THE MORPHOLOGY OF THE PITUITARY GLAND OF THE HAWAIIAN MONGOOSE *(HERPESTES AUROPUNCTATUS)*

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ABSTRACT

The pituitary gland of the female Hawaiian mongoose, Herpestes auropunctatus, is a small boat-shaped organ located in a relatively shallow sella turcica, attached to the anterior clinoid process by dura mater, and caudally partially covered by the posterior clinoid process. The pituitary has an ovoid pars distalis. The pars intermedia forms a cup-like structure around the pars nervosa open dorsally. A prominent intraglandular cleft separates the pars distalis from the pars intermedia.

Three discrete cell types can be distinguished in the pars distalis with periodic acid-Schiff (PAS)-Orange G and Mallory III staining techniques: acidophils, basophils, and chromophobes. The cytoplasm of acidophils stains orange-yellow in Orange G; basophils contain PAS-positive and aniline blue-positive material, and the cytoplasm of chromophobes has no affinity for either stain. Chromophobes are the most abundant cell type (69.1%), followed by acidophils (25.2%), and lastly basophils (5.7%). Acidophils were concentrated in the dorsal posterior and lateral regions of the pars distalis, while basophils and chromophobes were more numerous in the rostral and central regions.

Numerous follicles consisting of a single layer of unstained cells surrounding a PAS or aniline blue-positive colloid-filled lumen were observed throughout the pars distalis but were most abundant ventrally and rostrally.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

The small Indian mongoose, Herpestes auropunctatus, was introduced into Hawaii from the West Indies in 1883, to control the rodent population plaguing the sugar cane industry (Pearson and Baldwin, 1953). It has since become a pest as it has broadened its diet to include several species of endemic ground-nesting birds in the Hawaiian Islands (Walker, 1945). The most popular hypothesis explaining the mongooses' failure as a favorable predator is that it is actively hunting for prey during the day and rodents are nocturnal.

The mongoose is a seasonally breeding animal, active during the spring and summer months. Several investigators have described the pituitary gland of other seasonally breeding animals such as the ground squirrel (Hoffman and Zarrow, 1958); ferret (Holmes, 1960); five-striped palm squirrel (Dhaliwal and Prasad, 1965); and the vole (Clarke and Forsyth, 1964). The pituitary or hypophysis in these animals as in most mammals is an endocrine gland resting at the base of the brain in a deep depression in the sphenoid bone, the sella turcica. The dura mater of the brain extends across this area as the diaphragma sellae which forms a fibrous connective tissue capsule. There is a small opening in the diaphragm through which passes the infundibular stem.

The pituitary is composed of glandular and nervous tissue. The adenohypophysis is pinkish in color and composed of soft glandular tissue. It arises as a dorsal outpocketing (Rathke's Pouch) of the

roof of the embryonic pharynx. The neurohypophysis, which appears white and fibrous, is the nervous component of the pituitary. It develops as a ventral downgrowth from the floor of the diencephalon.

The adenohypophysis is usually subdivided into the pars distalis (anterior lobe), pars tuberalis, and pars intermedia. The pars distalis is the major glandular portion of the adenohypophysis. A small part of it extends upward as the pars tuberalis and forms a collar around the infundibular stem. A layer of cells, the pars intermedia, is present between the anterior and posterior lobes in some species. The neurohypophysis is subdivided into the pars nervosa (posterior lobe), infundibular stem, and median eminence. The brain is connected to the pituitary by the median eminence and infundibular stem which expands to form the pars nervosa of the pituitary.

Hanström (1952, 1966) has made extensive studies of the pituitary gland of mammals, including four members of the Family Viverridae to which the Indian mongoose belongs. Although the pituitary gland of Herpestes auropunctatus was not examined, hypophyses from a mongoose of the genus Myonax, a large-spotted genet (Genetta tigrina), a bushy-tailed miercat (Cynictis penicillata), and a ruddy mongoose (Galerella caesi) were studied. The pituitary of these four animals had anterior lobes in a pronounced rostral position and neural lobes in a caudal position. Hanström found considerable variation in the distribution of pars tuberalis and pars intermedia. In Myonax, the pars tuberalis does not form a complete collar around the infundibular stem, but a collar is present in the three other species studied. The pars intermedia in Myonax covers only the rostral flat surface of the pars nervosa. In Genetta, the pars intermedia is well developed and

covers the anterior, ventral, and ventral-posterior surface of the pars nervosa while the pars intermedia in Cynictis and Galerella is doubly folded caudally and ventrally. Well-defined hypophysial clefts were present in all animals but Genetta, and stainable substances (colloid-like material) were noticed in the clefts of Myonax and Galerella. Colloid-filled follicles were observed in the pars distalis of all species, in the pars intermedia of Genetta and Galerella, and in the pars tuberalis of Myonax and Galerella.

The anterior lobe is almost completely enclosed by a dense fibrous capsule. The pars distalis of mammals is composed of glandular epithelial cells arranged in irregular cords surrounding and intimately related to an extensive system of blood sinusoids. The nomenclature of the anterior pituitary cell types has proved to be the major source of perpetual confusion among investigators. Throughout the literature, it appears that whenever a new species is studied, the investigator must devise an entirely new system of classification based upon anything from the cells' particular affinity for a certain stain such as an erythrophil (for the dye erythrosin) to a letter in the Greek alphabet such as epsilon. It is, therefore, difficult to compare cell types found in one species with those found in another. Functionally equivalent cells may not stain consistently from species to species. This is understandable since some hormones differ chemically in different species and thus yield varying results.

With the aid of a few rudimentary differential stains and the light microscope, early investigators were able to distinguish only three major groupings of cells in the anterior pituitary - acidophils, basophils, and chromophobes according to their affinity for acid stains,

basic stains, or neither type of stain, respectively. However, as histologic techniques improved, researchers realized that there were several different cell types within each of the original three groups. For instance, it is now established that the pars distalis of the rat (Purves and Griesbach, 1951; Purves, 1961); the monkey (Dawson, 1954b); the dog (Purves and Griesbach, 1957); and the bat and cat (Purves, 1961) contain three distinct basophilic cell types identified as thyrotrophs for their positive periodic acid-Schiff reaction and positive aldehyde fuchsin staining, and FSH- and LH-secreting cells for their positive periodic acid-Schiff reaction but negative aldehyde-fuchsin staining. It should be remembered, however, that it is possible to distinguish cell types by differential staining reactions or distinct morphology without really "identifying" them, that is, correlating function with a distinguishable cell type. In fact, it is still not clear if FSH and LH are screted by the same cell or by different cells.

Similarly, the acidophil cell class can be broken down into two sub-classes of cells, the carminophil for red-staining cells (its granules show a greater tendency to be stained by the red dyes - asocarmine, acid fuchsin, and erythrosin) and the orangeophil which has a greater affinity for Orange G (Purves, 1961; Dawson, 1954a; Dawson and Friedgood, 1938; Holmes, 1960). The carminophil is believed to be a prolactin-secreting cell and the orangeophil a growth hormone-secreting cell.

There are nine recognized hormones secreted by the pituitary gland, six of these - growth hormone, prolactin, adrenocorticotrophin, thyrotrophin, follicle stimulating hormone, and luteinizing hormone are secreted by the anterior lobe. It is no wonder then that investigators have searched diligently for six cell types in the pars distalis responsible for the secretion of these hormones. Ideally, the cytologist and

histochemist hope to find a direct relationship between the morphology and staining reactions of a particular cell and its functional secretory activity. With the increasing availability of more sophisticated techniques such as electron microscopy, radioimmunoassay, and immunofluorescence, as well as improved microsurgical techniques for tissue removal and transplants, the functional role of particular cell types have been matched to their appearance in a few "favorable" species such as the rat (Purves, 1961). At least six differentially staining cell types have been claimed in the dog (Goldberg and Chaikoff, 1952); the cat (Purves, 1966); the ferret (Holmes, 1960); and the human (Bergland and Torack, 1969; Ciocca et al., 1979). It should be noted that, in most of these studies at least one of the six cell types identified was designated as chromophobic and degranulated, thus appearing to have no secretory activity. In addition, in the study by Ciocca et al. (1979), a total of nine cell types were identified based on granule size and electron microscope ultrastructure. Seven of these cell types were presumed to have overlapping secretory activity, and two cell types were non-secretory. As more information is obtained, the complicated process of relating pituitary morphology to its secretory activity will hopefully become easier.

The pituitary gland of the Hawaiian mongoose has not been studied either structurally or functionally. The purpose of this research is to conduct preliminary investigation of the cytology and gross morphology of the Hawaiian mongoose pituitary gland. A thorough study of the anterior pituitary gland would include electron microscope and sophisticated immunofluorescent and autoradiographic techniques. However, before such research can be conducted, a general description

of the morphology and cytology of the pituitary gland at the light microscope level is essential.

This study is designed to:

(1) Describe the gross morphology of the mongoose pituitary gland and its relationship to the brain;

(2) Describe the relationships of the various parts of the pituitary- pars distalis, pars intermedia, and pars nervosa;

(3) Identify and describe the three major cell types (acidophils, basophils, and chromophobes) present in the pars distalis;

(4) Determine the relative percentages of these three cell types in the population, and their distribution in the pars distalis; and

(5) Describe the internal structure of the pars distalis and intermedia.

METHODS AND MATERIALS

Animals

Twelve sexually mature female mongooses were trapped from September 22, 1979, to January 25, 1980, at the Kaneohe Marine Corps Air Station in Havahart #2 traps using freshly killed rodents or meat scraps as bait. The traps were checked daily and upon finding an animal in the trap, the mongoose was transported to the Department of Anatomy animal colony. The mongooses were transferred to 15" x 18" x 19" metal cages upon arrival and were given Purina Cat Chow and water *ad libitum* until autopsy.

The reproductive status of the mongooses was determined by post-mortem examination of the uterus. Seven of the twelve animals had old implantation sites on their uterus; mammary tissue was regressed and the animals were designated post-lactational and reproductively inactive. The remaining five mongooses had no implantation sites on their uterus and were not lactating; they were also designated as reproductively inactive.

Autopsy Procedure for the Cytology of the Pituitary Gland

To avoid prolonged stress, the female mongooses were autopsied within two days of capture. Each animal was transferred from its metal cage into a lidded can containing paper towels saturated with ether. The mongooses were weighed and immediately decapitated. The calvaria was removed and the brain gently lifted out to expose the

pituitary on the sphenoid bone. The average elapsed time from anesthesia to decapitation was about 5 minutes.

Fixation, Embedding, Sectioning and Staining of Pituitaries

The head of the mongoose with the pituitary *in situ* was immersed in 200 ml of fresh Zenker Formol fixative (see Appendix A) for 1 hour after which the pituitary was carefully dissected out of the sella turcica and placed in 2 ml of fresh fixative for an additional 2 - 2½ hours. The fixative was then discarded and the pituitary rinsed with distilled water until the solution was clear in a total time of 3 hours. This was followed by immersion in 50% alcohol for 30 minutes and storage in 70% alcohol.

Before dehydration, the pituitaries were immersed in iodized 70% alcohol for 1 hour to remove mercuric chloride crystals. Then the pituitaries were dehydrated in a graded series of alcohols and xylene followed by infiltration and embedding in Paraplast (see Appendix B).

The pituitaries were serially sectioned with a rotary microtome horizontally at 4 microns beginning dorsally. Three adjacent sections from each of three representative levels through the pituitary gland were mounted on slides (for mounting media see Appendix A). The levels selected for study were taken 1/4, 1/2 and 3/4 way through the organ by counting sections. An additional 10 slides were made from each pituitary by mounting those sections immediately adjacent to those on the previous slide.

Pituitary sections were differentially stained either with (see Appendix C):

1. Periodic acid - Schiff - Iron Hematoxylin - Orange G (PAS-OG) (modified from Evans, 1964);
2. Performic acid - Alcian blue - PAS - Iron Hematoxylin - Orange G (PPAS-OG) (modified from Pearse, 1968); or
3. Mallory III (modified from Koneff, 1938).

Sections from the pituitary gland of a male albino rat were processed as controls along with the mongoose pituitaries in order to validate the staining procedures.

Although the pituitaries from a total of twelve mongooses were processed, only three were suitable for study. A great deal of experimentation was necessary to work out the details of the histologic protocol.

Differential Cell Counts of the Pars distalis

Using slides stained by the PAS - OG technique, a differential count of 1000 pituitary cells from each of the three levels was made with an American Optical binocular microscope. Cell counts were taken under oil immersion with an ocular micrometer (10 x 10 squares). Only those cells that fell completely or partially into the four corners of the grid (composed of 16 squares each) were counted. Every third field of view was counted starting at the rostral end of the anterior pituitary. The differential counts of the three levels were averaged and expressed as the percentage of acidophils, basophils, and chromophobes present in the gland. The mean percentage of the three cell types at each level through the pituitary was also determined. All follicles that appeared in each complete field of view were also counted. A total of about 20 fields of view were counted for each pituitary section.

Mongoose Brain and Pituitary Anatomy

To obtain parasagittal whole-mount sections through the brain and pituitary gland, an additional female mongoose was anesthesized with ether and then perfused by the following technique. Blood was first withdrawn from the bifurcation of the abdominal aorta to facilitate the perfusion. A clamp was placed above the renal arteries and an incision made in the left ventricle through which the perfusion needle was pushed up into the aorta. The perfusion apparatus consisted of three IV bottles containing a rinsing solution, a 3% glutaraldehyde fixative solution, and a 6% solution suspended from a height of about 2 meters and connected to a 250 cm length of 4 mm rubber tubing joined by an adapter to the cannula. The rinsing solution was perfused through the animal until it appeared clear (free of blood) from a hole cut in the right ventricle. Following the rinsing solution, the 3% and then the 6% solutions were perfused through the animal until the head and upper body region hardened (modified from Forssmann et al., 1976) (as described in Appendix A). The calvaria was then removed and the entire brain together with the bony floor of the skull was placed in 6% glutaraldehyde fixative for 12-24 hours. The brain was washed in distilled water, immersed in 70% alcohol for 15 minutes and stored in fresh 70% alcohol.

Before dehydration was attempted, the brain was cut into 8 sections and immersed in RDO (Du Page Kinetic Laboratories, Inc.) for 4-5 hours to decalcify the sphenoid bone. The bone was tested periodically with a needle to determine when decalcification was complete.

Histologic Processing of Brain Specimens

The brain section containing the pituitary was dehydrated in a graded series of alcohols and chloroform followed by infiltration and

embedding in Paraplast (see Appendix B). The brain block containing the pituitary was then parasagittally sectioned at 10 microns with a rotary microtome and stained with the Harris Hematoxylin and Eosin (H&E) technique (see Appendix C).

Statistical Analysis

Students' "t" test was used to determine significant differences between acidophil percentages at the 3/4 level and the combined 1/4 and 1/2 levels (Fisher, 1958).

RESULTS

(1) Morphology of Mongoose Brain and Pituitary

The mongoose pituitary was found in the sella turcica of the sphenoid bone in the middle cranial fossa. The pituitary was anchored rostrally by dura mater to the anterior clinoid process and caudally, the posterior clinoid process arched over part of the posterior lobe (Figs. 1 and 2). The pituitary was generally boat-shaped, flattened on its dorsal surface and rounded on its ventral surface. It was connected to the brain (Figs. 3 and 4) by a brownish-colored infundibular stem. The pars distalis was visually distinct from the pars nervosa with the naked eye. The pars distalis appeared pinkish compared to the whitish-appearing pars nervosa and both lobes were mechanically easy to separate from each other.

The mongoose pituitary was very small in proportion to the size of the animal. The dimensions of the entire gland were about 3 mm in length and 1 mm in width and thickness. The 3 mongooses in the experiment weighed 430g, 448g, and 414g. The pituitary of these mongooses were not weighed, but Hoffmann (1979) reported that the anterior pituitary weighed 3.63± 0.17 mg in 53 reproductively inactive female Hawaiian mongooses.

(2) Morphological Relationships of the Pars distalis, Pars intermedia, and Pars nervosa

A striking feature of the Hawaiian mongoose pituitary was the encircling of the pars nervosa by the pars intermedia on all but the

dorsal surface. Thus, the pars intermedia formed a cup-like structure around the pars nervosa. The mongoose pituitary had a single pars distalis which rested on a relatively flat sella turcica (Fig. 5). The pars distalis consisted of cords of densely packed chromophilic and chromophobic cells supported by a network of connective tissue surrounding numerous irregular shaped blood sinusoids. An intraglandular cleft usually filled with stainable colloidal material was also present, separating the pars distalis from the pars intermedia on all but the lateral surfaces (Figs. 6-10).

At the 1/4 level through the pituitary from the dorsal surface, the pars nervosa accounted for about 70% of the whole organ in horizontal section (Figs. 6-8). Halfway through the gland, pars nervosa and pars distalis occupied about equal amounts of the section (Fig. 9). At 3/4 level, the pars nervosa represented only about 30% of the total organ. The anterior lobe had caudal extensions along the lateral aspects of the pars intermedia and nervosa which appeared midway through the gland and became more prominent ventrally (Fig. 10).

(3) Anterior Pituitary Cell Types

Three major cell types were identified in the mongoose pars distalis with the PAS-OG, PPAS-OG, and Mallory III differential staining techniques. No mitotic figures were observed in the pars distalis of any animal.

Acidophils

Acidophils were designated as those cells stained with Orange G. These cells had moderately large rounded nuclei, were usually oval-shaped, and had a substantial amount of cytoplasm. The cytoplasm of the acidophils stained brownish-orange in the PAS-OG and PPAS-OG procedures

(Fig. 12). With the Mallory III stain, however, the acidophils were a brilliant yellow-orange color with numerous reddish-orange granules in the cytoplasm (Fig. 13).

Basophils

Basophils were defined as those cells staining with PAS or aniline blue. They tended to be large polyhedral cells with coarsely granulated PAS-positive material in a large amount of cytoplasm. The cells stained a magenta-purple color with PAS-OG (Fig. 12) but failed to stain distinctly with the PPAS-OG technique (Fig. 7). The basophils in the PPAS-OG stain appeared a light-lavender color and could not be distinguished from chromophobes and pars intermedia cells. With the Mallory III stain, the basophils were a moderately dark blue color (Fig. 13) but again were difficult to distinguish from chromophobes. Mongoose basophils seemed to have little affinity for any of these commonly-used dyes and varying the pH or timing had little effect. Control slides of the rat pituitary used to test the staining procedures showed abundant basophil staining in all slides. Therefore, the techniques appeared to be working properly.

Chromophobes

Two general types of chromophobes were observed in the pars distalis. Chromophobes in the follicular structure were PAS-negative, ungranulated, and had irregular nuclei in a small amount of cytoplasm. The other type of chromophobe had a large round nucleus, a moderately large amount of cytoplasm and stained very faintly blue in Mallory III, and lavender in PAS-OG and PPAS-OG staining procedures. Both types of chromophobes were dispersed throughout the pars distalis.

(4) Distribution of Acidophils, Basophils and Chromophobes in the Pituitary

At the 1/4 level, acidophils were absent in the rostral area and concentrated laterally and caudally near the pars intermedia. Basophils and chromophobes were evenly distributed throughout the remainder of the gland where acidophils were less abundant (Figs. 6-8).

The section half-way through the gland also showed acidophils concentrated laterally and posteriorly in the pars distalis. A few more acidophils were present in the center of the pars distalis than in the 1/4 level (Fig. 9).

Near the 3/4 level, acidophils were more abundant in the central portions of the pars distalis. Basophils and chromophobes tend to be evenly distributed throughout the rest of the gland (Fig. 10).

The mean percentage for each of the three cell types found in the pars distalis of the three mongooses is given in Table 1. Chromophobes were found to be the abundant cell type at all three levels through the pituitary. The percentage of chromophobes at each level remained relatively constant. Acidophils were the most abundant chromophilic cell type at 25.2% compared to 5.7% basophils.

The percentage of acidophils was highest at the 1/4 level and declined at the 3/4 level. The difference in the mean acidophil percentages at the 3/4 level (17.5$\pm$ 2.0%) and the combined 1/4 + 1/2 levels (29.1 $\pm$ 1.7%) was highly significant ($P < 0.01$). Thus, acidophils appeared to be concentrated in the dorsal half of the pituitary. Basophils comprised a slightly larger percentage of the pituitary at the 3/4 level, but the results were not statistically significant.

Table 1. Percentage Distribution of the Cell Types in the Anterior Pituitary of the Mongoose

Cell Type	Anterior Lobe		% Distribution by Level		
	Number	%	1/4	1/2	3/4
Acidophils	2269	25.2	31.3	26.8	17.5
Basophils	511	5.7	3.4	5.1	8.6
Chromophobes	6219	69.1	65.1	68.1	74.0

(5) Internal Structure of the Pars distalis and intermedia

The anterior pituitary of the mongoose contained numerous follicles, each with a lumen of variable size containing PAS-positive colloid and surrounded by a single layer of epithelial cells. At the 1/4 level through the pituitary, the follicles were concentrated rostrally and laterally in two cords. In the 1/2 and 3/4 horizontal sections, the follicles were most numerous in the anterior and central regions of the anterior pituitary. With the PAS-OG and PPAS-OG stains, the follicles were composed of chromophobic cells encircling deep magenta-colored colloidal material. Occasionally, follicles appeared to include acidophilic cells. The follicular cells were also colorless in the Mallory III stain, but the basophilic colloid stained a deep royal blue instead of magenta.

While doing differential cell counts follicles at each of the three levels were also counted. There was an average of 66 follicles at the 1/4 level, 73 follicles at the 1/2 level, and 89 follicles at the 3/4 level. Thus, the distribution of follicles seemed to increase from the dorsal to the ventral region of the anterior pituitary. Follicles were completely absent in the pars intermedia of the mongoose and in the control slides of rat anterior pituitary.

The cells of the pars intermedia appeared to be basophilic. They stained a light lavender with PAS-OG and PPAS-OG and were a light sky blue with Mallory III. The cells stained homogeneously, and appeared to be of one type. The pars intermedia was arranged as densely packed cuboidal epithelium around the pars nervosa in a layer varying from 6-8 cells in thickness toward the ventral region of the pituitary to 15-20 cells near the dorsal aspect of the pituitary.

DISCUSSION

The gross morphology and location of the female mongoose pituitary was found to be similar to other mammals. The pituitary was boat-shaped, flattened dorsally, rounded ventrally, and located in a relatively shallow sella turcica of the sphenoid bone. The mongoose pituitary had an ovoid pars distalis similar to the human (Bloom and Fawcett, 1975), but in contrast to the bi-lobed pars distalis of the rat (Purves and Griesbach, 1951). However, unlike the human gland which has only the remnants of a pars intermedia, the mongoose had a very well-defined and distinct pars intermedia. The pars intermedia of the mongoose surrounded the pars nervosa on all but the dorsal surface, forming a cup-like structure (Fig. 5). The pituitary of Herpestes auropunctatus, however, most closely resembled the pituitary of members of its family Viverridae (Hanström, 1952). With some variation, Herpestes' pituitary was similar to that of Galerella in terms of the proportional sizes of the pars distalis and pars nervosa, but unlike Galerella, Herpestes does not have a double-layered pars intermedia. The pars intermedia of Genetta, Cynictis, and Galerella like Herpestes form a cup-like covering around the pars nervosa. Unlike Genetta which has no intraglandular cleft, Herpestes along with Myonax, Cynictis, and Galerella does have a cleft and it usually contains a basophilic staining substance (Figs. 6-8).

Three general cell types were identified in the Hawaiian mongoose with PPAS-OG, PAS-OG, and Mallory III staining procedures (Figs. 12-13).

The acidophils that stained orange in Orange G matched in description those orange cells found in the ferret (Holmes, 1960); the carminophil of the cat (Dawson and Friedgood, 1938); and the dog (Goldberg and Chaikoff, 1952). The two types of acidophils responsible for prolactin and growth hormone secretion, could not be distinguished with the stains employed.

The PAS-positive mongoose basophils corresponded well to the description of basophils found in the dog (Purves and Griesbach, 1957); the ferret (Holmes, 1960); and the rat (Purves and Griesbach, 1951). Holmes (1960), in a study of the pituitary gland of the female ferret, found three types of PAS-positive mucoid cells with PPAS-OG staining. He identified: (1) PAS +, Alcian blue -; (2) PAS +, Alcian blue +; and (3) PAS +, Alcian blue -, Orange G + mucoid or basophilic cells. Such distinctions were not apparent with the mongoose pituitary using the same staining procedures. In fact, there was tremendous difficulty finding a technique which would stain any of the basophils of the Hawaiian mongoose. Control slides of rat pituitary sections processed together with mongoose pituitaries stained basophil as predicted and thus verified that the procedure was working for that species. Holmes observed variation in the abundance of PAS + cells with the reproductive cycle of the seasonally breeding ferret. Generally, in seasonally breeding animals, reproductive cycles are accompanied by histological changes in the pituitary cells secreting gonadotrophins. Clarke and Forsyth (1964) found that during the breeding season of the vole, cells filled with PAS-positive granules were numerous, but in the non-breeding season PAS-positive material was present only in the walls of otherwise unstaining large vesicles. In another study, Hoffman and Zarrow (1958) correlated an increase in size and abundance of PAS-positive staining

basophils in the ground squirrel with an increase in thyroid and gonadal activity during the breeding season. During the non-breeding season they observed a decrease in basophil size and abundance and the loss of Schiff + material. Since the mongooses used in the present study were captured and autopsied in September, during the non-breeding season, the difficulty encountered in staining basophils may be partially explained. The next step would be to study the pituitary of the mongooses autopsied during the breeding season.

Two general types of chromophobes were seen in the mongoose. One was a follicular cell with an irregular sickle-shaped nucleus and a poor affinity for stain. The other type of chromophobe had a more regular rounded nucleus, slight affinity for the basophilic stains, and was scattered throughout the pars distalis with the other cell types. These two types of chromophobes were very similar to the two chromophobic cells described by Oldham (1938) in the armadillo hypophysis. One type was found scattered among chromophilic cells, stained a faint blue in hematoxylin-eosin-azure II, and had spherical nuclei. The second type was usually found in nests and had shriveled nuclei.

Chromophobes accounted for 69.1% of the cells present in the pars distalis of the mongoose as compared to 54.8% for the golden hamster (Hanke and Charipper, 1948); 50% for the non-pregnant human female (Rasmussen, 1933); and 49% for the rat (Schooley et al., 1966). Basophils were very scarce in the mongoose pars distalis (5.7%). The percentage of basophils, however, tends to be low in several other species, 7% in the human (Rasmussen, 1933) and 9% in the rat (Schooley et al., 1966). In several species of animals studied, the predominant chromophilic cell is the acidophil. Low basophil numbers and high chromophobe numbers may be due to de-granulated

or degenerating basophils that have lost their ability to take up stain and thus appear to be chromophobes. Electron micrographs of rodent pituitaries seem to support this hypothesis. Halmi (1963) found that most of the chromophobes seen with the light microscope were poorly-granulated chromophils by electron microscopy and, therefore, true chromophobes may be an infrequent cell type.

The distribution of cell types within the mongoose pars distalis was very similar to that found in the rabbit, cat (Dawson, 1937); dog, pig, beef, man (Baker, 1974); ferret (Holmes, 1960); and the monkey (Dawson, 1948). Basophils and chromophobes were found to localize primarily in the rostral and central zones of the pars distalis and acidophils tend to be more numerous in the posterior and lateral regions.

The pars intermedia of the mongoose consisted of closely packed basophilic cells and varied from 6-8 cells in thickness to 15-20 cells. Dhaliwal and Prasad (1965) found the pars intermedia in the five-striped palm squirrel to be 3-4 cell layers thick. An explanation for the greater thickness of the mongoose pars intermedia is not known. In a study by Purves and Bassett (1963), the pars intermedias of 12 mammals were found to be similarly basophilic. Holmes (1960) described two types of pars intermedia cells in the ferret. One type of cell was ependymal and had an elongated nucleus and little cytoplasm. The other cell had a faintly PAS-positive cytoplasm, round nuclei, and formed the bulk of the pars intermedia. Such a distinction could not be made in the mongoose, and probably only the latter type of cell was seen.

Follicles composed of a single layer of chromophobic cells surrounding a PAS-positive-staining colloidal material were frequently observed in the pars distalis of the mongoose. Such colloid-filled

structures have been described in several species of animals: the armadillo (Oldham, 1938); potto (Holmes and Ball, 1974); ground squirrel (Hoffman and Zarrow, 1958); dog (Kagayama, 1965); man (Bergland and Torack, 1969; Ciocca et al., 1979); and in rats raised at high altitude (Nelson, 1973). Hanström (1952) found colloid-filled follicles in the pars distalis of all the members of Viverridae studied. Although the function of these cells is not really known, Farquhar (1971) suggested that these cells can function as phagocytes since pituitary slices incubated in vitro engulfed and phagocytized degenerating cells and cell processes. Oldham (1938) hypothesized that these cells with colloid may be pinched off diverticuli of the pharyngeal Rathke's pouch.

The present study has described the morphology of the pituitary gland of the reproductively inactive female mongoose. The project was limited by the number and type of experimental animals utilized. Future studies should use increased numbers of test animals, particularly female mongooses at different seasons, and should compare light microscopy with electron microscopy to correlate cytology with functional secretory activity of the various cell types.

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APPENDIX A

SOLUTIONS

I. Pituitary Fixative (after Evans, 1964)

Zenker Formol

Zenker Base	40.0 ml
Glacial acetic acid	2.5 ml
37% Formalin	7.5 ml

Made immediately before autopsy.

Zenker Base

Potassium dichromate	25.0 g
Mercuric chloride	45.0 g
Distilled water	1000 ml

II. Perfusion Fixation for the Pituitary (modified from Forssmann et al., 1976)

Rinsing Solution

Sodium chloride	9.0 g
Polyvinylpyrrolidone (PVP)	25.0 g
Heparin	9.25 g

Just prior to autopsy add 1000 ml of distilled water and mix thoroughly. Adjust the pH to 7.35 with 1N Sodium hydroxide using a pH meter.

0.2M Monosodium Phosphate (NaH_2PO_4)

$NaH_2PO_4.H_2O$	27.60 g

Bring to 1000 ml with distilled water.

0.2M Disodium Phosphate (Na_2HPO_4)

$Na_2HPO_4.H_2O$	28.39 g

Bring to 1000 ml with distilled water.

3% Fixative

0.2M NaH_2PO_4	45.0 ml
0.2M Na_2HPO_4	405.0 ml
25% Formalin	60.0 ml
25% Glutaraldehyde	60.0 ml
PVP	25.0 g

Mixed just prior to autopsy. Bring the solution to 1000 ml with distilled water and adjust the pH to 7.35.

6% Fixative

0.2M NaH_2PO_4	45.0 ml
0.2M Na_2HPO_4	405.0 ml
25% Formalin	120.0 ml
25% Glutaraldehyde	120.0 ml
PVP	25.0 g

Mixed just prior to autopsy. Bring the solution to 1000 ml with distilled water and adjust the pH to 7.35.

III. Tissue Mounting Media (after Evans, 1964)

Gelatin	0.2 g
Potassium dichromate	0.2 g

Dissolve in 1000 ml of cold distilled water. Heat and boil solution for 5 minutes uncovered. May be re-used and stored in the refrigerator indefinitely.

APPENDIX B

DEHYDRATION, INFILTRATION, AND EMBEDDING

I. Pituitaries (modified from Evans, 1964)

Day 1

75% alcohol	- 1 hour
80% alcohol	- 1 hour
85% alcohol	- 1 hour
90% alcohol	- 1 hour
95% alcohol	- 2 changes (30 min each)
Absolute alcohol	- 2 changes (30 min each)
Xylene	- 20 min
Paraplast	- 2 changes (30 min each)
Paraplast	- Overnight

Day 2

Melt off paraplast in oven at 60° C.

Paraplast vacuum. - 30-45 min

Embed.

Cool paraplast block for at least 2-3 days.

Store blocks in the refrigerator until ready to section.

II. Brain (modified from Luna, 1968)

Day 1

95% alcohol	- 3 changes (2 hours each)
Absolute alcohol	- Overnight

Day 2

Absolute alcohol	- 2 changes (1 hour each)
Chloroform	- 2 changes (1.5 hours each)
Paraplast	- 2 hours
Paraplast	- 1 hour
Paraplast	- Overnight

Day 3

Melt off paraplast in oven at 60° C.

Paraplast vacuum.

Embed.

Cool paraplast blocks for at least 2-3 days.

Store blocks in the refrigerator until ready to section.

APPENDIX C

STAINING PROCEDURES

I. Brain Stain

Harris Hematoxylin and Eosin (modified from Evans, 1964)

Sections affixed to slides with tissue mounting media.

Xylene	- 3 changes (5 min each)
Absolute alcohol	- 2 changes (5 min each)
90% alcohol	- 5 min
70% alcohol	- 5 min
Iodized alcohol (70%)	- 10 min
70% alcohol	- 5 min
Distilled water	- 2 changes (3 min each)
Harris Hematoxylin	- 30 sec
Distilled water	- 2 changes
Tap water	- 15-30 sec
Distilled water	- 2 changes (1 min each)
Alcoholic Eosin (85%)	- 30 sec
Absolute alcohol	- 2 rapid rinses
Xylene	- 2 changes (5 min each)

Mount in Permount.

II. Pituitary Stains

(a) Periodic acid-Schiff, Iron Hematoxylin, Orange G

(modified from Evans, 1964)

Proceed to 70% alcohol as above.

Distilled water	- 2 changes
0.6% Periodic acid	- 10 min
Distilled water	- 5 changes
Schiff's Reagent	- 30 min
Running tap water	- 30 min
Distilled water	- 5 changes
5% Iron alum	- 1 min
Distilled water	- 2 changes
Harris Hematoxylin	- 1 min
Distilled water	- 5 changes
Orange G	- 5 min
Distilled water	- rinse
Running tap water	- 2 min (hard)
Absolute alcohol	- 3 rapid changes
Xylene	- 2 changes (5 min each)

Mount in Permount.

0.6% Periodic Acid

Periodic acid	0.6 g
Nitric acid	0.3 ml
Distilled water	100 ml

Schiff's Reagent

Dissolve 1.0 g of basic fuchsin in 200 ml of boiling distilled water. Stir for 5 minutes and cool to exactly 50° C. Filter and add to the filtrate 20 ml of 1N Hydrochloric acid. Cool the solution to 25° C and add 1.0 g of Sodium metabisulfite ($Na_2S_2O_5$). Stand this solution in the dark for 14-24 hours. Add 1.0 g of activated charcoal (Norite) and shake for no more than 1 minute. Filter and store the filtrate in the dark at 0-4° C. Allow the solution to reach room temperature before using. The solution should remain clear, but if any reddish color appears, it is denaturing and should be discarded.

5% Iron Alum

Ferric ammonium sulfate	5.0 g
Distilled water	100 ml

Orange G

Orange G	2.0 g
Phosphotungstic acid	5.0 g
Distilled water	100 ml

Shake solution occasionally and let stand overnight. Centrifuge about 5 minutes and decant clear supernatant.

(b) Performic acid-Alcian blue-PAS-Iron Hematoxylin-Orange G (modified from Pearse, 1968)

Proceed to 70% alcohol as above.

Distilled water	- 2 changes

Blot carefully around tissues with a cheesecloth.

Performic acid	- 5 min
Tap water	- 10 min

70% alcohol	- rinse
Absolute alcohol	- rinse
Blot carefully.	
Tap water	- rinse
Air dry slides.	
Absolute alcohol	- rinse
Tap water	- 1 min
Alcian blue	- 1 hour
Tap water	- 5 min

Stain by the PAS-Iron Hematoxylin-Orange G Sequence.

Performic Acid

Add 4.5 ml hydrogen peroxide (30%) and 0.5 ml concentrated sulfuric acid to 45 ml of 98% formic acid. Let stand for 1 hour. Stir well before use. Use for 24 hours only.

Alcian Blue

3% (w/v) in 2N sulfuric acid. Dissolve the dye by heating to 70° C and filter when cool. The pH should be 0.2-0.3.

(c) Mallory III (modified from Koneff, 1938)

Proceed to 70% alcohol as above.

Distilled water	- 2 changes
5% Phosphotungstic acid	- 1.5 hours
Distilled water	- 2 thorough rinses
Mallory III	- 30-45 min
Distilled water	- rinse quickly

Shake excess water off slide and blot dry.

Absolute alcohol	- 2 rapid changes
Xylene	- 2 changes (10 Min each)

Mount in Permount.

Mallory III

Aniline blue	0.5 g
Orange G	2.0 g
Oxalic acid	2.0 g
5% Phosphotungstic acid	1.0 ml
Distilled water	100.0 ml

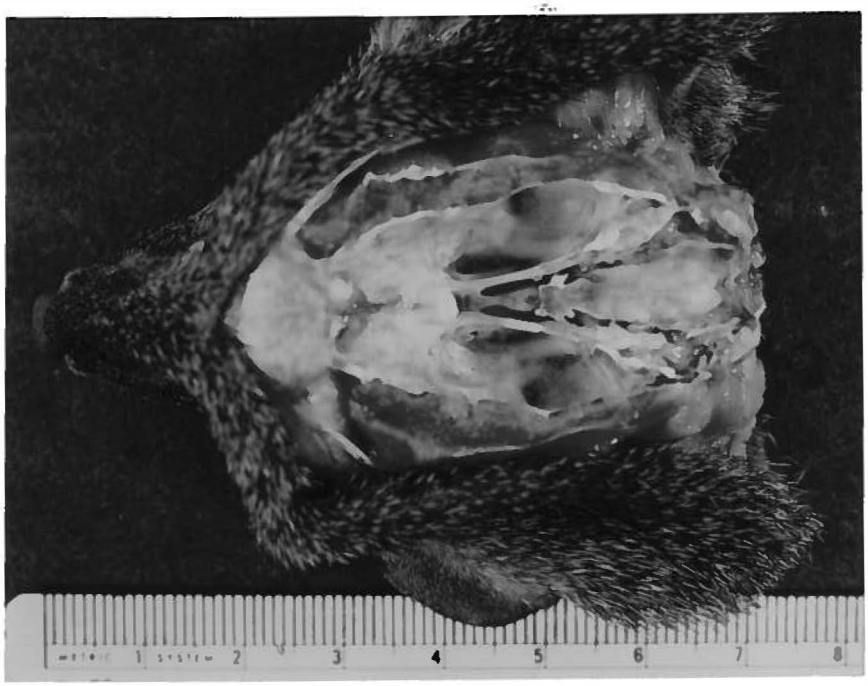

Figure 1

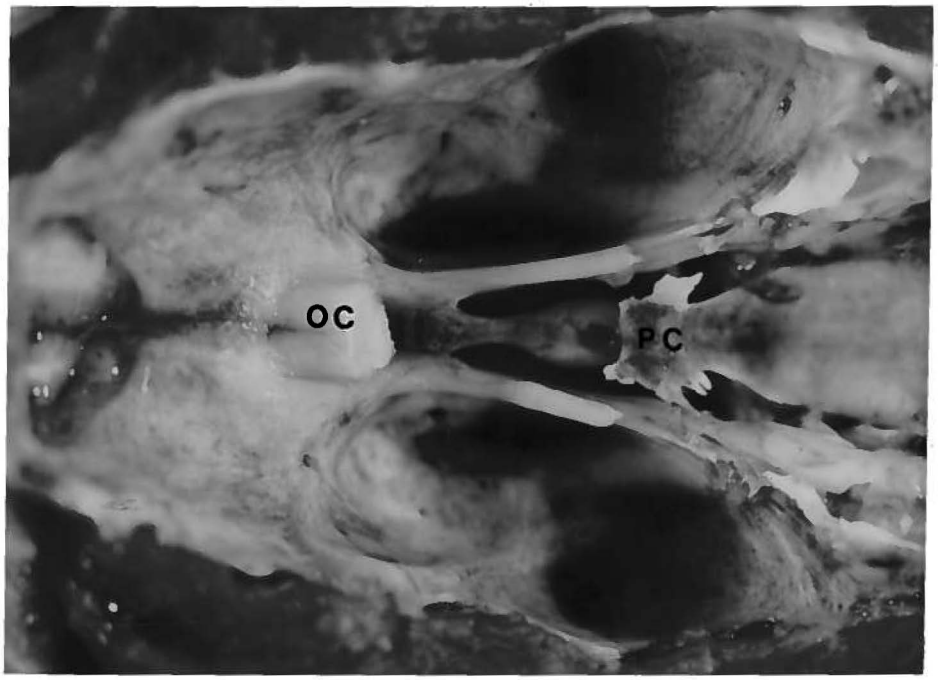

Figure 2

Figure 5. A parasagittal section through the hypothalamus and pituitary of the mongoose. The pituitary is located in a relatively flat sella turcica (ST). The pars distalis (PD) is rostral (to the right) of the pars nervosa (PN) and the pars intermedia (PI) surrounds the pars nervosa on all but the dorsal surface. The infundibular stem and pars tuberalis are not visible in this section. PC, Posterior clinoid process; ME, median eminence; OC, optic chiasm. (H & E; magnification X10)

Figure 3. Dorsal view of the mongoose brain.

Figure 4. Ventral view of the mongoose brain. The pituitary is located immediately ventral to the floor of the hypothalamus (the area between the two arrows).

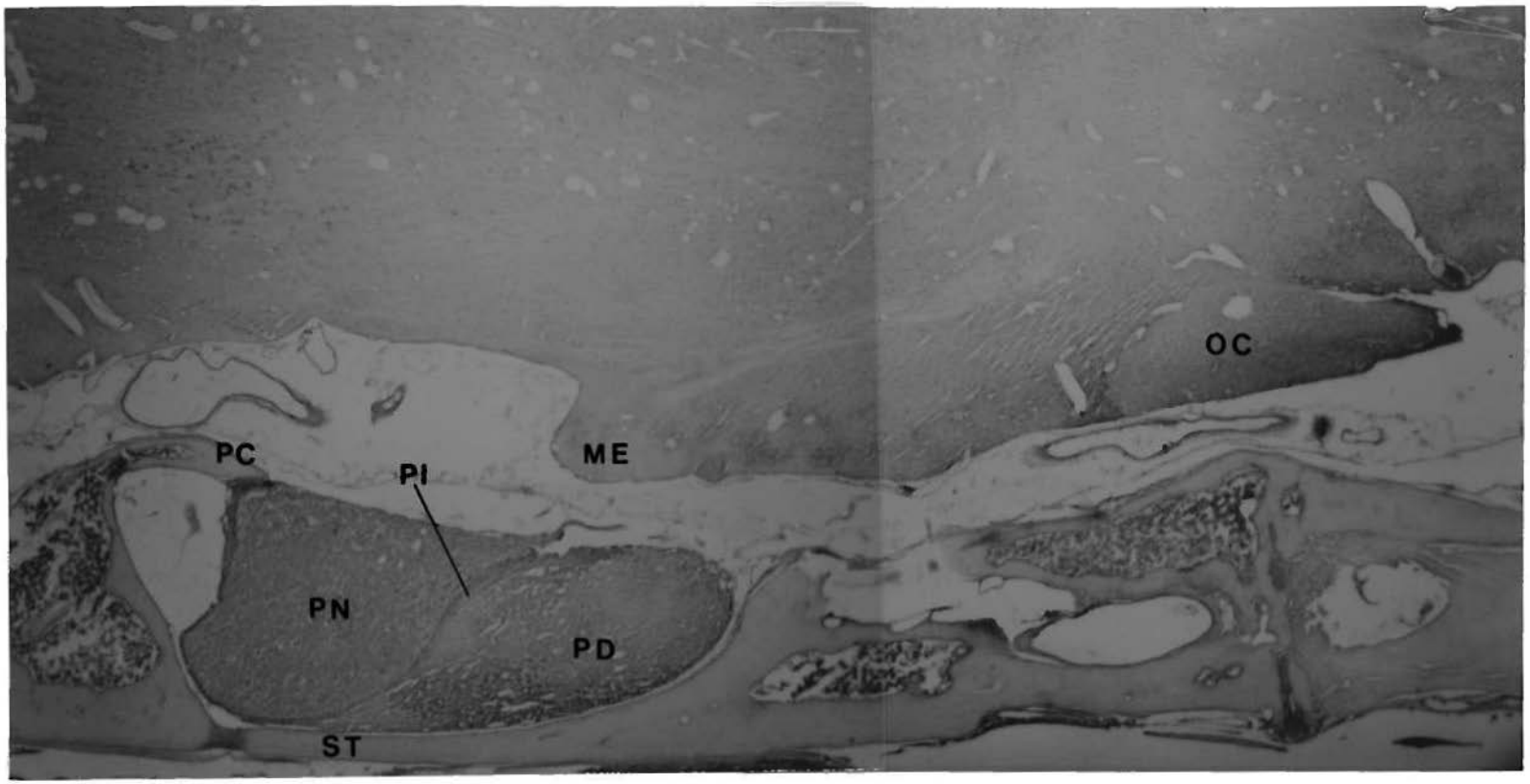

Figure 5

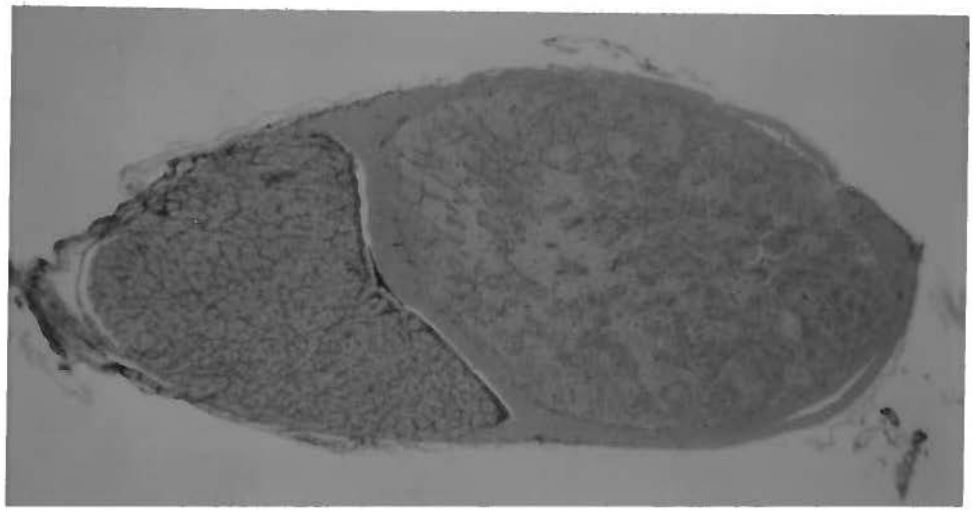

Figure 6

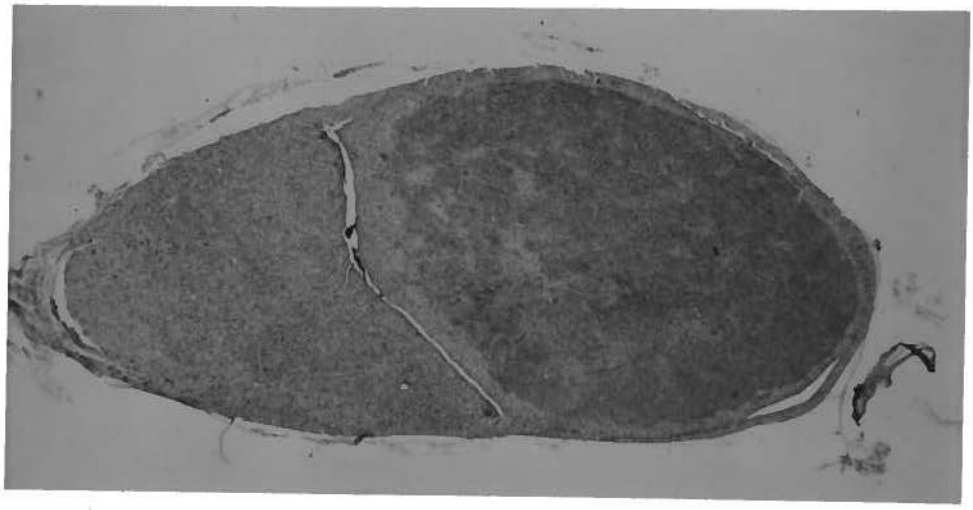

Figure 7

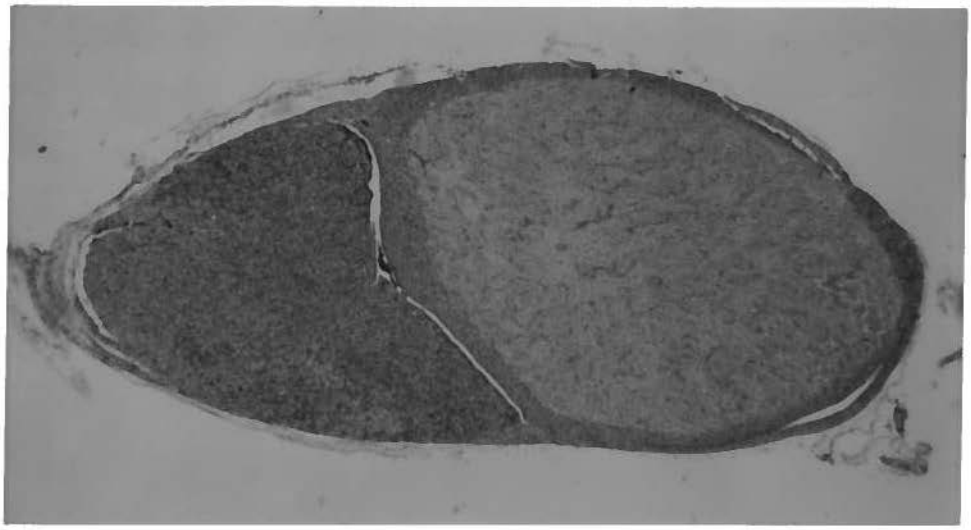

Figure 8

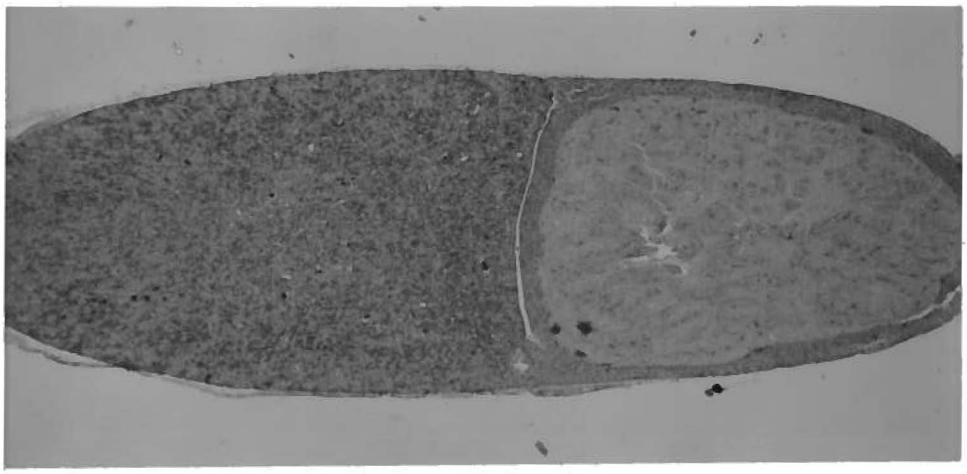

Figure 9

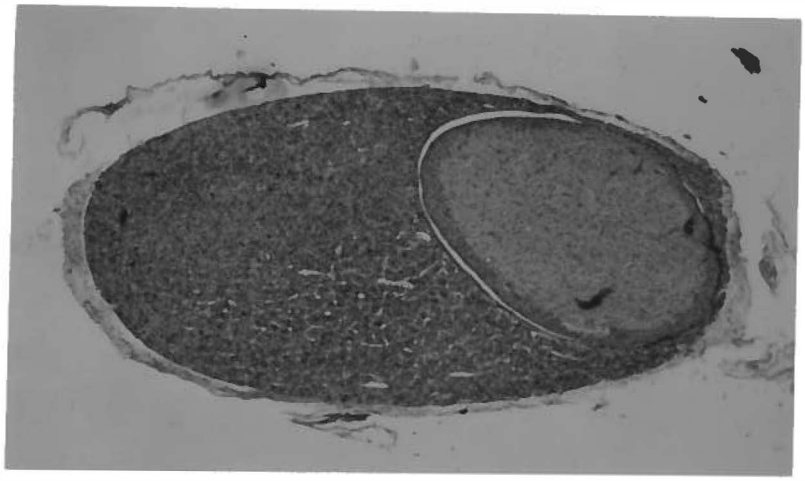

Figure 10

Photomicrographs of the anterior pituitary cells of the rat and mongoose under oil immersion and stained with PAS-OG.

Figure 11. Anterior pituitary of the rat showing orange acidophils (A), bright purple granulated basophiles (B), and unstained chromophobes (C). A blood sinusoid filled with erythrocytes is present in the upper left corner.

Figure 12. Anterior pituitary of the mongoose showing brownish-orange acidophils (A), purple granulated basophils (B), and unstained chromophobes (C).

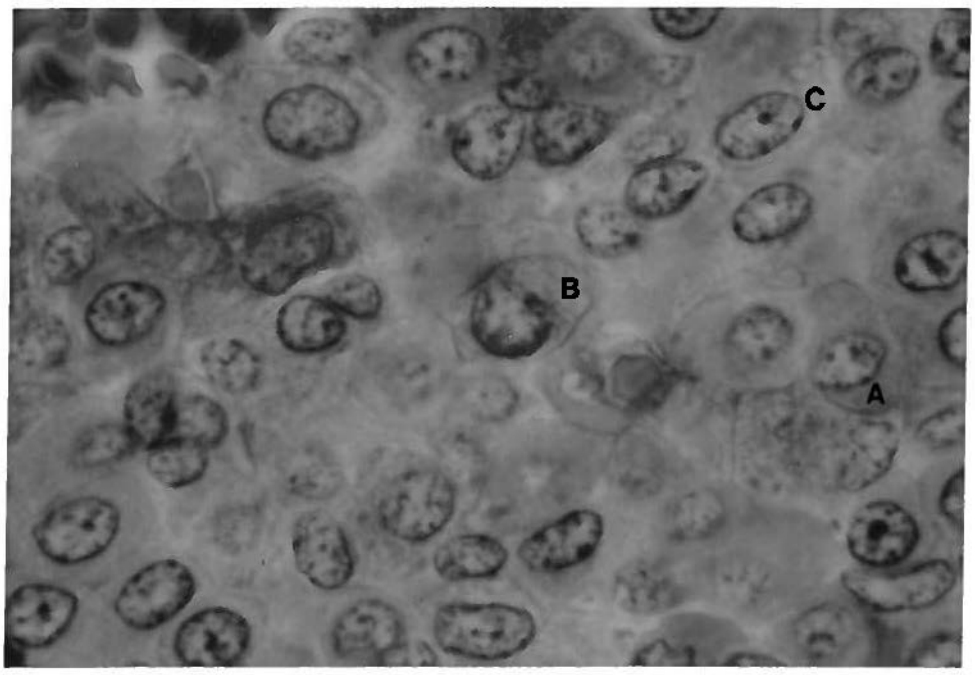

Figure 11

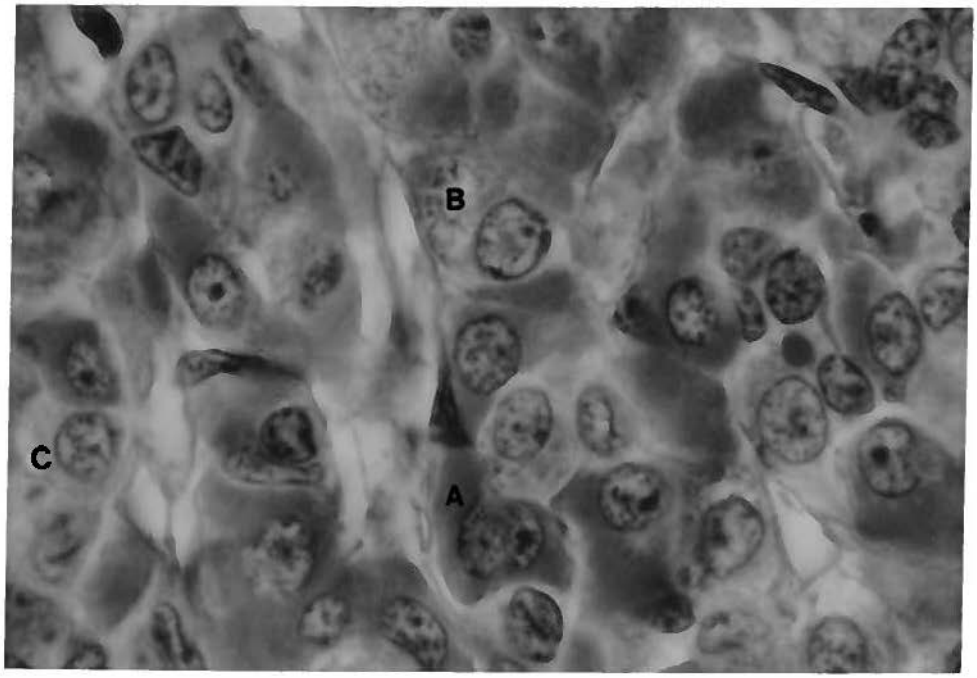

Figure 12

Figure 13. Anterior pituitary of the mongoose showing bright yellow-orange acidophils (A), dark blue basophils (B) and colorless chromophobes (C). Note the presence of two dark-blue stained colloid-filled follicles (F). (Mallory III; magnification X1000)

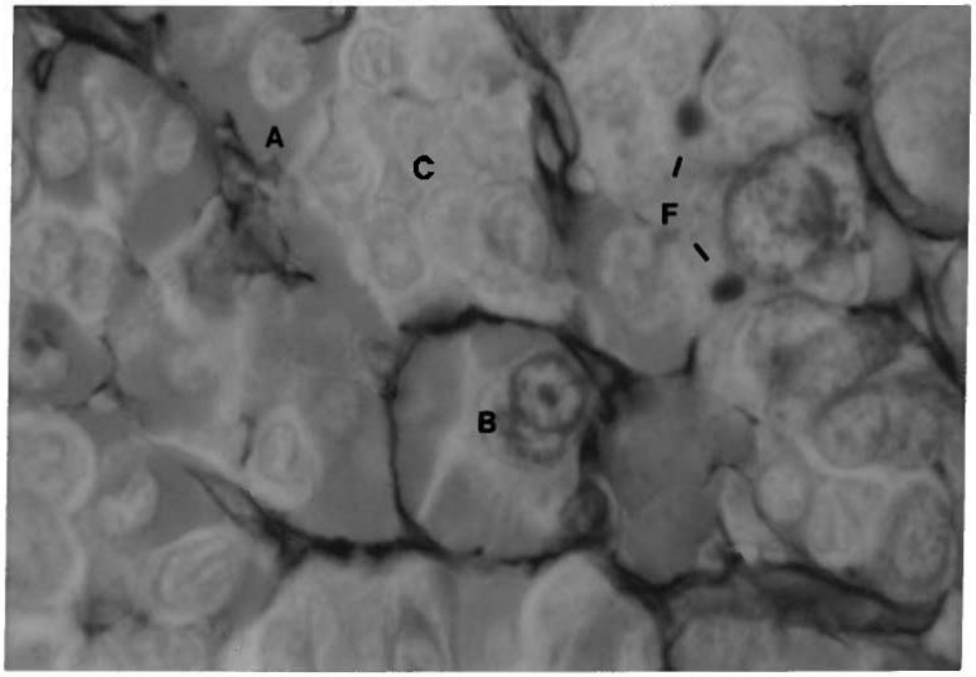

Figure 13