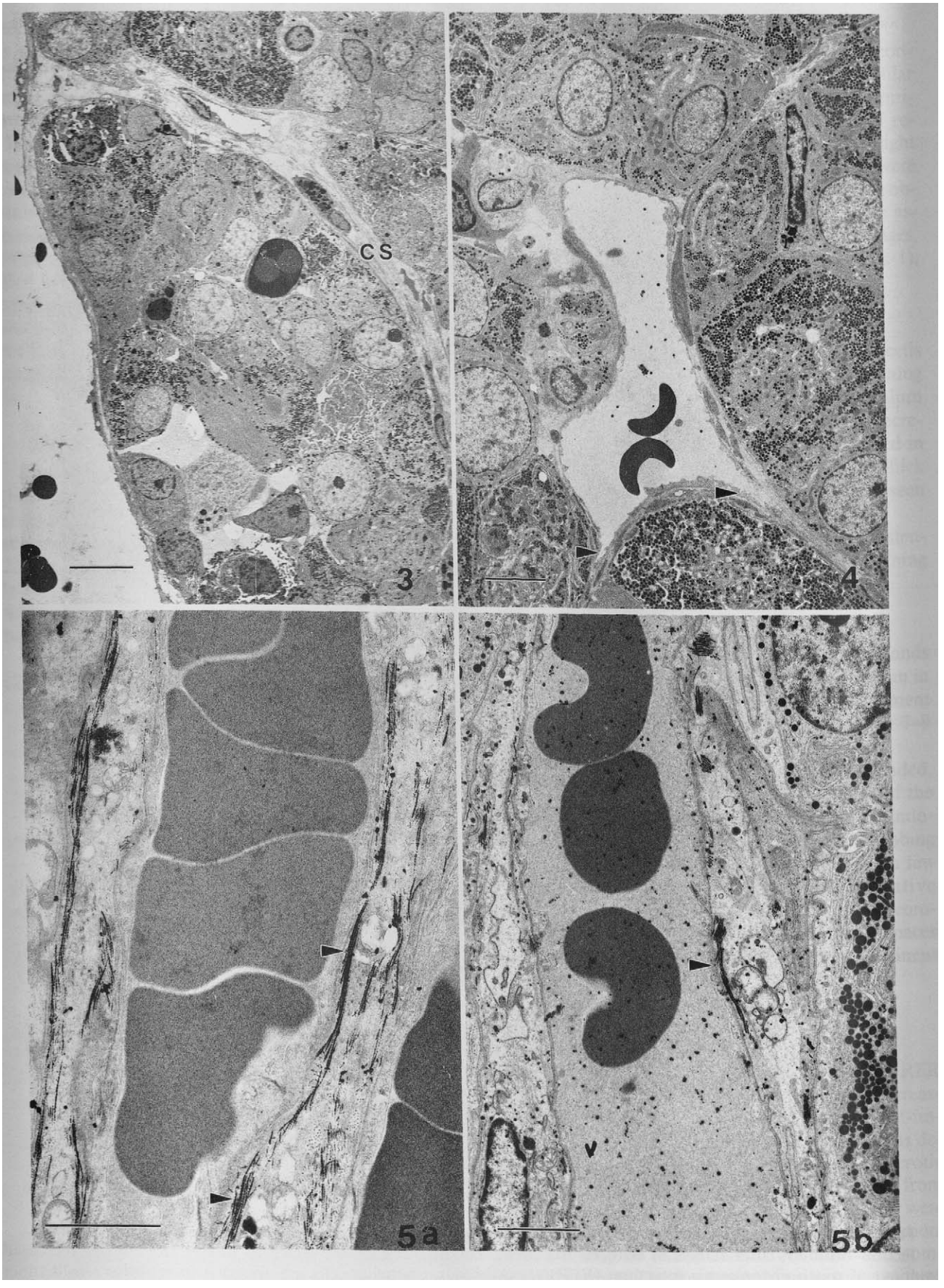

TABLE I.—Presence and severity of epithelial and perivascular alterations in rat pituitaries at different times after the start of estradiol treatment.

Alteration	Time-period (days after start of estradiol treatment)					
	7–9	11–13	16–81	114–133	150–217	241–272
Epithelial compartment						
Cell size	↑	⇈	⇈	↓	⇊	
RER proliferation	+	++	+	→	→	±
Degeneration/necrosis			+	+	+	+
Immature cells			+	++	++	+++
Lipid droplets				+	++	++
Cells directly on sinusoidal lumen				+	++	+++
Hemorrhagic areas				±	+	++/+++
Growth pattern:						
Trabecular	+	++	+			
Islands		+	++	+++	++	+/++
Nodular					++	+++
(Peri)vascular compartment						
Distention of pericapillary spaces	+	++	++			
Collagen dispersion	+	++	++			
Sinusoidal distention		+	++	→	→	±
Endothelial blebbing		+	++			
Endothelial degeneration and necrosis		±	+	++	+++	+++
Localization of sinusoids within islands/nodules		+	++	→	→	+++
Sinusoidal compression		±	+	++	++	++
Loss of collagen			±	+	++	+++
Loss of basement membrane			+	→	→	++
Multiple basement membranes				+	++	+++

Change in cell size is indicated as: ↑ moderate, ⇈ marked increase; ↓ slight, ⇊ moderate decrease.
The degree of severity of alteration is indicated: ± minimal; + slight; +/++ slight to moderate; ++ moderate; ++/+++ moderate to marked; +++ marked; → gradual change.

tween 16 days and 272 days, which was the last time point in this study. There were also some scattered cells with an immature character, lacking secretory vacuoles and with few RER and Golgi structures.

Blebbing of endothelial cells was now very frequent, and from day 16 to day 81 post-implantation there were increasingly more degenerated/necrotic cells than at 11–13 days. There was also scattered loss of basement membrane material and some loss of pericapillary collagen. Distention of pericapillary spaces and dispersion of pericapillary collagen was similar in severity to what was seen at days 11–13. However, both distention and compression of sinusoids were distinctly more prominent, as was the location of sinusoids within epithelial cell islands. The distended sinusoids were located in areas of epithelial cell trabeculae, whereas compressed sinusoids occurred in both islands and trabeculae of epithelial cells.

Days 114 and 133 Post-Implantation (Figs. 5–7)

Some epithelial cells were now directly lining a sinusoid, due to the loss of endothelium and basement membrane, and there were some small hemorrhagic areas, i.e., distended, blood-filled areas lined with epithelial cells (Fig. 7). The epithelial cells were now decreasing in size and seemed to contain less RER as compared with days 16–81, whereas several cells now appeared to contain lipid droplets. There were more immature cells than seen at days 16–81, and their frequency of occurrence did not change much between days 114 and 217. Arrangement of epithelial cells in islands predominated, and many of these islands included one or a few sinusoids.

There were now many necrotic and degenerated endothelial cells, and blebbing endothelium without any more advanced degenerative changes was rare.

←

FIG. 3.—Pituitary after 11 days of estrogen treatment. Some cells are arranged in islands often surrounding a compressed sinusoid (CS). Other sinusoids are distended (lower left hand corner). Bar = 10 μm.

FIG. 4.—Pituitary after 16 days of estrogen treatment. A sinusoid with compression surrounded by epithelial islands (arrowheads). Bar = 5 μm.

FIG. 5.—Pituitary after 114 days of estrogen treatment. Distention of sinusoids and loss of pericapillary collagen [compare arrowheads in (a) control and (b) estrogen-treated rat pituitary]. Bar (a) = 2.5 μm, and (b) = 2.5 μm.

Multiple basement membranes were occasionally observed for the first time (Fig. 6), but there was also somewhat more severe scattered loss of basement membrane than on days 16–81. Distention of sinusoids was somewhat less prominent than at earlier time points, whereas loss of pericapillary collagen was slightly more prominent (Fig. 5a, b). Compression of sinusoids was more frequent and severe than at days 16–81.

Days 150, 168, and 217 Post-Implantation (Fig. 8)

Several epithelial islands now contained cells that were slightly pleomorphic, i.e., showed variation in their size and in size and shape of their nucleus. These islands were demarcated from and caused compression of the surrounding islands that did not have pleomorphic cells, and they were therefore classified as nodules, perhaps representing early tumors. Approximately one third to half of the pituitary mass consisted of nodules. The decrease in epithelial cell size first seen between days 16 and 81 gradually continued, and the presence of lipid droplets was more prominent than earlier. There were more epithelial cells directly lining sinusoids and there were more and larger hemorrhagic areas, which were more frequent in nodular areas than in islands.

Necrosis and degeneration of endothelium were abundant, and there were more areas with multilayered basement membranes, as well as more loss of basement membrane material in other locations than on days 114–133. Loss of pericapillary collagen and sinusoidal compression were more prominent than on days 114–133, and distention of sinusoids was less conspicuous.

Days 241 and 272 Post-Implantation (Figs. 9a, b, and 10)

The number and the size of nodules and hemorrhagic areas continued to increase. On day 272, approximately two thirds of the pituitary consisted of large nodules containing sinusoids, remnants of sinusoids, multiple basement membranes, and hemorrhagic areas. These hemorrhagic cavities were lined by epithelial cells and were filled with erythrocytes, a few macrophages, and some cellular debris. Immature cells were very frequent, occurring more often than at the previous time points, but there were no further changes in epithelial cell size or the amount of lipid droplets they contained.

There was a further increase in the extent of endothelial necrosis and loss of their basement membrane material, and, particularly on day 241, the presence of areas of multiple basement membranes. Loss of pericapillary collagen was progressive with virtually no collagen left on day 272. Sinusoidal compression and location of sinusoids within epithelial islands or nodules were very frequent, whereas distention of sinusoids was further reduced and almost absent on day 272.

DISCUSSION

This study demonstrates that the development of hemorrhagic areas during estrogen-induced rat pituitary tumor formation is preceded by degenerative and necrotic changes in endothelium and loss of basement membrane and pericapillary collagen. Coinciding with these endothelial and pericapillary changes, some sinusoids are distended whereas others are compressed. Epithelial cell swelling and arrangement of these cells in trabeculae occurs prior to these sinusoidal changes. This sequence of events suggests that swelling of pituitary epithelium leads to interference with local circulation followed by ischemic injury to the endothelium. The result is loss of sinusoidal lining and degradation of basement membrane and pericapillary collagen. Ultimately, epithelial cells line sinusoidal lumina.

There are 2 potential mechanisms by which epithelial cell swelling can interfere with pituitary circulation. i) The entire pituitary gland may enlarge which would result in compression of the draining venous system located at the pituitary surface (11). This leads to general pituitary congestion, subsequent distention of sinusoids, and finally compression of afferent stalk blood vessels. This process would not result in sinusoidal distention. ii) Sinusoids within the pituitary are locally compressed which may result in local reduction of blood flow and distention of proximal parts of the affected sinusoids. Stalk compression by the tumor and hypoxic endothelial damage due to reduced blood supply has been proposed as the mechanism for hemorrhagic lesions in human pituitary adenomas (7, 14). In the estrogen-induced pituitary tumor model used in this study, stalk compression is unlikely until 16 days following estrogen treatment. We have previously shown, using Magnetic Resonance Imaging (MRI) techniques (20), that the subarachnoid space between the pituitary and the brain (diencephalon and pons) did not become smaller by that time. Swelling of the pituitary was first apparent by MRI on day 9 in that study. Distention of sinusoids was first seen on days 7 and 9 in the present study, together with the first signs of compression of sinusoids. Therefore, compression of draining veins on the surface of the pituitary and local compression of sinusoids, rather than stalk compression, may impair pituitary blood circulation and cause ischemia. The occlusion of sinusoids and pituitary circulatory congestion, however, are not so severe that

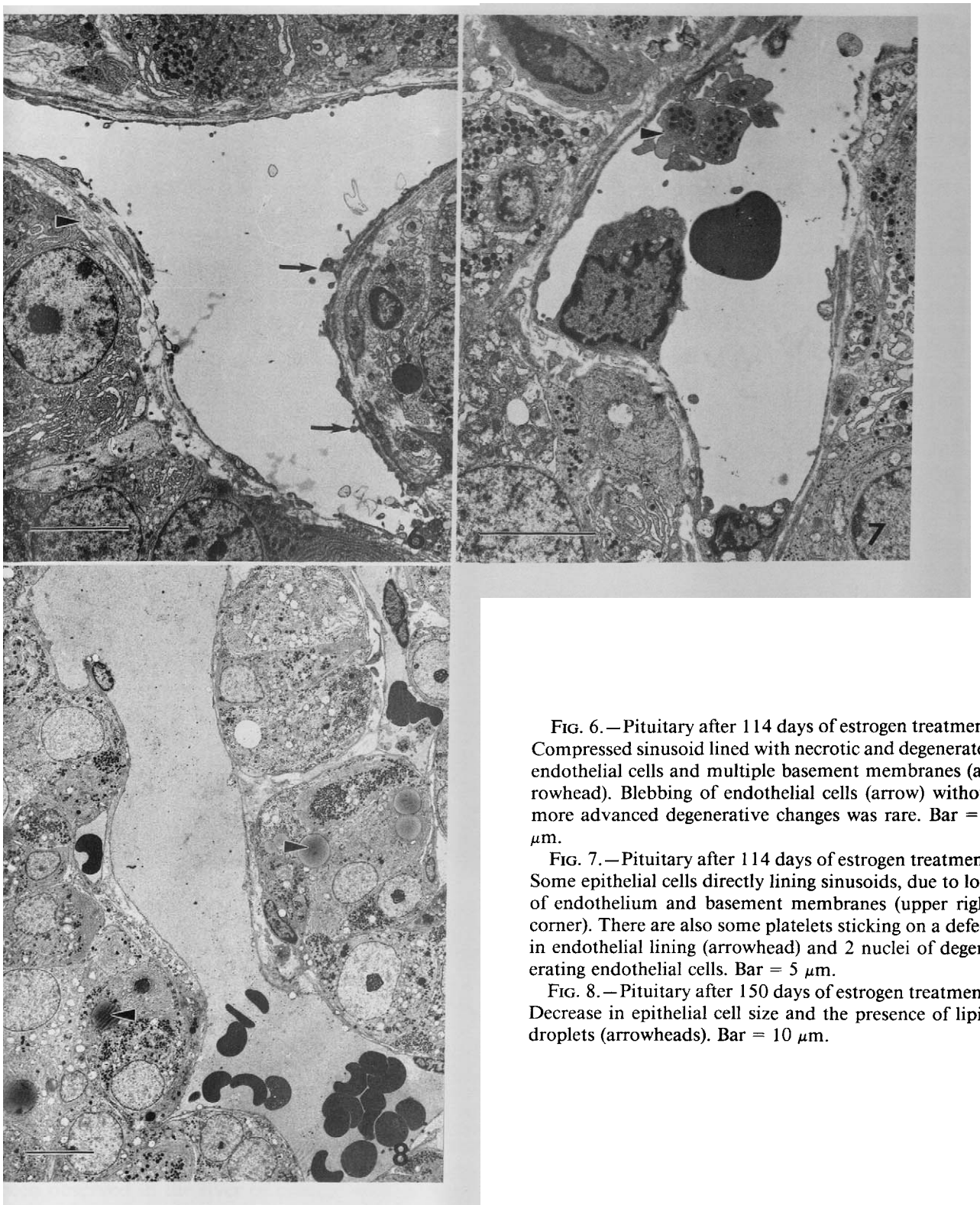

FIG. 6.—Pituitary after 114 days of estrogen treatment. Compressed sinusoid lined with necrotic and degenerated endothelial cells and multiple basement membranes (arrowhead). Blebbing of endothelial cells (arrow) without more advanced degenerative changes was rare. Bar = 5 μm.

FIG. 7.—Pituitary after 114 days of estrogen treatment. Some epithelial cells directly lining sinusoids, due to loss of endothelium and basement membranes (upper right corner). There are also some platelets sticking on a defect in endothelial lining (arrowhead) and 2 nuclei of degenerating endothelial cells. Bar = 5 μm.

FIG. 8.—Pituitary after 150 days of estrogen treatment. Decrease in epithelial cell size and the presence of lipid droplets (arrowheads). Bar = 10 μm.

stasis occurs, since no signs of coagulation or thrombus formation were found.

Hemorrhagic lesions are present in even very small spontaneous rat pituitary tumors (1, 16, 17) that cannot possibly cause compression of afferent or efferent blood vessels. This observation provides strong support for an important role of local ischemia due to local obstruction of blood flow in the pathogenesis of the hemorrhagic areas. The estrogen-induced rat pituitary tumor model differs from

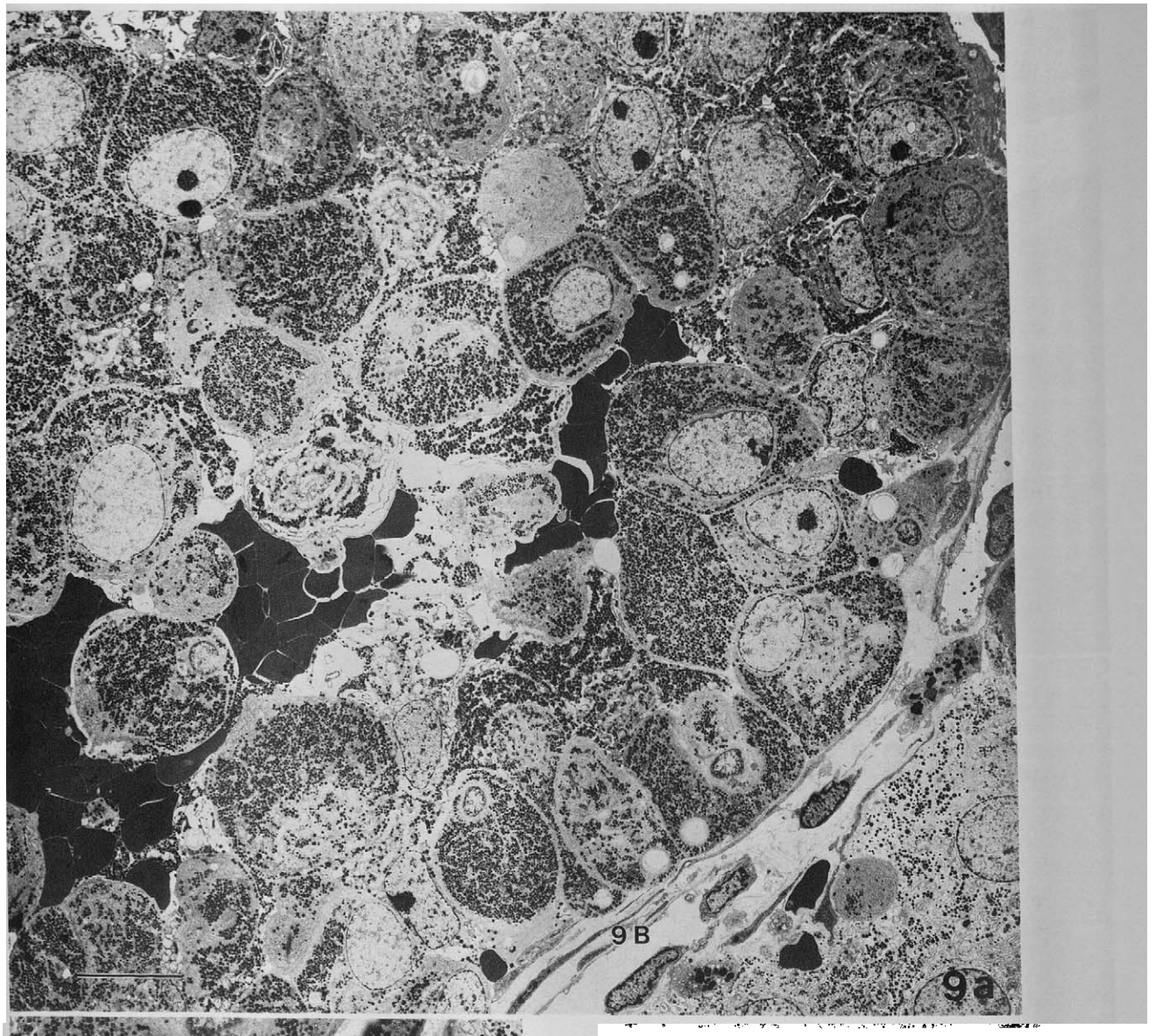

FIG. 9.—Pituitary after 241 days of estrogen treatment. a) Nodule with hemorrhagic areas and multiple basement membranes (arrowhead in b which is a higher magnification of area labeled 9B in a). Bar a = 10 μm, and b = 5 μm.

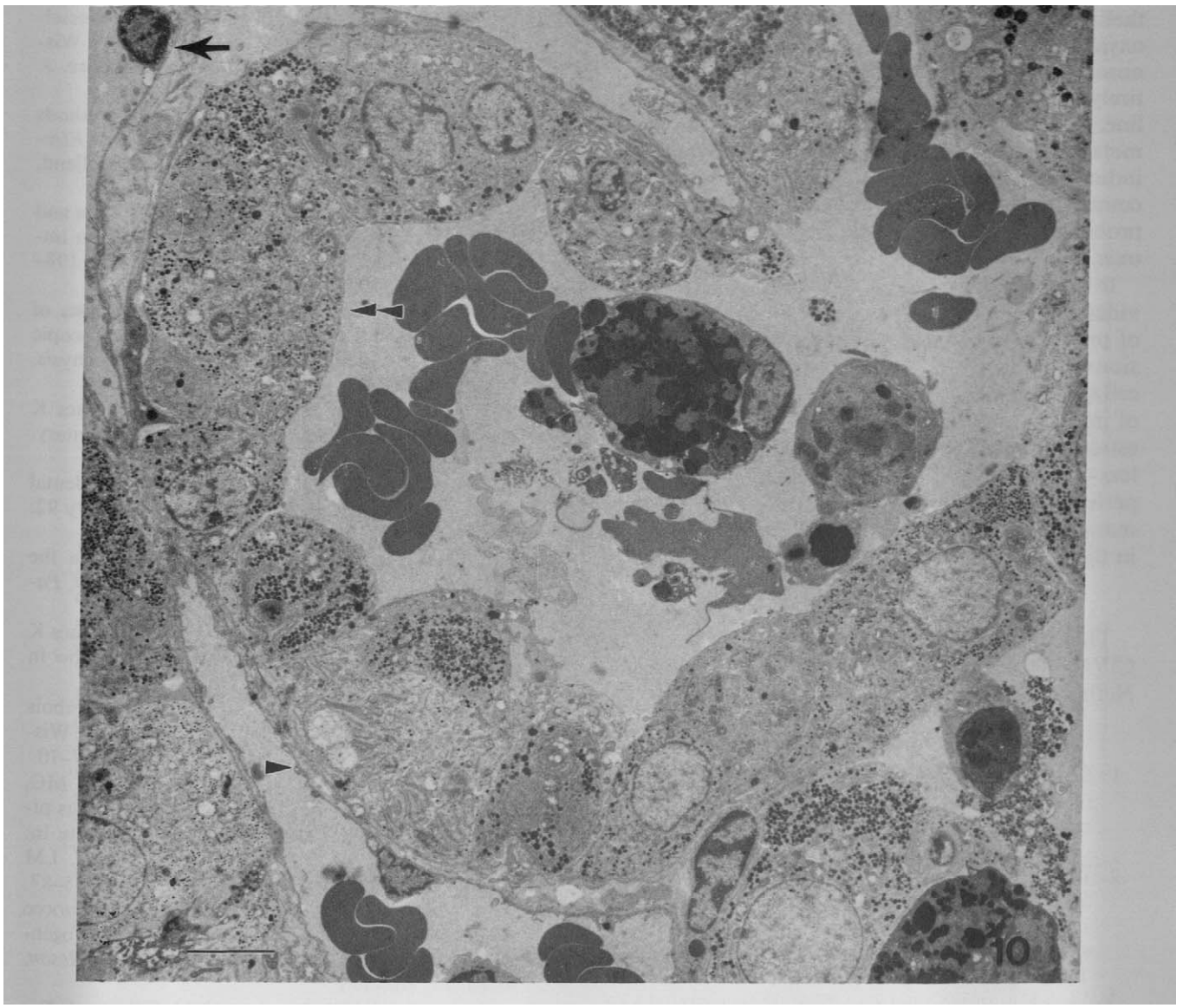

FIG. 10.—Pituitary after 272 days of estrogen treatment. Nodule with hemorrhagic cavity filled with erythrocytes, a few macrophages and some cellular debris and lined by epithelial cells (double arrowhead). The nodule is surrounded by sinusoids lined with endothelial cells (single arrowhead) some of which are degenerated (arrow). Bar = 5 μm.

spontaneous rat tumors in that the model produces enlargement and eventual tumor formation involving the entire pituitary, rather than the spontaneously occurring focal process. The tumorous nodules seen in the present study were, however, morphologically indistinguishable from spontaneously occurring hemorrhagic tumors in aged rats described elsewhere (1, 8, 16, 17). Angiogenesis has been observed in the liver of estrogen-treated rats (21), and angiogenesis may occur in the pituitary of estrogen-treated rats (15). Signs of increased amounts of sinusoidal structures were not found in the present study. Regeneration of endothelium following recurrent degeneration/necrosis as indicated by the presence of multiple basement membranes, which did not occur until hemorrhagic areas were first encountered, was observed, however.

Contribution of central necrosis, in epithelial cell islands or tumorous nodules, to the development of hemorrhagic areas is theoretically also possible. Necrosis occurred in a scattered fashion (single cell necrosis) and no indications of central necrosis were observed until the last time point (272 days), when cellular debris and mononuclear inflammatory cells, predominantly macrophages, were observed in the hemorrhagic areas. There were no early signs of inflammatory reactions, and thus, endothelial necrosis probably occurred gradually enough for vascular clearance of debris. Epithelial cells appeared less susceptible to the hypoxic conditions than endo-

thelial probably because the former cells obtain oxygen from erythrocytes in several surrounding sinusoids, whereas endothelial oxygen supply is entirely derived from the blood in the sinusoid they line. As the epithelial cells apparently underwent a metabolic change with ongoing estrogen treatment, indicated by the decrease in cell size, change in RER content and the appearance of lipid vacuoles, they probably also became somewhat resistant to hypoxia.

In summary, this serial sacrifice study has provided morphologic evidence that local compression of pituitary sinusoids and probably also compression of pituitary surface veins, both due to epithelial cell swelling, play a primary role in the development of ischemic endothelial damage in the pituitary of estrogen-treated rats. This process in turn, causes loss of endothelial lining, basement membrane, and pericapillary collagen, thereby, resulting in the formation of blood-filled spaces lined by epithelial cells in the developing tumors of the anterior pituitary.

ACKNOWLEDGMENTS

This work was supported in part by Grant No. CIVO 87–2 from the Dutch Cancer Society, and The Netherlands Ministry of Welfare and Public Health.

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