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Seasonal Changes in the Pituitary Gland of the Feral Hawaiian Mongoose (Herpestes auropunctatus)

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The Hawaiian mongoose (Herpestes auropunctatus) is a ABSTRACT seasonally breeding mammal whose pituitary gland resembles that of other Viverridae. Certain features, such as a prominent pars tuberalis interna and a doublelayered pars intermedia forming a cup for the neurohypophysis, are unique.

With the light microscope, five different cell types can be recognized in the pars distalis after staining with periodic acid-Schiff (PAS)-orange G. Two types of acidophils are seen, a small yellow-staining cell and a large angular orange cell. Two basophilic cells are also seen, one with fine PAS-positive cytoplasmic granules and the other with coarse PAS-positive granules in the cytoplasm. The last cell type seen is the chromophobe. Differential cell counts indicated an altered distribution of chromophils in the ventral pars distalis of the female mongoose with changing season and reproductive status, but the most striking change was a decreased percentage of basophils in the pars distalis during the nonbreeding season.

The morphology and cytology of the pituitary gland of the Hawaiian mongoose (Herpestes auropunctatus) have not been studied. Hanström ('66) compared the anatomy of the pituitary glands of three members of the Viverridae to which Herpestes belongs - the largespotted genet (Genetta tigrina), the bushytailed meerkat (Cynictis penicillata), and the ruddy mongoose (Galerella caesi) - but the cytology of the gland was not described.

The Hawaiian mongoose is a seasonally breeding mammal and exhibits annual fluctuations in the size and weight of its reproductive organs in both sexes (Pearson and Baldwin, '53; Soares and Hoffmann, '81). During the nonbreeding season from September to January, the ovaries and pituitary gland of the female mongoose decrease somewhat in weight. Herpestes has not been successfully bred in captivity in Hawaii and little is known about its reproductive physiology. As part of an investigation of reproductive function in the Hawaiian mongoose, a study of the seasonal changes in the microanatomy of the pituitary gland was conducted.

MATERIALS AND METHODS Animals

Feral Hawaiian mongooses captured in Kaneohe on the windward side of the island of Oahu, were housed in wire cages and given

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Purina cat chow and water ad libitum. Adult female animals with body weights over 400 gm were used in the study. Unless otherwise indicated, the animals were autopsied within 2 days of capture.

Six mongooses captured during the breeding season were placed at autopsy into either group I, pregnant or lactating females with recent implantation scars in the uterus (body weight, 477 ± 24 gm), or Group II, nonpregnant females lacking implantation scars and with a uterine weight of less than 5 gm (body weight, 449 ± 23 gm). One mongoose from group II was ovariectomized (OVX), and the other two animals in this group were laparotomized (sham OVX) to verify their reproductive status two weeks prior to autopsy.

During the nonbreeding season, 12 female mongooses were captured. Their reproductive status was determined at autopsy, and adults (body weight, 431 ± 10 gm) with uterine implantation scars and inactive mammary tissue formed group III.

Histologic methods

Two animals from group III were used to examine the microanatomy of the pituitary gland. The mongooses were ether-anesthe-

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tized, exsanguinated, and perfused with gluteraldehyde solutions by a modification of the method of Forssman et al. ('76). The brain in situ on the cranial floor was placed in 6% gluteraldehyde for an additional 18 hours before storage in 70% ethanol. After decalcification in RDO (DuPage Kinetic Laboratories, Inc.), a block of tissue containing the preoptic region, diencephalon, infundibular stalk, pituitary gland, and sphenoid bone was embedded in paraffin and sectioned parasagittally at 10 μ m. Sections containing the pituitary gland were mounted on slides, stained with hematoxylin and eosin, and a sagittal-section line drawing of the pituitary was made (Fig. 1).

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All of the remaining animals were etheranesthetized, weighed, and decapitated within 5 minutes of initial handling. The pituitary gland in situ on the cranial floor was immersed in freshly prepared Zenker formol for 1 hour, followed by an additional 2 hours in fresh fixative for the isolated gland. During alcohol dehydration, the pituitaries were passed through a saturated iodine solution in 70% alcohol to remove mercuric chloride deposits. The glands were then embedded in paraffin and blocks of tissue were serially sectioned in a horizontal plane at 4 μ m. The number of sections for each gland was determined, and three representative sections each from $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ of the way through the gland dorsally to ventrally were mounted on a slide. Duplicate slides were made with sequential sections to those on the initial slide.

Since the tinctorial properties of cells in the pars distalis of the Hawaiian mongoose were unknown, several techniques were used to determine the most effective method of staining. The most satisfactory results were obtained with periodic acid-Schiff reagent (Purves and Griesbach, '51) with an iron hematoxylin orange G-phosphotungstic acid counterstain (PAS-OG) according to Pearse ('68), or performic acid-Alcian blue-PAS-OG (modified from Pearse, '68). The descriptions of cytology are largely based on the PAS-OG method.

Differential cell counts

Pituitaries from three animals in group I, the sham OVX animals of group II, and three animals from group III were used in cell counts. Although cell counts were determined for the OVX mongoose, this animal was not included in the statistical analysis. A differential count of anterior pituitary cells at each of the three levels $(\frac{1}{4}, \frac{1}{2}, \text{ and } \frac{3}{4})$ through the



Fig. 1. Diagram of the pituitary of the Hawaiian mongoose (Herpestes auropunctatus). Brain and neurohypophysis, vertically striated; pars intermedia, solid black; pars distalis, large dots; sphenoid bone, oblique striations; l, infundibular recess; M, mammillary body; t, pars tuberalis.

gland was made using an ocular micrometer grid $(10 \times 10\mu m)$ and a magnification of \times 1000. Those cells in every third field of view were counted to a total of 200 cells from each mounted section of the pars distalis. Counts from the three sections at each level were averaged and expressed as a percentage for that level. The counts for the three levels through the gland were likewise averaged and expressed as a percentage for the gland (Schooley et al., '66). The Student t-test was used to determine the significance of differences in the percentages or distribution of cells in the pars distalis (Fisher, '58).

OBSERVATIONS

The pituitary gland of the Hawaiian mongoose lies ventral to the mammillary bodies of the hypothalamus (Fig. 1). In the adult, the posterior clinoid processes frequently overhang the gland and form a bony roof over the pituitary. The pars distalis lies rostral to the spherical pars nervosa and ventral to the infundibular stalk. The infundibular recess of the third ventricle does not extend far into the stalk. The pars intermedia, a double-layer of cells cupping the pars nervosa, is separated from the pars distalis by a prominent residual cleft which extends between the layers of the intermedia. Short anterior extensions of the lateral part of this cleft partially separate the pars intermedia from the pars distalis (Fig. 2A), but rostrally the two soon become indistinguishable. The pars tuberalis, limited to the ventral surface of the infundibular stalk, is a layer of cells intermingled with pial membranes. In the pars distalis, a large flattened Rathke's cyst peripherally caps the anterior

end of the gland (I posteriorly from this c distalis on either side of columns of cells form a Numerous follicles cor colloid are present in th

A. Pars distal.

Based upon their sta and size, five clearly dia cells can be seen in th Hawaiian mongoose: tw two types of basophils type.

Acidophils

Oval cells. Numero brilliantly yellow with They average about 1(dimension and display clearly defined cell boun appears homogeneous, fine refractile granules a

Angular cells. A se which stains with orang finity for PAS. Conseque orange color with PAS-C formic acid-Alcian blue acidophils are less nur cells and are usually lau μ m in diameter. The cy stains diffusely and the centrically. These cells groups of oval acidophi appear to be embedded i the boundaries of angul unclear.

Basophils

Oval cells. These granulated cytoplasm w PAS stains (Figs. 3B,C throughout the gland b peripherally and rostr similar to that of the ov are slightly smaller, ave diameter. The nucleus the cell boundaries ma basophils usually occur with chromophobic cells

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ng an ocular micrometer nd a magnification of \times every third field of view tal of 200 cells from each the pars distalis. Counts tions at each level were ssed as a percentage for ints for the three levels ere likewise averaged and ercentage for the gland). The Student t-test was the significance of difentages or distribution of talis (Fisher, '58).

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A. Pars distalis: cell types

Based upon their staining affinity, shape, and size, five clearly distinguishable types of cells can be seen in the pars distalis of the Hawaiian mongoose: two types of acidophils, two types of basophils, and a chromophobe type.

Acidophils

Oval cells. Numerous oval cells stain brilliantly yellow with orange G (Fig. 3A). They average about 10 μ m in their longest dimension and display a single nucleus and clearly defined cell boundaries. The cytoplasm appears homogeneous, although occasionally fine refractile granules are seen.

Angular cells. A second and larger cell which stains with orange G also has weak affinity for PAS. Consequently, these cells are an orange color with PAS-OG with or without performic acid-Alcian blue treatment. Angular acidophils are less numerous than the oval cells and are usually larger, ranging up to 20 μ m in diameter. The cytoplasm of these cells stains diffusely and the nucleus is located eccentrically. These cells are often located with groups of oval acidophils, and oval cells may appear to be embedded in an angular cell since the boundaries of angular cells are sometimes unclear.

Basophils

Oval cells. These cells contain finely granulated cytoplasm with a weak affinity for PAS stains (Figs. 3B,C). They are dispersed throughout the gland but are most numerous peripherally and rostrally. Their shape is similar to that of the oval acidophils but they are slightly smaller, averaging about 8 μ m in diameter. The nucleus is often eccentric and the cell boundaries may not be clear. Oval basophils usually occur in clusters together with chromophobic cells.

Angular cells. The least numerous cells in the pars distalis are the angular basophils (Figs. 3B,C) which contain coarse cytoplasmic granules staining strongly PAS-positive. The granules are a blue-purple color with PAS in contrast to the magenta color of the oval basophils. The nucleus may be eccentric, an

oval negative Golgi image is often seen in the cytoplasm, and cell boundaries may not be distinct. These are the largest cells in the gland averaging up to $20-25 \ \mu m$ in diameter.

Chromophobes

Cells of varying shapes and sizes and showing little affinity for PAS, Alcian blue, or orange G are found throughout the pars distalis. Their cytoplasm appears clear, although a few may contain a single clump of PASpositive material. The nucleus is generally central and contains finely dispersed chromatin granules with one or more nucleoli.

Distribution of cell types within the pars distalis

The five cell types are distributed evenly throughout most of the pars distalis except in the extreme rostral area and adjacent to the pars intermedia where basophils and chromophobes predominate. A few angular basophils may be found in the pars tuberalis interna, but finely granulated oval basophils and chromophobes constitute the majority of cells in this region.

Table 1 compares the distribution of chromophils at the three levels through the pituitary gland previously described. From dorsal to ventral, there were no differences in the distribution of acidophils in the glands from groups I and II captured during the breeding season. However, females captured during the nonbreeding season (group III) had significantly more stainable acidophils in the dorsal half of the gland than in the ventral. The dorsal pars distalis of the pregnant or lactating animals (group I) contained slightly fewer acidophils than did the same region in the sham OVX females (group II) and in animals captured during the autumn (group III). Similar results were obtained when numbers of acidophils were compared midway through the gland for all three groups, but a striking difference in acidophil distribution was found when the ventral regions of the pituitaries were compared. Significantly fewer acidophils were found in the ventral quarter of the glands from group III animals than in either of the other two groups.

The distribution of basophils dorsally in the pars distalis did not change in any group. However, when the groups were compared with one another, several differences were found. The most significant change was seen in the animals of group III where the smallest percentage of basophils was found. In addi-



Fig. 2. Horizontal sections through the pituitary gland of the Hawaiian mongoose taken from $\frac{1}{4}(A)$, $\frac{1}{2}(B)$, and $\frac{3}{4}(C)$ of the way through the gland from dorsal to ventral. 1,

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mongoose during the breeding season; a small follicle filled with colloid (e) is part of the pars tuberalis interna. All sections at 4 μ m; stained with PAS-OG. Scale line = 10 μ m.

lis; 3, pars intermedia; 4, neurohy-AS-OG. × 10.

tion, increased numbers of basophils were present in the ventral half of the gland when group II animals were compared to those in group I.

Seasonal variations

Table 2 gives the mean percentages of chromophils and chromophobes throughout the pars distalis for each group. There was no difference in the total percentage of acidophils when animals trapped in the spring and fall of the year were compared (groups I + II vs. group III). However, when basophil percentages were compared, a striking decrease was found in group III with few stainable basophils evident and an increased percentage of chromophobes. During the breeding season, more basophils were present in the sham OVX females than in either the pregnant or lactating mongooses. The greatest percentage of basophils and the fewest acidophils were found in the OVX female (22.2% acidophils, 21.6% basophils, 56.2% chromophobes).

B. Pars tuberalis and pars tuberalis interna

The pars tuberalis in the Hawaiian mongoose lies on the ventral surface of the distal half of the infundibular stalk. It does not encircle the stalk and consists of a layer of chromophobes closely associated with the overlying meninges. An occasional palestaining basophil may be seen, but no colloid follicles were found in the pars tuberalis.

Within the pars distalis, two broad columns of cells extend from rostral to caudal ends through the gland on either side of the midline. The columns are most noticeable in the midhorizontal plane of the gland, although they extend vertically through most of the thickness of the pituitary. This region corresponds to the pars tuberalis interna or zona tuberalis (Hanström, '52; Holmes and Ball, '74). The predominant cell is a chromophobe with a single central nucleus. The cells are arranged in cords along sinusoids. Many follicles, composed of simple cuboidal epithelium surrounding a lumen filled with PAS-positive colloid, are scattered throughout the zones and vary in diameter with most averaging less than 50 μ m. Follicles are most numerous in the ventral pituitary, where they averaged 89 follicles per section.

C. Pars intermedia

The pars intermedia cups the neurohypophysis in two sheets of cells, each sheet three to four cells thick and separated incompletely from the other by a narrow cleft. Adjacent to the pars distalis, the anterior wall of the

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TABL	D I. Distribution of	Percentage acidophils at each level of gland (%)			
Group	N	1/41	1/2	3/4	
A. Acidophil distribu I II III	tion 3 2 3	31.0 ± 2.9 38.3 ± 1.6 39.0 ± 1.8	34.4 ± 2.0 35.1 ± 1.1 39.7 ± 1.6	36.2 ± 3.2 34.8 ± 0.2 21.6 ± 2.5	
B. Basophil distribut I II III	ion 3 2 3	13.9 ± 3.4 19.4 ± 1.1 0.8 ± 0.4	$\begin{array}{c} 10.8 \pm 1.2 \\ 18.3 \pm 1.4 \\ 1.0 \pm 0.7 \end{array}$	$\begin{array}{c} 10.0 \pm 1.6 \\ 19.7 \pm 2.6 \\ 1.8 \pm 1.4 \end{array}$	
Significance levels		I vs. II	I vs. III	II vs. III	
1/4	A. B	$\overline{P < 0.05}$ ns	P < 0.02 P < 0.01	$\mathbf{P} < 0.001$	
1/2	A. B	$\mathbf{P} < 0.01$	P < 0.05 P < 0.001	P < 0.05 P < 0.00	
3/4	A. B	ns P < 0.05	P < 0.02 P < 0.01	P < 0.01 P < 0.01	

See text for explanation. Values given are the mean \pm SEM.

TABLE 2. Differential cell counts in the pars distalis of the Hawaiian mongoose

		Pe	Percentage of cells in pars distalis (%)			
Chain	N	Acidophils	Basophils	Chromophobes		
I 3 I 2 III 3		$\begin{array}{c} 33.1 \pm 2.5 \\ 36.1 \pm 0.5 \\ 33.4 \pm 0.6 \end{array}$	$ \begin{array}{r} 12.2 \pm 2.2 \\ 19.1 \pm 0.0 \\ 1.2 \pm 0.8 \end{array} $	$54.7 \pm 0.4 \\ 44.8 \pm 1.0 \\ 65.4 \pm 1.2$		
Signifi cance levels		I vs. II	I vs. III	ll vs. III		
Acidophils Basophils Chromophobes		$\mathbf{P} < 0.02$ $\mathbf{P} < 0.001$	P < 0.01 P < 0.001	ns P < 0.001 P < 0.001		

Values given are the mean \pm SEM.

residual cleft is composed of a single layer of pars distalis cells. The pars intermedia forms the posterior wall of this cleft and is 15–20 cells thick dorsally, thinning to about 6–8 cells ventrally (Fig. 2). Cells of the pars intermedia are clearly separated from the neurohypophysis, are homogeneous in appearance, and stain a basophilic lavender with PAS. No follicles or colloid were seen in the pars intermedia; however, homogeneous acellular material present in the residual cleft had affinity for PAS, Alcian blue and aniline blue stains.

D. Neurohypophysis

The neurohypophysis of the Hawaiian mongoose has two distinct regions. Infundibular stalk fibers extend vertically through more than half of the lobe as a central pale area (Fig. 2B), while the bulk of the gland around this core consists of scattered cells, nerve fibers, and vessels. Slightly PAS-positive material is distributed along blood vessels throughout the lobe but is most noticeable near the periphery. With performic acid-Alcian blue staining, clumps of intensely stained material fill the neurohypophysis except in the central core region.

DISCUSSION

There was little variation in the morphology of the pituitary gland among the Hawaiian mongooses examined. Their pituitary gland has certain unique features, although there are similarities to pituitaries of other Viverridae. The long infundibular stalk of *Herpestes* overlying the pars distalis resembles that of the ruddy mongoose (*Galerella caesi*) and the large-spotted genet (*Genetta tigrina*) (Hanström, '52), but the Hawaiian mongoose pituitary has a short infundibular recess and the pars tuberalis does not surround the stalk. The only other member of the Viverridae in which the pars tubera is the mongoose My double-layered pars Herpestes has also be ('66) in Genetta and t (Cynictis penicillata). pars intermedia is genera studied (Myo pestes), but the prese separating the interlayers throughout, animals in this study, Galerella (Hanström,

A pars tuberalis in many species ranging hedgehog to the g Among the Viverric developed pars tubera only in *Cynictis*. The *Herpestes*, in contrast and contains many f wide range of varia phology reported a (Hanström, '66), th pituitary gland is neit most complex in its n

In the pars distal resemble the typical most species. These sidered to be soma although no attempt functions to any cells i acidophils described i spond to the red acide and Greisbach, '57), th the ferret (Holmes, '60 vole (Clarke and Forsy been given different 1 affinities for different they are morphologica similar functions. In t there were no seasona number of pituitary a distribution of stainal tral pars distalis varie reproductive status (parent absence of an group I may reflect en able material. The deg during pregnancy and investigation with niques and electron m

A dramatic seasona basophilic population *Herpestes.* The large seen in the spring of crease during pregnar

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PITUITARY	GLAND	OF THE	HAWAIIAN	MONGOOS
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ach level of g	land (%)
	3/4
2.0	36.2 ± 3.2
1.1	34.8 ± 0.2
1.6	21.6 ± 2.5
1.2	10.0 ± 1.6
1.4	19.7 ± 2.6
0.7	1.8 ± 1.4
11	II vs. III
12	ns
51	P < 0.001
15	P < 0.05
001	P < 0.001
)2	P < 0.01
21	P < 0.01

 $\frac{\text{diian mongoose}}{\text{pars distalis (\%)}}$ $\frac{\text{Chromophobes}}{2}$ $\frac{54.7 \pm 0.4}{44.8 \pm 1.0}$ $3 \qquad 65.4 \pm 1.2$ $- \qquad \frac{\text{II vs. III}}{\text{ns}}$ P < 0.001 P < 0.001

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A pars tuberalis interna has been found in many species ranging from the South African hedgehog to the giraffe (Hanström, '52). Among the Viverridae, however, a poorly developed pars tuberalis interna has been seen only in *Cynictis*. The pars tuberalis interna of *Herpestes*, in contrast, is large, well-developed, and contains many follicles. Considering the wide range of variations in pituitary morphology reported among the *Viverridae* (Hanström, '66), the Hawaiian mongoose pituitary gland is neither the simplest nor the most complex in its microanatomy.

In the pars distalis, the oval acidophils resemble the typical yellow acidophil seen in most species. These cells are usually considered to be somatotrophs (Purves. '66). although no attempts were made to ascribe functions to any cells in this study. The angular acidophils described in this study may correspond to the red acidophils of the dog (Purves and Greisbach, '57), the orange mucoid cells of the ferret (Holmes, '60), and the red cells of the vole (Clarke and Forsyth, '64). These cells have been given different names based upon their affinities for different staining methods, but they are morphologically similar and may have similar functions. In the Hawaiian mongoose, there were no seasonal differences in the total number of pituitary acidophils; however, the distribution of stainable acidophils in the ventral pars distalis varied significantly with the reproductive status of the animal. The apparent absence of an increase in acidophils in group I may reflect enhanced turnover of stainable material. The degranulation of these cells during pregnancy and lactation needs further investigation with immunocytologic techniques and electron microscopy.

A dramatic seasonal change occurs in the basophilic population of the pars distalis of *Herpestes*. The large numbers of basophils seen in the spring of the year appear to decrease during pregnancy and lactation. Many of these basophils must be gonadotrophs, since this cell population in other species produces the glycoprotein hormones, follicle-stimulating hormone, luteinizing hormone, and thyrotrophin (Purves, '66). In this regard, it is interesting to note that the single ovariectomized mongoose in this study had the greatest percentage of basophils in its pituitary. In future studies, differentiation of the basophilic cell population will be investigated by correlating light and electron microscopy with the results of immunocytochemistry.

Seasonally breeding animals, such as the 13-lined ground squirrel (Citellus tridecem*lineatus*), the male vole (Microtus agrestis), and the male Columbian black-tailed deer (Odocoileus hemionus columbianus), show decreased granulation of basophils during the anestrus season, but some PAS-positive reaction persists in these cells throughout the year (Clarke and Forsyth, '64; Hoffman and Zarrow, '58; West and Nordan, '76). However, the Hawaiian mongoose, like the ferret (Holmes, '63), loses almost all granulation in the basophils during the anestrum. Correlating changes in basophil numbers with hormonal levels in the pituitary gland has not yet been attempted in the Hawaiian mongoose. Such studies together with immunocytochemistry, conducted throughout the year and with animals in different reproductive states, can yield valuable information about reproduction in seasonally breeding mammals.

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ABSTRACT companied in osteoclasts of rats by transfmation of oste In this inv been determin treated with vnumbers of or soon after cell disease. No ch not cure the d These dat in the recover phenotype is : store bone res



disease characterized sive accumulation of s Walker, '76). The mec believed to be a gener resorption (Marks and rameter has only beer be reduced in the (Marks, '73, '74) and mouse (Raisz et al., '7 to date. In *ia* rats and bone resorption are sence or poor deve border of osteoclasts, foldings next to the al., '81; Marks, '73, '78

Osteopetrosis is an

The skeletal scleros petrosis is eliminated after spleen or bone from normal siblings (Coccia et al., '80). Du covery from osteopetr osteoclasts appears i y-body analysis in hu and by morphological et al., '80; Marks and (Marks, '78; Marks a

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