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THE COMPARATIVE HISTOMORPHOLOGY AND CORTICOSTEROID PROFILE OF ADRENAL GLANDS IN SOME AFRICAN ANTELOPES.

by

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Suggested short title

ADRENAL GLANDS IN AFRICAN ANTELOPES

ABSTRACT

The comparative histomorphology and corticosteroid profile of adrenal glands in some African antelopes.

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Adrenal glands from five species of South African antelope; cape eland (*Taurotragus o.oryx*), gemsbok (*Oryx g.gazella*), southern greater kudu (*Tragelaphus s.strepsiceros*), red hartebeest (*Alcelaphus buselaphus caama*), springbok (*Antidorcas marsupialis hofmeyri*), were collected from 43 trophy-hunted males for histology and corticosteroid analysis. The gross anatomy of the adrenal glands are species-specific, with the left gland being most variable. There were differences found in the number of cortex capsular layers and zona glomerulosa between species. Extensive capsular trabeculae penetrate deep into the cortex in only the largest antelope, i.e. eland and gemsbok, and are representative of these species. In all species the zona glomerulosa form variations in types of cellular cord structures, with the greater kudu having the most unique architecture of horizontally stratified, highly columnar cells that form winding cords which arches at the capsular end, and resemble those observed in equine species. Medullary capsules were observed in the eland, and incomplete capsules in the gemsbok and greater kudu. The medulla is characterized by an outer, adrenaline secreting zone that encapsulates a inner noradrenaline secreting zone in all species. The corticosteroid patterns are typical of bovids, with cortisol and corticosterone present, however significantly larger amounts of 18-hydroxy-corticosterone were found in all species of antelope. The total identified corticosteroid contents had interspecies differences, which are possibly based on species body size.

RÉSUMÉ

Étude comparative de l'histomorphologie et des corticostéroïdes des glandes surrénales de quelques antilopes africaines.

M.Sc. Andrew S. Fazekas Sciences des Ressources Naturelles

Les glandes surrénales de cinq espèces d'antilopes d'Afrique du Sud ont été collectées sur 43 individus mâles en vue d'une analyse histologique et des corticostéroïdes. L'anatomie générale des glandes surrénales varie d'une espèce à l'autre, la glande gauche présentant plus de variabilité. Les espèces différent au niveau des zones glomérulaires et dans le nombre de couches capsulaires des zones corticales. Les trabécules capsulaires, larges et nombreux, pénètrent profondément dans la zone corticale, et ce seulement chez les grandes antilopes, l'éland et l'oryx. Pour toutes les espèces la zone glomérulaire présente des variations dans l'apparence des cordons cellulaires. Le grand koudou se distingue par une structure unique de cellules très allongées et stratifiées horizontalement, formant un cordon qui s'arque à l'extrémité de la capsule, structure ressemblant à celle observée chez les équidés. Des capsules médullaires sont présentes chez l'éland, et des capsules incomplètes chez l'oryx et le grand koudou. La médullo-surrénale se caractérise par une zone externe de sécrétion de l'adrénaline qui encapsule une zone interne de sécrétion de la noradrénaline. Les profils corticostéroïdiens sont typiques des bovidés, avec présence de cortisol et de corticostérone, la 18-hydroxycorticostérone étant la plus abondante chez les cinq espèces d'antilopes. La quantité totale de corticostéroïdes identifiés varie entre les espèces, possiblement en relation avec la masse corporelle.

Dedicated in loving memory of my grandfather, Dr. I. Gyula Fazekas, and his pioneering work on adrenals

" ***Ex Africa semper aliquid novi.***"

Ancient Rome ca.300 B.C.

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I. INTRODUCTION

The adrenal glands are found in all levels of vertebrates with variations in their structure and hormonal secretions. The effects of these adrenal hormones range from salt and water metabolism to inflammatory regulation and stress adaptation, all of which are essential to life.

The histology of the adrenal gland of most domestic animals has been widely studied (Bourne 1949; Chester Jones & Henderson 1978), but little work has been carried out on the histomorphology and endocrinology of adrenals in wildlife species. It may seem reasonable to assume that the histology would be fairly uniform throughout the mammals since they belong to the same taxonomic class, but this is not the case. There are documented differences which do occur in adrenals between orders of mammals, such as variations in cellular architecture and adrenal cortex zonation (Hartman & Brownell 1949; Chester Jones 1957).

The *Bovidae* is a large family which contains several domestic species and some 90 species of antelopes. Many of these antelopes are endangered and their populations are restricted to reserves. The stress of captivity and management can cause changes in the adrenal glands (Young 1973; Bothma 1989). This documentation on the endocrinology of normal, healthy individuals, and the differences between species would be of value to researchers in the field of capture myopathy, and conservation biology in general.

To date work has been done mostly on domestic bovine species, and only rudimentarily for a handful of their wildlife counterparts. In antelopes, the adrenals

have not been studied to any great extent (Chelik & Ley 1977; Bernert 1981; Spinage 1982). Bernert (1981) conducted a brief overview of ruminant adrenal glands, including antelope, mostly from museum collections, and found histological differences between different species. Teixeira et al.(1993) in their study on the histology of the adrenals of the African buffalo (*Bovidae*) concluded there to be significant differences in the histology of the gland, when compared to domestic ruminants.

The aim of the present study was to compare the histomorphological structure of the adrenal gland and its corticosteroid profile in different species of African antelope. It is hoped that the results will not only broaden the comparative endocrinological and histological database but help increase our understanding of the general biology of the many antelope species.

II. LITERATURE REVIEW

A. History of Research on Adrenals

The adrenal gland of mammals is composed of adrenocortical and chromaffin tissues. The columnar epithelial cells of the mesoderm which line the coelom form the cells of the adrenocortical tissue. The chromaffin cells originate from paraganglion cells of the neural crest complex and migrate to lie beside the cortical cells.

In the adult eutherian mammals the adrenal gland consists of two parts, the cortex and medulla. The medulla is composed of chromaffin tissue which is centrally located. The three main zones found in the adrenal cortex, the zona glomerulosa, zona fasciculata, and zona reticularis, were named by Arnold in 1886. Deane and Greep (1946) later developed the hypotheses that these three zones were not only morphologically distinct but had separate secretions. This was later termed the "zonal theory" by Chester Jones in 1948. It was Brown-Sequard (1856) who first demonstrated that the presence of adrenals was essential for life by conducting adrenalectomies on various animals, which proved fatal. Thus, it was concluded to be involved in the regulation of "internal secretions". At the beginning of this century adrenaline was isolated by Aldrich (1901) which later led to the discovery of its chemical structure and its synthesis by Stolz in 1904.

The first observations of adrenal structure and function were made on humans and then on a constantly widening variety of mammals and other vertebrates. In

some cases, species possessed "true" adrenals (with cortex and medulla), while others had their homologues.

The separate function of the adrenal cortex was elucidated slowly because of the difficulty of preparing extracts free of adrenaline. In 1927 Hartman et al. showed that cortex extracts could preserve life of adrenalectomized cats. Within a few years six major steroids were isolated from adrenal tissue, and these, when administered collectively, prolonged the life in adrenalectomized animals. Today their trivial names are deoxycorticosterone, corticosterone, 11-dehydrocorticosterone, cortisol, 11-deoxycortisol, and cortisone. The development of paper chromatography in the 1950's made it possible to show that either cortisol or corticosterone was the major steroid product secreted in a variety of mammals (Bush 1953; Tait and Tait 1979).

The study of the physiological role of adrenal cortical secretions was pioneered by Hans Selye (1936), who developed the concept of the "General adaptation Syndrome" in which the response of the endocrine system, particularly the adrenals to chronic stress was described along with the onset of various diseases.

B. Gross Anatomy and Weight of Adrenals

The eutherian mammals have paired adrenal glands, each lying at the anterior pole of the kidneys. They tend to be surrounded with varying amounts of both white and brown fat. The glands themselves take on different shapes such as spheroid, oval, elliptical, cylindrical or rod-like (Hartman and Brownell 1949). They may be somewhat angular, occasionally flattened and irregular, rarely folded. Bourne (1949)

published the first attempt of systematically describing available adrenal glands from museums and zoo specimens of many animal species. Hartman and Brownell (1949) further described in detail the gross anatomical differences in many species of mammals. For example, they described that the adrenals of the North American porcupine (*Erethizon dorsatum*) differ from most mammals in their shape, being elongated rods, often curved, and closely applied to the kidneys. Hartman and Brownell (1949) found that in a majority of mammals the shape of the medulla conforms to that of the whole gland. If the gland is spheroid, so is the medulla; if elongated the medulla is extended. In the human the extensive folding of the cortex creates a large surface of contact between the cortex and medulla (Symington 1969).

The general plan for the blood supply to the gland is similar in all mammals. The dorsal aorta provides directly most of the arterial blood to the gland through a number of small arteries, and in some species from branches of the renal and inferior phrenic arteries. From these, the blood is dispersed in a network of arterioles throughout the connective tissue of the adrenal capsule. A single vein provides the discharge into the renal vein or the vena cava. These connecting vessels may be long, so that the gland is easily separated from the vena cava, as in the mouse (*Mus spp.*), and rat (*Rattus spp.*), or they may be short, as in the cat (*Felis catus*) or dog (*Canis familiaris*). In some species like the rabbit (*Oryctolagus spp.*) and guinea pig (*Cavia porcellus*), the right adrenal may be closely applied to the vena cava so as to make an adrenalectomy without injury impossible (Hartman and Brownell 1949).

The adrenal gland weights have been found to show variations according to factors such as species, age, sex, season, population density and physiological states, which all provide information for estimating the functional status of the gland (Krumrey and Buss 1969). There seems to be a general trend for an increase in weight with age (Goldzieher 1946), although some data indicate the contrary (Yamauchi 1965, Belloni et al. 1992). The weight of adrenals change with such physiological episodes as oestrus and pregnancy, and in proportion to the body weight varying from strain to strain and from species to species (Chester Jones 1957). Rogers and Richter (1948) showed that the wild rat has a much heavier adrenal, both absolutely and relative to body weight, than the albino and other laboratory strains, which some believe to be distinct subspecies. Christian (1953) showed that relative adrenal atrophy is common in captive, closely confined mammals, therefore data obtained from these specimens do not reflect feral conditions. Benedict (1938) showed that there was a direct relationship of the adrenal weights to the surface area of the animal rather than body weight. The general formula is $S = Kw^{2/3}$ where S is the surface area, w the body weight and K a constant with a value of about 10 for many animals. The guineapig and mongolian gerbil (*Meriones unguiculatus*) were found to have large adrenal weights for their relative body sizes (Chester Jones 1957). The ratio between adrenal weight and body weight appears to be approximately the same among most mammals, about 0.15×10^{-3}. In many mammalian species variations in size seem to be determined to the larger extent by the cortex, whether it be in different species or in different individuals of the same

species (Hartman and Brownell 1949). Myers (1967) described that the 30 - 50 % fluctuation in adrenal weight and size for wild rabbits was solely restricted to changes in the cortical tissue. Kojima (1928) showed that left and right adrenals are not necessarily the same weight, and Donaldson (1924) noted that the left adrenal was 10 % to 20 % heavier than the right in the rat. The same was found in two strains of cattle (Chester Jones and Henderson 1976) and in the dog (Baker 1937). Rats and mice are sexually dimorphic in adrenal weight (Chester Jones 1957), with the females being the heavier. McKeever and Tomich (1963) found that in the adult mongoose (*Herpestes auropunctatus*) the relative weights of the glands in the females were greater than those of males. Histological examination demonstrated that this dimorphism was attributable to the development of a pronounced inner fasciculata in females. Krumrey and Buss (1969) found that in African elephants (*Loxodonta africanus*) that there was a pronounced increase in relative adrenal weight, related to pregnancy and lactation, and that females had higher adrenal weights than their male counterparts. Sexual differences are not as marked in other animals such as the rabbit (Christian 1953) and golden hamster (*Mesocricetus auratus)* (Meyers and Charipper 1956).

Seasonal variation has been correlated with adrenal hypertrophy and atrophy, particularly for mammals inhabiting areas of marked climatic fluctuations. Adrenal glands weigh less during winter months than in other seasons (Christian 1962; Myers 1967). Blair-West (1968) reported that adrenals of alpine rabbits were heavier in the spring than in other seasons due to the marked climactic fluctuations, which caused

an increase in the total area occupied by the zona glomerulosa of the adrenal gland. In addition to seasons, well defined breeding periods in mammals have been reported to significantly increase adrenal weight in both sexes, with the changes primarily in the zona reticularis (Christian 1962). African elephants, on the other hand exhibited no marked variations in adrenal weights when exposed to a lengthy dry season with harsh conditions, indicating an alternate mechanism to cope with this stress (Krumrey and Buss 1969).

Stresses of various kinds have the ability to change the size of the adrenals, with causes ranging from heat, cold, exercise, toxins or infections (Fazekas 1939; 1941).

C. Histology

1. General

The pattern found in the adrenal cortex is caused by the growth and organization of the arterial vessels. The zona glomerulosa (z.glom.), a narrow concentric band, found just below the outer connective tissue capsule, generally forms balls or loops of cells. The zona fasciculata (z.fasc.), usually the widest zone, has cords of cells which are radially oriented, and which breakup into less organized cell tissue in the zona reticularis (z.ret.). The volume of the gland occupied by these various zones depend on the species and physiological state. For example, Chester Jones and Henderson (1976) compared the rat where z. glom.= (38 %), z.fas.= (15 %), and z.ret.= (5 %), to the ox (*Bos spp.*) where z.glom. = (20 %). z.fas. = (51 %),

and z.ret.= (29 %). In addition, other cortical layers are sometimes found, such as the zona intermedia (between the z. glom. and z. fasc.), or the X zone.

2. Capsule

Variations in the histological appearance of the adrenal in different species are only modifications of the same cellular architectural plan. There is varying predominance of the three major cortical zones, but these are almost always present, except in disease (Symington 1969). The adrenal is enclosed by a thick or thin capsule, composed mostly of collagenous connective tissue that contain occasional smooth muscle fibers. The capsular tissue may extend to varying depths into the cortex as trabeculae. Frequently clusters of undifferentiated cortical cells are seen in the capsule (Dellman 1993). Greep and Deane (1949) reported that some capsules may consist of an outer coat of dense fibrous tissue and an inner layer of areolar tissue containing a meshwork of arterioles.

Prasad and Yadava (1974) and Das et al. (1965) reported that the capsule of the Indian buffalo (*Bubalus arnis bubalis*) is a blend of elastic and reticular fibres, with some presence of smooth muscle fibres as well. In the African buffalo (*Syncerus caffer*), the capsule is described as well developed and containing eosinophilic staining collagenous fibres towards the surface. Smooth muscle fibres are more numerous closer to the cortex (Teixeira et al. 1993). Bernert (1981) noted that the eland (*Taurotragus oryx*) and oryx (*Oryx beisa*) possess 4 layers of capsule, as opposed to 3 layers in most other ruminants studied.

3. Zona Glomerulosa

The cells of the zona glomerulosa, arranged in groups by the connective tissue trabeculae, have basophilic nuclei which possess one or two nucleoli. The nuclei may vary in shape from round, oval to sausage-shaped. The cytoplasm has a varying number of liposomes (lipid droplets) under normal conditions, depending on the species (Chester Jones and Henderson 1978). Descriptions of this zones' architecture in domestic cattle (*Bos spp.*) vary in the literature, although indications are that there are no discernable histological differences between the sexes in mature bovine species (Weber et al. 1950). Cupps et al. (1954) described cords and cylinders of columnar cells making up the glomerulosa layer in adult cattle, whereas Elias (1948) explained that the cells are cuboidal and are in small balls. Prasad and Sinha (1984) showed that the zone was made up of irregular cell groups. The zona glomerulosa is the most variable zone in the cortex, not only individually but locally within the same gland (Yamauchi 1961).

The literature contains a few studies in adrenal histology on wild bovines and relatives. Teixeira et al. (1993) described the zona glomerulosa of the African buffalo , where the cells are arranged in curved cords or arcades of columnar cells. These arch structures sometimes lead to the naming of this zone as the zona arcuata, as found in horses and donkey species (*Equus spp.*), (Elias 1948; Dellman 1993), the nuclei of which were oval. Histological studies on Indian buffalo showed distinct cortical zones with a thick connective tissue capsule. The zona glomerulosa was composed mostly of high cuboidal cells, arranged in cords connected at the capsular

end by the familiar arch structures. The cells had lightly staining basophilic cytoplasm (Prasad and Yadava 1974). Instances of prominent trabeculae of reticular fibres from the capsule penetrating through the zones of the cortex are also noted in the Indian buffalo (Prasad and Sinha 1984), and in domestic cattle species (Cupps et al. 1954). In antelope (Bernert 1981) the cytoplasm of the glomerulosal cells was highly acidophilic for some species as the lesser kudu (*Tragelaphus imberbis*), and Thomson's gazelle (*Gazella thomsoni*), while the zone was lighter stained in impala (*Aepyceros melampus*) and oribi (*Ourebia ourebia*). In oryx (*O.gazella callotis*), mountain reedbuck (*Redunca fulvorufula*) and steenbuck (*Raphicerus campestris*), a lighter region was found adjacent to the zona fasciculata. The glomerulosa showed an unordered structure in general, exhibiting primarily a bundle-shaped cellular pattern. The nuclei in this zone of most African ruminants showed up to four distinct nucleoli. Throughout all species studied much of the capillaries scattered in the different regions of the gland were filled with pockets of erythrocytes.

4. Zona Intermedia

Sometimes a transitional zona intermedia is described, appearing between the zona glomerulosa and zona fasciculata, as a narrow band of cuboidal or undifferentiated cells with small, darkly staining nuclei. This zone has been found to occur in the dog (Bloodworth and Powers 1968), ferret (Holmes 1961), horse, cat, rabbit, guineapig, mouse and hamster (Nicander 1952), and to a lesser extent in the goat (*Capra spp.*), sheep (*Ovis spp.*), and cow (Dellman 1993). The true interpretation

of this zone is bound up with consideration of the two adjacent zones (Chester Jones and Henderson 1978). Therefore, identification of this zone is dependent on the authors' subjective classification scheme. Nicander (1952) points out that this zone may consist of atrophied cell groups originally constituting the innermost layers of the zona glomerulosa. Some literature makes no mention of any such zone in domestic cattle (Elias 1948; Cupps et al. 1954). It is speculated that this zone may appear in aged individuals, as Yamauchi (1965) had described. The zona intermedia in the Indian buffalo was identified by a region exhibiting closely packed nuclei, and being sudanophilic (Sohal and Chaturvedi 1962; Prasad and Yadava 1974). No such zone was evident in the African buffalo (Teixeira et al. 1993). In many but not all antelope species (Bernert 1981), a narrow dark band between the glomerulosa and fasciculata zonae can be seen. It appears to show more of a transitional fascicular arrangement, while the nuclei are centrally located and smaller and closer together.

5. Zona Fasciculata

The typical mammalian zona fasciculata profile consists of polyhedral cells arranged in radial rows, and are considerably larger than those found in the glomerulosa. The cells have round nuclei with one or two nucleoli and the cytoplasm is lipid rich. The nuclei of the female tend to be larger, and less so in the zona reticularis. The cells in the zona fasciculata have often been referred to as spongiocytes because of the foamy appearance caused by the lipid being dissolved by routine histological procedures leaving artefactual vacoules. The lipid droplets

themselves tend to be all of the same size in any one cell. In some species the outer fasciculata may be more lipid-rich than the inner part. The cytoplasm in the latter region therefore may take up more acidophilic stains, which is correlated to the lower number of vacuoles (Chester Jones 1957). As a consequence, in some cases, there is an absence of a distinct boundary between the zona fasciculata and zona reticularis.

Descriptions for cattle of this zone include that the cells are the largest in the cortex and are mostly arranged in straight cords separated by sinusoids which contain connective tissue fibres. The cytoplasms are highly vacuolated in the outer half of the zone, while the inner region contain smaller, darker nuclei (Nicander 1952). Symington (1969) described in the human that a unified zone of cells constitute the fasciculata and reticularis zonae, indicating that it was difficult to discern the juncture of the two. In the Indian buffalo the cells in the zona fasciculata were polygonal and arranged in characteristic radiating columns. There was no distinct demarcation between this zone and zona reticularis, which had relatively small cells arranged in irregular groups. To the contrary Das et al.(1965) described the cells as being irregularly placed, with no distinct sinusoids or longitudinal cords. In the African buffalo, Teixeira et al. (1993) described the zone as consisting of an outer portion having an extensive sinus network with vacuolated, cuboidal cells forming cords. An inner portion contained more compact cells not as vacuolated yet more eosinophilic. Bernert (1981) explained that African antelope exhibited the characteristic radial cell columns of single cell width, and separated by sinusoids and connective tissue.

Particularly wide sinuses were found in eland, waterbuck (*Kobus ellipsiprymnus*), mountain reedbuck, white bearded wildebeest (*Connochaetes taurinus mearnsi*), and Thomson's gazelle (Bernert 1981). The cell shapes were mostly cuboidal , but were also ovoid. In the wildebeest, impala and steenbuck, the cells and their nuclei were smaller than those found in the glomerulosa. The nuclei usually lay centrally and contained distinct nucleoli and chromatin. The zona fasciculata of antelope seem to be richer in erythrocytes compared to their adjacent zona glomerulosa (Bernert 1981).

6. Zona Reticularis

The zona reticularis exhibits variations between species, as the other zones, but is never as wide as the zona fasciculata. The anastomosing networks of reticular cell cords surround the large blood sinuses. The cells are smaller than in the other zones, with less liposomes, making the cytoplasm more acidophilic with less vacuoles present (Chester Jones and Henderson 1978).

Nicander (1952) reported that the zona reticularis in the domestic cattle was inconspicuous and not continuous. The cells were more compressed than in the inner fasciculata, and were arranged in approximate horizontal, freely anastomosing cords. Cupps et al. (1954) observed the penetration of glomerulosal cells into the zona reticularis along with the septa from the capsule in cattle. In the Indian buffalo cells of the zone were arranged in irregular groups with strongly eosinophilic cytoplasm (Prasad and Yadava 1974) and prominent sinusoids (Das et al. 1965). A very distinct

demarcation existed between the cortex and medulla, and there were two layers which comprise the medulla (Prasad and Yadava 1973; 1974). The African buffalo, like the indian buffalo, includes deeply staining cell nuclei and the clear demarcation between the cortex and medulla (Teixeira et al. 1993). In African antelope the transition to the zona reticularis was in general difficult to see, especially in those specimens examined with irregular cords in the inner fascicula (Bernert 1981). A very distinct network structure was observed for this region in steenbuck, bushbuck (*Tragelaphus scriptus*), klipspringer (*O. oreortagus*), Thomson's gazelle, and eland antelope. Vacuoles were absent in the inner reticularis in reedbuck (*R. redunca*), impala, and steenbuck (Bernert 1981).

7. Adrenal Medulla

The medulla for many mammalian species in general is composed of irregular epithelioid cells collectively called chromaffin cells, which are arranged in rounded groups or short cords surrounded by blood capillaries and venules. Two types of cells can be found making up the medulla, norepinephrine and epinephrine producing, both of which are primarily involved in physiological homeostasis functions (Dellman 1993).

In the African buffalo, no medullary capsule was found. The cells were divided into two zones, with outer one consisting of large columnar cells arranged in circular forms and seeing eosinophilic, while the inner region had smaller, less intensively staining cells. A distinct boundary exists between the two regions. Prasad and Yadava

(1974) and Das et al. (1965) observed that the medulla of the Indian buffalo also had similar arrangement as its African counterpart, with the cells reported to have large spherical nuclei. In earlier studies, Prasad and Yadava (1972; 1973) identified that the adrenaline-secreting cells were arranged in the outer layer and noradrenaline cells in the inner or central portion.

Bernert (1981) found that klipsringer, steenbuck, and dik-dik (*Madoqua spp.*) had no medullary capsule, while wildebeest, greater kudu (*T. strepsiceros*), reedbuck, Grant's gazelle (*G. granti*), Thomson's gazelle, gerenuk (*Litocranius walleri*), and suni (*Neotragus moschatus*) all had a capsule consisting of tightly packed reticular cells. In parts this capsule was separated from the peripheral medulla cells by robust collagen fibres. The outer medulla was described as having radially oriented cell columns, similar to the zona fasciculata, in many species of antelope including, oryx, Jackson's hartebeest (*Alcelaphus buselaphus jacksoni*), and wildebeest (Bernert 1981). In contrast, in wild sheep species the cell rows ran parallel. Two sections were found in the medulla, with an outer, lighter staining region with large, "highly prismatic" cells and a central darker staining area, with smaller polyhedral cells (Bernert 1981). In most antelope described, except for the Thomson's gazelle and bushbuck, a distinct border existed between the two regions of the medulla (Bernert 1981).

D. Adrenal Gland Hormones and Their Role in Adaptation

Steroid biochemistry development was slow due to methodological problems. Both corticosteroids and sex hormones are produced in minute amounts (typically between 10^{-6} and 10^{-12}M) and therefore extremely difficult to quantify and chemically analyze (Chester Jones and Henderson 1976). The full potential of steroid biochemistry came about with the development of chromatography. Chromatography not only separates complex mixtures of steroids, but at the same time provides an indication about structure. Methodologies of *in vitro* studies of steroids involves extracting the tissue and characterising and quantifying the steroids present. The preparation of the tissue may include cutting into slices, mincing, or preparing a cell-free homogenate. The homogenate may be utilized as such or further fractionated by centrifugation (Chester Jones and Henderson 1976). Adrenocortical steroid hormones are biosynthesized in an orderly sequence of consequent reactions from a distant precursor, cholesterol. The biochemical end-products of these biosynthetic pathways are not the only physiologically effective compounds. The reaction intermediates in some instances may be more important than the last member in the chain (Chester Jones and Henderson 1976).

E. Adrenocortical steroids in mammals

Over 50 different steroids have been isolated from adrenal tissue, but only a few are secreted and have a known physiological function (Chester Jones and Henderson 1976). The corticosteroids themselves are classified into two main

groups: Glucocorticoids play a role in protein and carbohydrate metabolism and have anti-inflammatory effects. The principal ones are cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, 11-deoxycortisol. Mineralocorticoids, control the electrolyte balance of Na^+ and K^+ and water metabolism. The main compounds in this second group are aldosterone and 11-deoxycorticosterone. Other major steroids may be found in adrenals such as androgens and estrogens (Tait and Tait 1979; Vinson et al. 1992).

Most vertebrates secrete cortisol with varying proportions of corticosterone and aldosterone. Some species of rodents, particularly the rat and mouse, differ from the general mammalian steroid pattern in that they secrete primarily corticosterone (Chester Jones and Henderson 1976). Species differences are evident in production of 18-oxygenated steroids (Fazekas and Webb 1966). The rabbit is one of the few species which synthesizes 18-hydroxy-11-dehydrocorticosterone and 11-dehydroaldosterone (Fazekas and Sandor 1969).

The principal mineralocorticoid of mammals is aldosterone, which favours the retention of sodium ions and the excretion of potassium and hydrogen ions. Aldosterone accomplishes the regulation of ion balance by acting variously on the kidneys, intestine, sweat glands and salivary glands (Wilson and Foster 1985). For example, aldosterone's primary site of action in the kidney appears to be on the distal convoluted tubule of the nephron. It increases the active transport of sodium from the urine to the blood plasma. Removal of the adrenals results in the inability of the kidneys to retain sodium. The loss of sodium causes dehydration and collapse

of the peripheral circulation, so that not enough oxygen is supplied to the tissues and death follows (Vinson et al. 1992). Clearly, aldosterone has a widespread sodium-conserving action in the body based on previous evidence in a range of species (Chester Jones and Henderson 1978; Griffen and Ojeda 1988).

Many wildlife species can be classified as being water dependent or independent, and antelope are no exception (Wilson 1989; Grenot 1992). Some species such as the gemsbok (*O.g.gazella*) and springbok (*Antidorcas marsupialis*) are able to withstand drought conditions (Skinner and Smithers 1990). As far back as 1857 (Cummings), the gemsbok was noted for its water independence and its inhabitance of dry, desert terrain of the Kalahari in southern Africa. The ability to cope with the lack of water is due in part to physiological mechanisms (i.e. kidney anatomy, and mineralocorticoids). The waterbuck, for instance, has only a very limited ability to concentrate its urine when exposed to dehydration, therefore making it unable to conserve water and in turn limiting the species' range to water abundant habitats (Taylor et al. 1969; Spinage 1982). Many antelope species are not as specialized as the above mentioned, but are able to inhabit sub-optimal habitats and survive short periods of harsh environmental conditions. This is based on their ability to utilize and conserve water (Grenot 1992). For example, the eland and red hartebeest (*A.b.caama*) are able to withstand long periods of drought without any serious ill effects (Spinage 1986), while the waterbuck will not survive more than a few days if deprived of sufficient drinking water (Spinage 1982). Ghobrial (1974) found that the dorcas gazelle (*G.dorcas*), which lives in desert-like conditions in

North- East Africa, is able to reduce water loss when deprived of water by reducing urine output 3 to 4 fold and is able to double its concentration.

Studies done on the camel (*Camelus dromedarius*) have shown that aldosterone plays a key role in this specie's ability to survive and flourish in the hot arid environment of the deserts (Wilson 1989). The normally low volumes of urine voided by the camel are reduced even further under dehydration by increasing the plasma sodium levels, which is under the control of aldosterone. These same physiological mechanisms of water balance through hormones are most probably found in antelope as well, but have not been studied (Wilson 1989).

The glucocorticoids secreted by the adrenal cortex also play a major role in the physiological adaptation of animals to certain demands of the environment. For example, seasonal fluctuations in cortisol levels have been found in some deer species (Nilssen et al. 1985). Circadian rhythms of cortisol levels have been detected in many species including pigs (*Sus scrofa*) and horses (Bottoms et al. 1972). The primary function of glucocorticoids in many hoofed mammals involves various stress responses. Some of their direct effects include inhibition of ACTH secretion, acting as an immunosuppressant, and maintaining the functional capacity of skeletal muscles, among others (Young 1973).

In recent decades the importance of adrenal steroids in capture operations of many wildlife species, particularly hoofed mammals, has been studied. Plasma cortisol levels were measured in some species as an indicator for acute stress in response to handling procedures (Kock et al. 1987; Hastings et al 1992). Variations in the cortisol

levels had occurred due to different handling procedures. Capture Myopathy, a disease which causes mortality associated with capture operations, has been found to afflict many antelope species (Young 1973; Bothma 1989). It is caused by extreme, unaccustomed muscular exertion. Symptoms include torticollis, myoglobunuria, ataxia and paralysis, which can develop within one day to four weeks after capture. Young (1973) also found the adrenals to be congested and haemorrhagic and the cortex atrophied with this disease.

The present investigation will describe the morphology and endogenous corticosteroid content of the adrenal glands in healthy antelope of selected species. It will serve as a baseline study to be used in further research in population management and capture stress.

III. MATERIALS AND METHODS

A. Research Area

The sampling site was located on the Rooipoort Nature Reserve, which is about 60 km due west of the city of Kimberley, Northern Cape Province, Republic of South Africa. The privately owned reserve is 45, 000 hectares, and is bordered by the Vaal river on its north and west borders. The habitat is arid and according to White (1983), the two distinguishable vegetation types are the Bushy Karoo-Namib shrubland and Kalahari Acacia wooded grassland.

B. Animals and Sampling

Adrenal glands were obtained from the following six species and sample sizes; cape eland (*Taurotragus o.oryx*), n = 6; southern greater kudu (*Tragelaphus s. strepsiceros*), n = 6; gemsbok (*Oryx g. gazella*), n = 8; red hartebeest (*Alcelaphus buselaphus caama*), n = 12; springbok (*Antidorcas marsupialis hofmeyri*), n = 11. All individuals sampled were older adult males, shot as trophies by sport hunters during the winter season, a four month period, over three sampling seasons for the years of 1993, 1994, 1995. Carcasses were returned to the skinning facility and both left and right side adrenals were removed from each animal promptly with removal of fat and connective tissues surrounding it. Glands were cleaned of fat, longitudinally cut into three pieces, with the right adrenal preserved in 70 % ethanol for hormone analysis, and the left adrenal immersed in 10% neutral-buffered formalin for histological

analysis using light microscopy. Approximately one hour elapsed between time of death and tissue fixation, to ensure cellular structures remain intact.

C. Histology

After shipment back to the laboratory at McGill University, all glands were photographed, weighed, and measured for gross morphological study before any processing. The formalin fixed gland was then routinely processed through a graded series of alcohol and embedded in paraffin wax. Sections 5μm thick were stained with Haematoxylin and Eosin (Drury and Wallington 1980). Observations were made of the structural architecture of the capsule, zona glomerulosa, zona fasciculata, zona reticularis and medulla. Morphological measurements made of the longitudinal sections included cortex zone widths and cell density (Yamauchi 1961). All measurements were made using a morphological Analysis System (MOP, Zeiss), consisting of a light pen attached to a point counter for density and length measurements. All histological work was carried out at the Department of Pathology of the Montreal General Hospital.

D. Hormone Analysis

1. Extraction of steroids

The adrenals were preserved in 70 % ethanol in 60 ml nalgene bottles for transportation and storage. First, the alcohol was decanted into a 100ml Erlenmeyer flask. The gland itself was cleaned of all fat and chopped with scissors into fine

pieces and weighed. The gland was then ground with a mortar and pestle with 30 ml of 70 % ethanol. The suspension was then mixed on a magnetic plate for 10 min.. The adrenal homogenate was then centrifuged at 3 000 rpm for 10 min.. The supernatant was then filtered into the same Erlenmeyer flask as before through Whatmann No.4 paper, while the sediment was extracted once more with 30 ml of 70 % ethanol. This process was repeated for a third time. The combined adrenal extract was then transferred to a separatory funnel for extraction with equal volumes of petroleum ether, twice, for removal of lipids. The alcohol was then evaporated from the extract using a rotary evaporator (Buchi) under vacuum, with a water bath at 45^{O}C, for 30-50 min. until the extract had gone down in volume to about 20 ml. The watery extract was then transferred to a separatory funnel and extracted twice, using equal volumes of methylene chloride to extract the corticosteroids. The combined methylene chloride extract was desiccated by the addition of anhydrous sodium sulphate and then the extract was filtered through Whatmann No.1 filter paper. The extract was evaporated to dryness in the rotary evaporator. The extract was taken up in methanol and transferred to test tubes where one ml of methanol was equal to two g. of adrenal tissue extracted. This extraction procedure yields 70 % efficiency (Fazekas et al. 1968). This methanolic extract was used for thin layer chromatographic analysis.

2. Thin layer chromatography

An aliquot of the adrenal extract from 0.5 g tissue was spotted onto a Whatmann, 20 x 20 cm Silica gel TLC plate (250 μ thick) with built-in florescent indicator, along with standard corticosteroids, and developed in various solvent systems in standard TLC glass tanks. The standard corticosteroids used were the following: corticosterone (B), cortisol (F), aldosterone (Aldo), deoxycorticosterone (DOC), 11-deoxycortisol (S), 18-hydroxycorticosterone (18-OH-B), cortisone (E), 11-dehydrocorticosterone (A) (Sigma Chemical Co.,Missouri). The standard steroids were dissolved at 1 mg/ml concentration in methanol. Quantities of plated standards for semi-quantitative estimation ranged from 0.5 to 5.0 μg/spot. The adrenal extract spots were concentrated with a filter paper ring prior to running (Fazekas and Kovacs 1961). The solvent system used for the first run was Chloroform: Methanol: Water at 150:10:0.5 ratio. The elapsed time for runs in this system was about 1 h. 30 min.. Following each run the plates were dried with air blower and analyzed under a Spectroline shortwave UV lamp (Fisher Scientific) at 243 um band-width. The R_f values were recorded for all standards and sample spots. A semi-quantitative analysis was made of the quantity of steroids present by the intensity and size of each spot in comparison with a series of standards used (0.5μg, 1μg, 2μg, 3μg, 4μg, 5μg). The steroids were further identified by running them in a different solvent system (TLC-4 ; Ethyl Acetate:Methanol, 20:1).

E. Statistical Analysis

The mean (x), and standard deviations (SD) for each variable of interest in each species was calculated. Differences between species for each parameter were tested by analysis of variance using the general-linear analysis of variance (PROC GLM - SAS Institute, Inc. 1985). Because of unequal sample sizes, least square means was used for the multiple comparisons of averages. The levels of statistical significance was corrected for multiple comparisons with the sequential Bonferroni test (Rice 1989).

Since sample sizes (n) were small and the standard deviations relatively large in comparison to the corresponding mean values for each variable within a species, the homogeneity of each species was examined using multivariate statistical analyses. Step-wise discriminant analysis (PROC STEPDISC - SAS Institute, Inc., 1985) was used to produce a reduced set of parameters that would provide the best separation of individuals among the five species. Discriminant-function analysis (PROC DISCRIM - SAS Institute, Inc., 1985) using a jackknife procedure evaluated the accuracy of the resultant species classification by removing an individual from each species and reclassifying it back into the incomplete data set. This procedure was carried out for 50 repetitions using a randomly selected individual from the complete data set in each repetition.

IV. RESULTS

A. Gross Anatomy

The adrenal glands of the antelope species studied are reminiscent of their counterparts found in domestic bovids, but do have evident variations. The paired adrenal glands are both positioned anterio-posteriorly near the dorsal abdominal wall. The left adrenal gland is located anterior to the cranial pole of the kidney at its medial side without direct contact. The distance of the gland from the kidney appeared to vary not only between individuals of the same species but between species as well. The adrenal is entirely embedded in retroperitoneal adipose tissue, therefore making it difficult to extract without damaging gland tissue in some species. For instance, extraction was difficult in the eland and kudu and red hartebeest, while the gemsbok and springbok glands were readily visible in the tissue and detached from the surrounding tissue with relative ease. The right gland is at a more medial angle to the right kidney and less anterior than the left adrenal, and also has less fat surrounding it. In the red hartebeest, the right gland itself is found just adjunct to the vertebral column and adheres to its anterior longitudinal ligament. There were no accessory glands found in the tissue surrounding the adrenals in any of the species examined.

In all species, gross morphological dimorphism exists to varying degrees, between the left and right glands (Fig.1A,B,C and Fig.2D,E). Color differences found are were artifact of preservation. The right adrenals were preserved in 70 % ethanol

and therefore darkening and shrinkage of tissue occurred, while retaining their original shape. Due to the inconsistent percent in shrinkage in the right glands, weights of the formalin preserved left glands were used only in Table 1 (page 79) and Appendices 1. through 5. The largest glands were found in the eland, followed by gemsbok, greater kudu, red hartebeest and springbok, respectively. A similar order was followed by the adrenal weights (Table 1). For the greater kudu and gemsbok the adrenal weight differences were not significant ($P>0.05$).

In the four largest species examined, the left adrenal consistently showed species-specific gross morphological differences, while the right remained more or less ovoid and non-discript in shape. All glands from each species were slightly elongated and dorsoventrally compressed. The apical regions are rounded and the dorsal and ventral surfaces are flat. In the red hartebeest (Fig.1A), the left gland possesses an unusual morphological aspect of a thumb-like extension which is at a right angle to the remainder of the gland. The cross section of this extension revealed that both adrenal cortex and medulla was present in the same proportion and general configuration as in the rest of the gland. The right adrenal is pear-like in shape. In the greater kudu (Fig.1B), the left gland is most dorsoventrally compressed of all species, and is kidney-shaped with a larger , wider base and a thinner apical region. The right gland has a wider base and a rounded, thinner apical region, while lacking the bend in the central portion of the main body of the gland. In the gemsbok (Fig.1C) the left gland shape appears to be long and cylinder-like, and therefore quite distinct from those of other species. This uniqueness was found consistently

throughout all individuals sampled in this group. Again dorsoventral compression was evident but not as great as in the greater kudu. The right gland is also slightly elongated and in some individuals possesses a lobe-like extension.The springbok (Fig.2D), which has the smallest adrenal size had very little or no dimorphism present between the pairs of adrenals. The familiar broad base with a thinner pointy apical region was found in glands of both sides with dorsoventral compression as well. Finally, in the Cape eland (Fig.2E), with the largest adrenals, the left gland is moderately dorsoventrally flattened to the same degree as that found in the red hartebeest, and is an elongated ovoid shape, while the right is similar but more highly compressed laterally. Again, small lobe-like extensions are sometimes evident.

Figure 1. Pairs of adrenal glands.

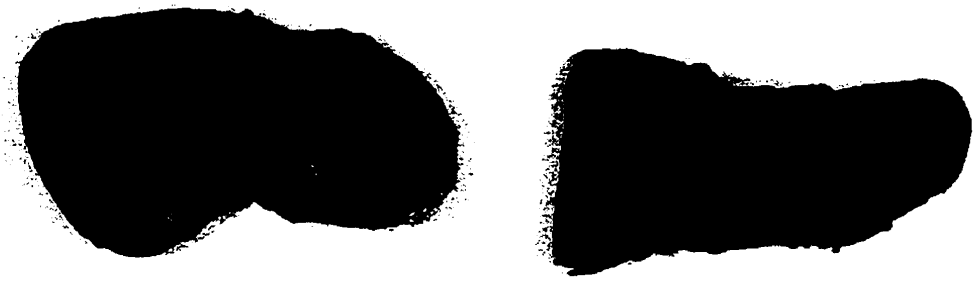
A. red hartebeest

B. greater kudu

C. gemsbok

A

B



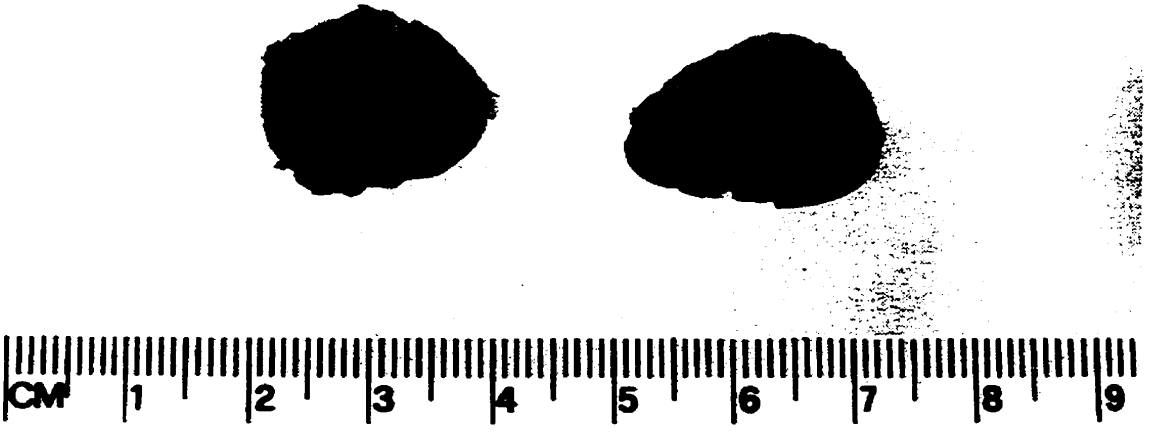
C

Figure 2. Pairs of adrenal glands.

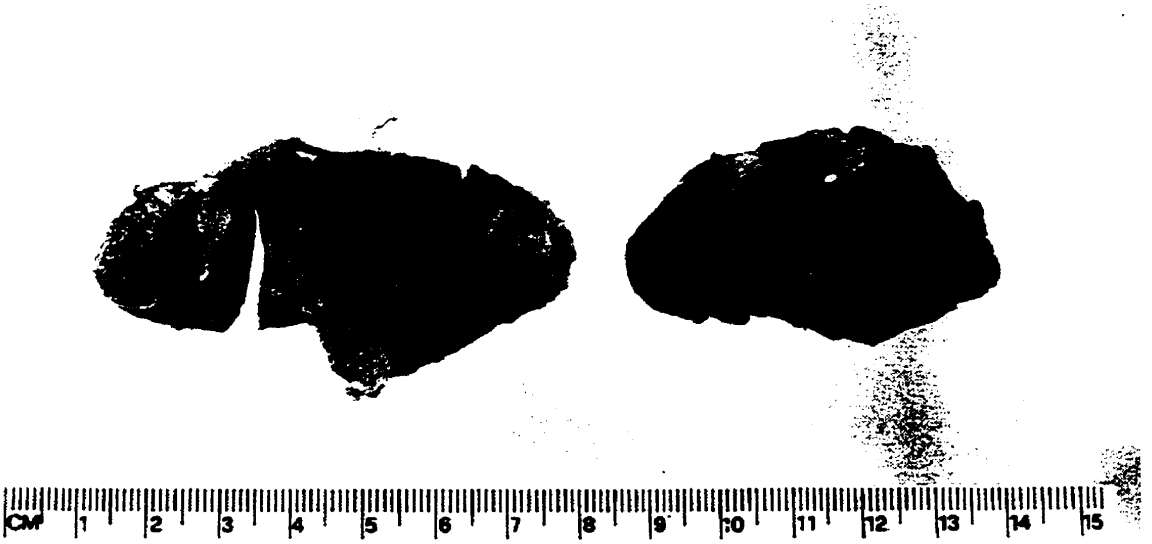
D. springbok

E. cape eland

D



E



B. Histology

1. Springbok

i) Capsule

The thick connective tissue capsule is composed of two cellular layers. The outer layer is relatively thin at a few microns in thickness, and more eosinophilic with highly scattered, few nuclei. The inner second layer is more areolar and has a higher density of nuclei, most of which are rounded and oval (Fig.3a).

ii) Zona glomerulosa

A distinct zona glomerulosa was found in all samples. The zone appeared to be more eosinophilic than the remainder of the cortex. The cells vary from highly to moderately columnar and are horizontally stratified and arranged in cord formations that run perpendicular to the capsule. The cords are composed of pairs of cells that are found end to end. The cell nuclei are heavily dark staining and are rounded oval in shape. Many nuclei appeared to be located centrally in the cords, forming close pairs down its median, with large areas of cytoplasm towards outer side (Fig.3a). No nucleoli were detected in any individuals examined. Large sinusoids were evident throughout the glomerulosa, running parallel and dividing each column. In some areas, undifferentiated cells were also found inside cords, in which case the structures width was composed of three or four cells. Horizontal sinusoids were frequently found to occur, dividing some of the strata of columnar cells.

iii) Zona fasciculata

In the springbok the outer fasciculata appears to be directly abutted to the base of the zona glomerulosa, with no connective capsular tissue present. The zone is the lightest eosinophilic staining of all the cortex, and the widest. The cells are mostly strong cuboidal and arranged in single cell width columns which run radially towards the medulla (Fig.3b). Moderate sized sinusoids separate the columns which become fragmented every 20 to 40 μ. The inner fasciculata exhibits wider sinusoids and longer intact cell columns. Scattered along the sinusoids throughout the zone are fibrocytes. In some samples small clumps of erythrocytes were found inside the sinusoids. The cell nuclei were lightly stained and exhibited one or two nucleoli.

iv) Zona reticularis

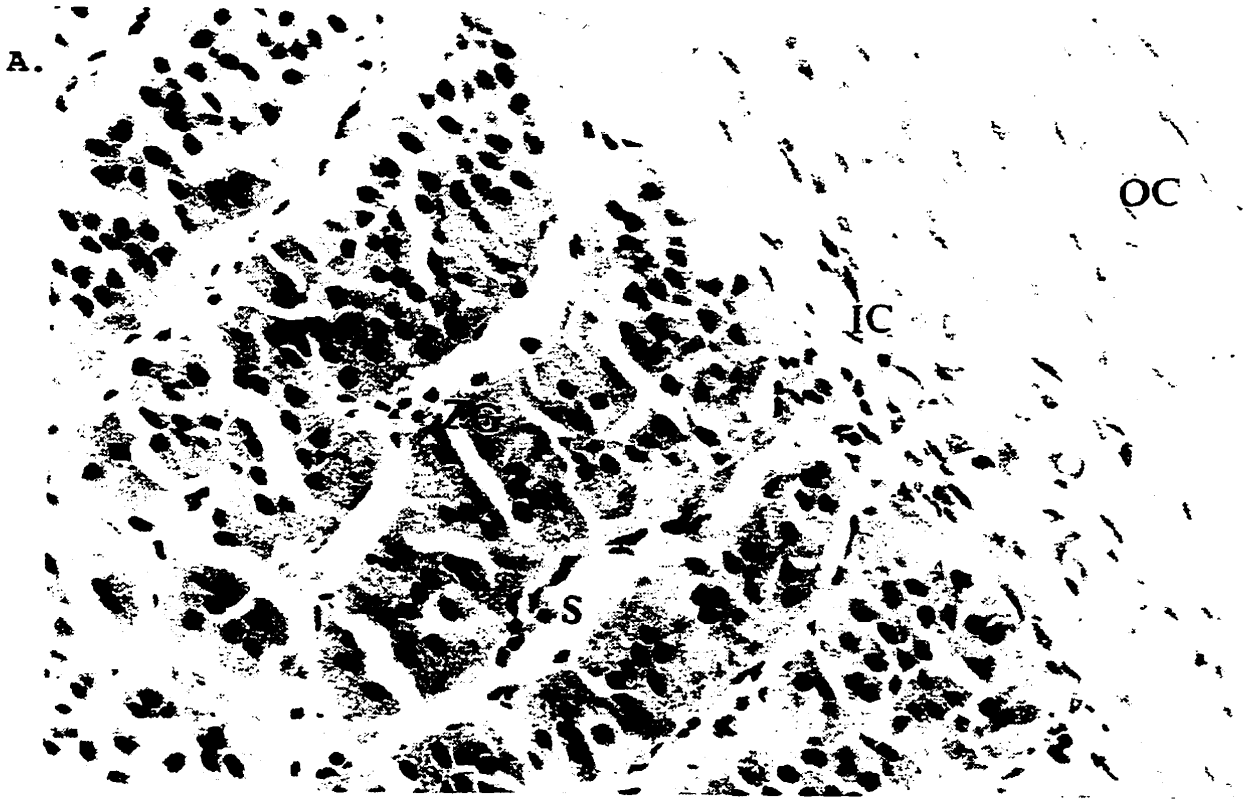
The reticularis directly borders the inner fasciculata and is difficult to distinguish exactly where the zone begins. As is characteristic of the reticularis zone, the cells have lost their cord structures and rearrange into an anastomosing network of cells (Fig.4a). Clear evidence of sinusoids are present and are arranged perpendicular to those found in the zona fasciculata. Fibrocytes are scattered within the sinusoids. The constituent cells are cuboidal and undifferentiated. Two types of cell nuclei can be observed, strongly basophilic, dark staining and a lighter staining variety with one nucleolus. The former appears to predominate the zone.

v) **Medulla**

The adrenal medulla is clearly demarcated from the cortex. There is no evidence of a medullary capsule separating the cortex from the medulla. It can be differentiated into two distinct regions, with an outer and inner medulla (Fig.4b). The outer medulla completely encapsulates the inner region, and the cells are larger, rounded and have highly vacuolated, light staining cytoplasms. The cells range in shape from low columnar to cuboidal. The cell nuclei are both dark staining and light, with the latter possessing one or two nucleoli. The nuclei occur in aggregates, scattered throughout the outer medulla, indicating acini cell structures. Sinusoids were found to separate these rounded structures. The inner medulla consists of higher density of cells with darker, smaller basophilic nuclei. The cells are undifferentiated and have pale, vacuolated cytoplasms. The sinusoids present were shorter and the region resembles in architecture, the zona reticularis of the cortex.

Figure 3 a. The springbok has a thin outer capsule (OC) which encompasses a thicker areolar inner capsule (IC). The zona glomerulosa (ZG) is composed of cord structures that are separated by sinusoids (S) which infiltrate them. Magnification 250 x

b. Springbok zona fasciculata (ZF) is composed of distinct, single cuboidal cell wide cords. Magnification 100 x


A.
OC
IC
S

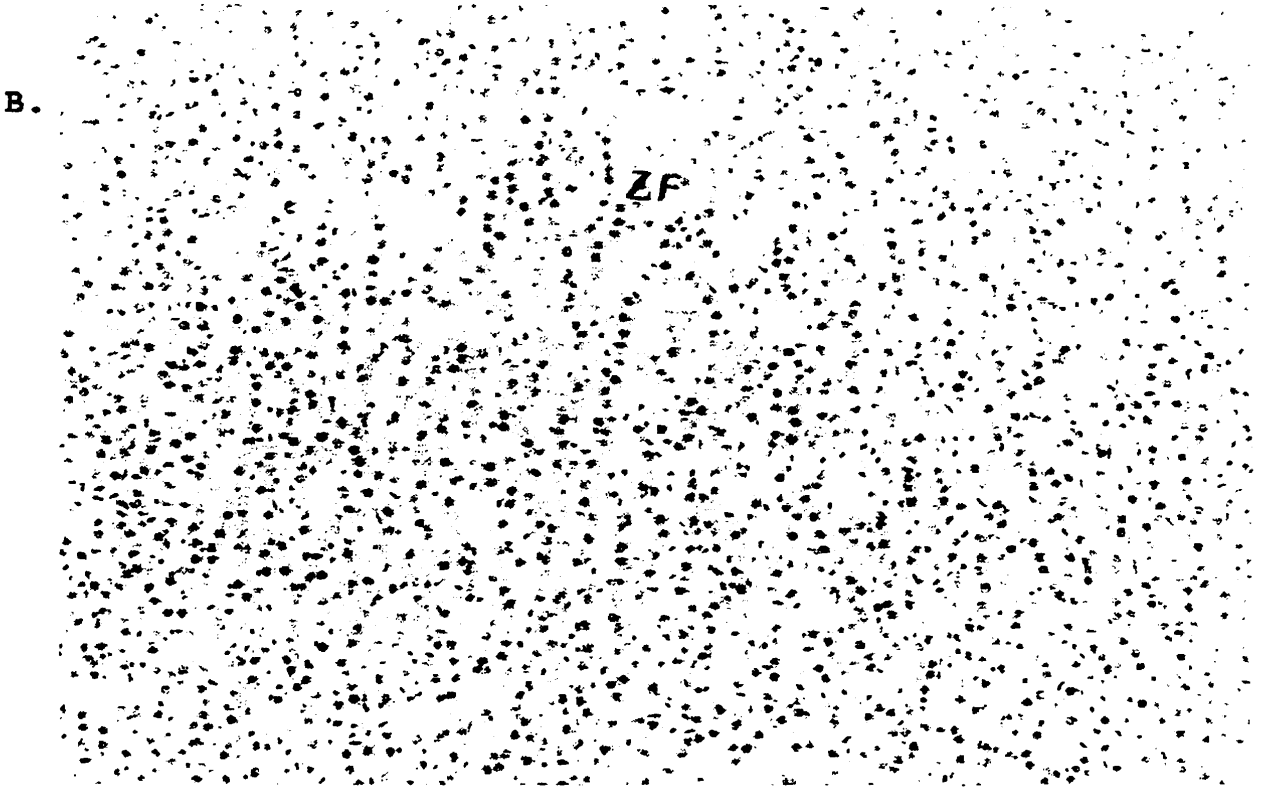
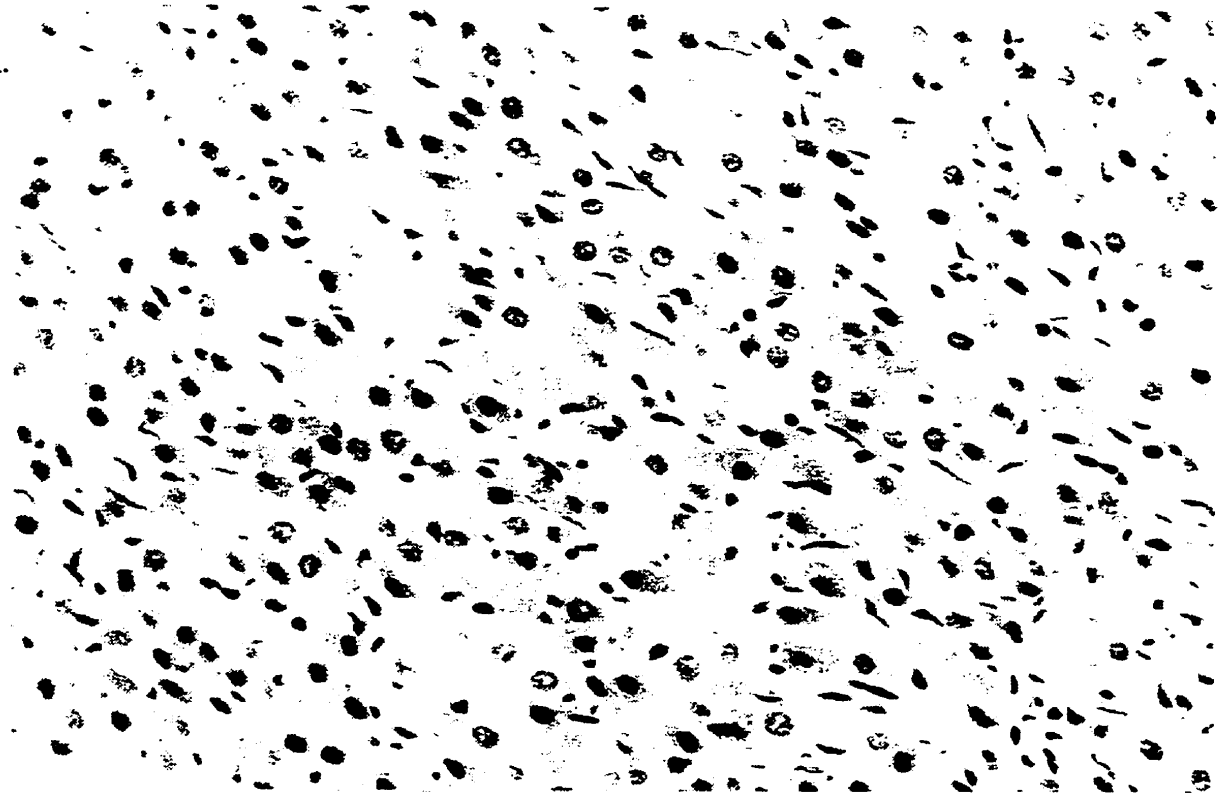

B.
ZF

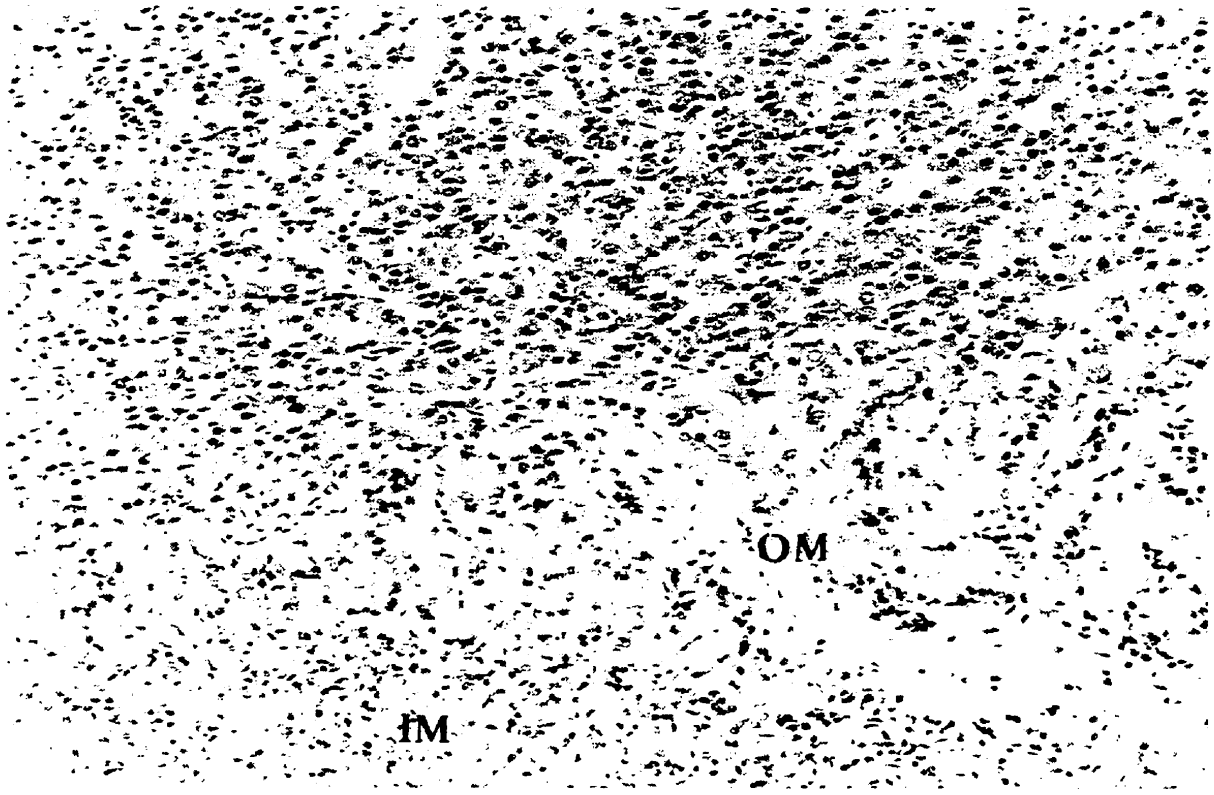
Figure 4 a. The springbok zona reticularis is made of anastomosing network of cells and is predominated by eosinophilic cell nuclei. Magnification 250 x

b. Directly abutted to the zona reticularis, the outer medulla (OM) encompasses the inner medulla (IM) completely. Magnification 100 x

A.



B.



2. Red hartebeest

i) Capsule

The thick, connective tissue capsule surrounding the gland consists of two distinct layers, with the outer capsule staining darker than the inner, therefore giving a highly contrasting appearance. The outer layer is relatively thin, being only a few microns thick, and is formed of dense fibrous tissue, with the cells having flattened oval nuclei. The inner layer is made up of areolar tissue containing a meshwork of arterioles, and the constituent cell nuclei are circular (Fig.5a).

ii) Zona glomerulosa

The zona glomerulosa is distinctly delineated from the capsule. This zone stains darker (i.e. eosinophilic) in comparison to the other zones in the cortex and as a consequence, is very prominent and easily identifiable. The cells in this region are arranged in irregular and interconnecting cords that are constructed of stratified, mostly paired cells which are joined by an arch near the capsule (Fig.5b). In some sections these arcade formations, which have their convexity directed towards the capsule, may appear as circular masses. Distinct sinusoids are found to separate these cords. Small groups of blood cells in the sinusoids were found to be scattered throughout the zone but were found mostly in the distal half. The cords are comprised of cells which are mostly cuboidal and undifferentiated at the capsular end and become horizontally stratified columnar at the base of the zone. The glomerulosa cells which are just adjunct to the zona fasciculata tend to be predominatly columnar.

The cells contain one centrally located spherical nuclei which tend to contain multiple nucleoli, mostly ranging in number from three to four. These glomerulosal cell nuclei are more intensely staining and robust than those of the zona fasciculata.

iii) Zona fasciculata

There is no evidence of a zona intermedia between the zona glomerulosa and zona fasciculata. This zone was the widest amongst the three cortical zones. The cells are arranged in long straight cords which are oriented radially with respect to the medulla (Fig.6a). The cords generally are one or two cells in diameter and are flanked by sinusoidal blood vessels, as in the glomerulosa. The sinusoids appear to be thinner and more irregular and not as extended in appearance, as in the zona glomerulosa. These narrow spaces which border the cell cords and extend parallel to them, sometimes join the sinusoids of the zona reticularis. There are again red blood cells scattered in small groups within the sinusoids as well as rod-like fibrocyte nuclei, which are strongly basophilic, appearing individually throughout the zone. Thin trabeculae running between the cell cord appear randomly in different regions. Cells are both undifferentiated and polyhedral with most exhibiting a cuboidal shape. Many cells appear to be large and swollen, thereby making the cytoplasm appear faintly basophilic. The cytoplasm retained a homogenous appearance at all levels of the zone, but near the junction with the zona glomerulosa, the cytoplasm of cells are more vacuolated giving this outer region a low affinity for staining. Most cells appear to have a centrally located nucleus which is circular, but appear vesicular due to the

chromatin being widely dispersed. The nuclei in each cell contain three to four nucleoli. Cell density appears to slightly increase toward the medulla, where the cell nuclei appear to be more compacted together and the cell cords are less distinguishable as well.

iv) Zona reticularis

There is no distinct demarcation between zona fasciculata and zona reticularis. The zona reticularis appears to be the second largest in thickness and lacks the rigid organization of the previous two zones of the cortex. The main structure of cells are not arranged in distinct cords but consists of irregular anastomosing cords of cells and a prominent meshwork of blood sinusoids. This meshwork structure appears oriented at a right angle to the cords in the fasciculata. The cells themselves are similar in shape, but are smaller and more compressed than those found in the zona fasciculata. The cell nuclei are central and some are highly eosinophilic, while the surrounding cytoplasm is less vacuolated. Dark and light staining cell nuclei are found scattered (Fig.6b). The nuclei are circular, and contain one or two nucleoli. Red blood cell clusters are scattered throughout the zone but tend to be more numerous in deeper areas. The zona reticularis region adjunct to the adrenal medulla is more compact, forming a ring-like structure or pseudo-capsule. Otherwise, no distinct boundary between the cortex and medulla was observed. Some specimens had thin strands of connective tissue on the boundary with the medulla in scattered areas, which never completely encircled the medulla. These regions contained spindle

shaped fibrocyte nuclei.

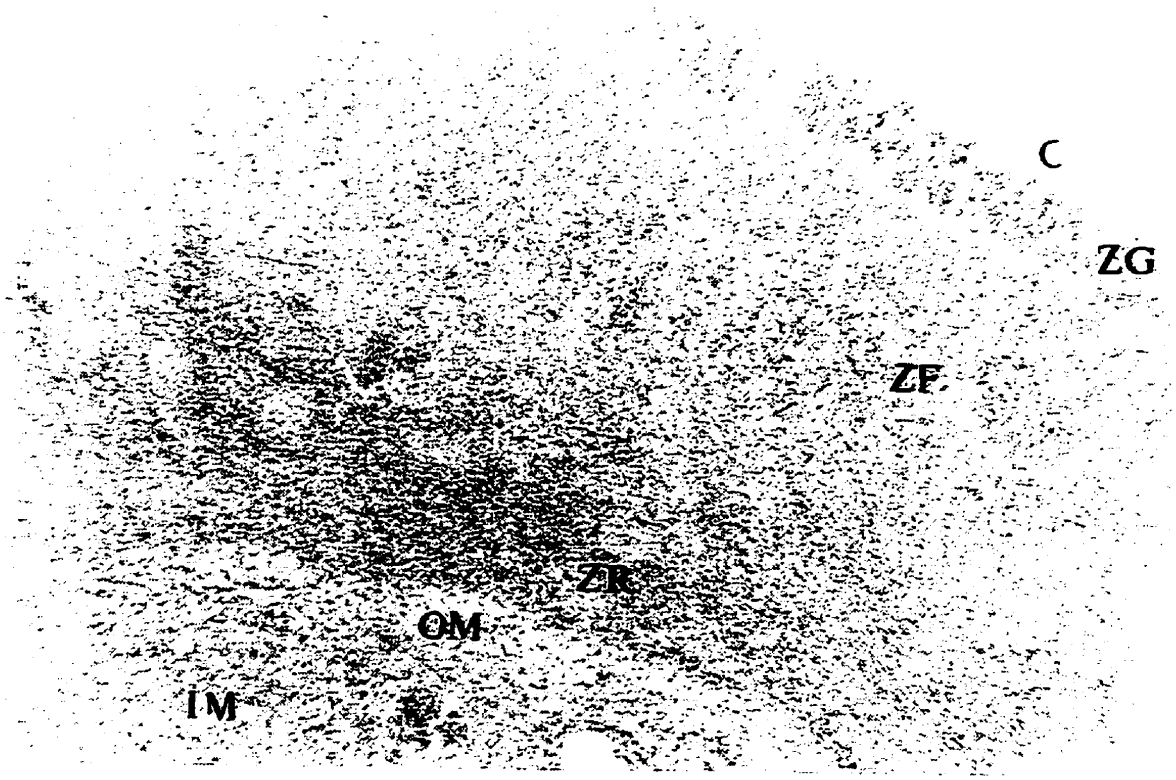
v) **Medulla**

The adrenal medulla appears to be clearly demarcated from the cortex with a distinct boundary. The medulla itself can be divided into two clearly defined zones, i.e. an outer and an inner layer (Fig.7a). The outer region completely encapsulates the inner layer and consists of cells which have highly vacuolated cytoplasms and are large and widely spaced. The cells vary in shape from low columnar to cuboidal, and form acini structures. The cell nuclei are larger than those which can be found in the cortex or inner medulla, and contain one or two nucleoli. The inner medulla contain undifferentiated cells, which possess a single highly compact nuclei, with a variable one to four nucleoli. Some areas in the inner medulla are highly compact with little cytoplasm, while other areas are highly vacuolated, and therefore contain relatively low cell densities when compared with the outer medulla.

Figure 5 a. Cross-section of red hartebeest adrenal with the capsule (C), distinct zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR), and outer (OM) and inner medulla (IM). Magnification 25 x

b. Red hartebeest zona glomerulosa with tightly packed cords of cells, with large oval nuclei. Magnification 250 x

A.



B.

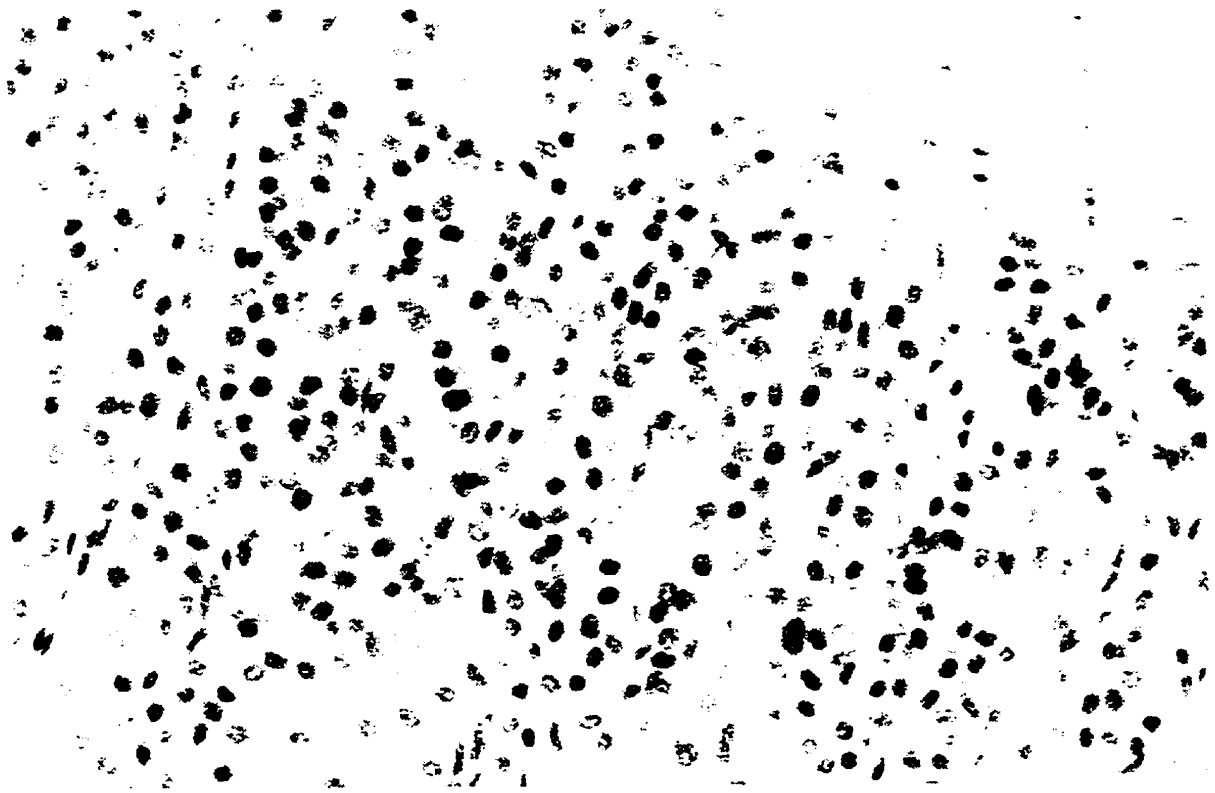


Figure 6 a. Red hartebeest zona fasciculata has cuboidal cells, with highly vacuolated cytoplasms, forming tightly packed cords.
Magnification 100 x

b. Dark and light staining cell nuclei are found in the zona reticularis. Sinusoids run perpendicular to the fascicular cell cords.
Magnification 250 x

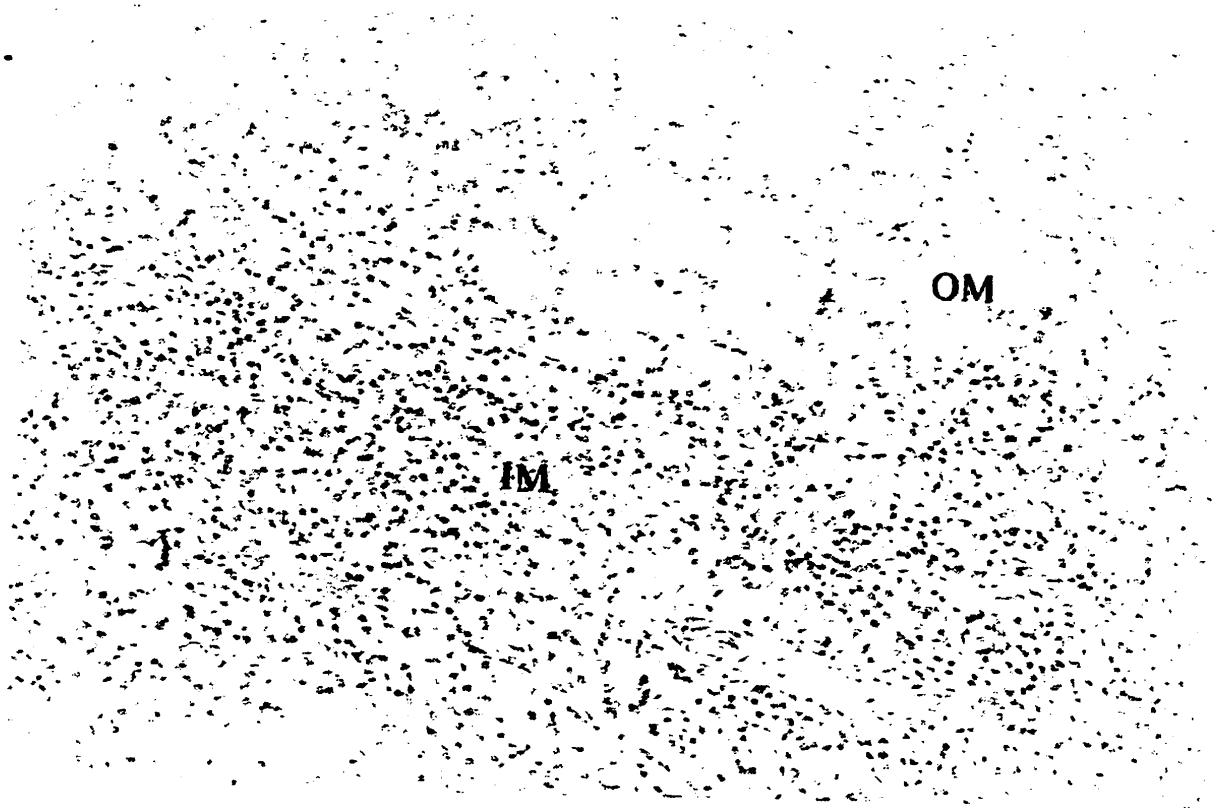
A.

B.

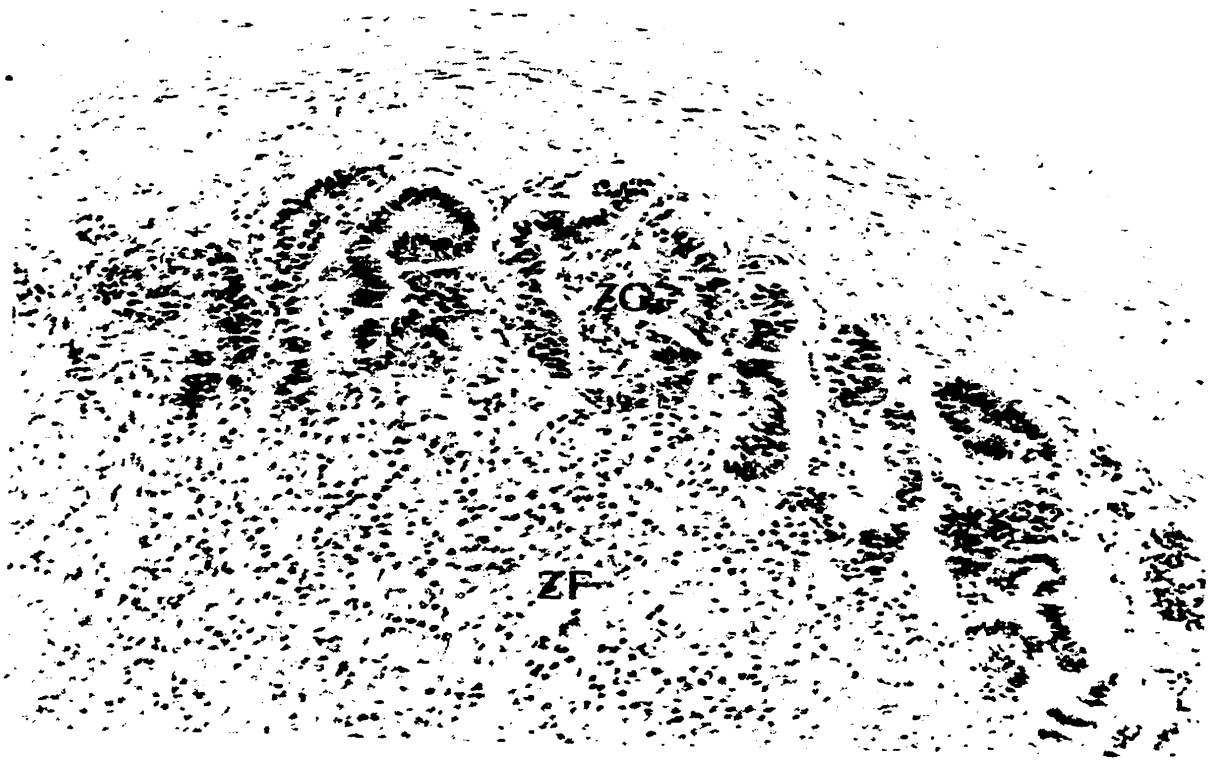
Figure 7 a. In red hartebeest the large cells of the outer medulla (OM) form patchy distributions around the smaller, undifferentiated cells of the inner medulla (IM). Magnification 100 x

b. The thick capsule of the greater kudu is composed of three layers. The zona glomerulosa (ZG) is thin, but distinct with winding cell cords forming arcades at the capsular end of zone, and the zona fasciculata (ZF) underneath. Magnification 100 x

A.



B.



3. Greater kudu

i) Capsule

The thick capsule is composed of three layers (Fig.7b). The outermost layer consists of thick connective tissue strands with reticular fibers and with fibrocytes and compact elongated nuclei. The mid-layer is of areolar connective tissue with large rounded, pale nuclei. The innermost third layer is extremely thin at only a few microns in thickness, and is only one or two cell layers thick. It resembles the outer capsule in that it is reticular fibrous and follows the outline of the outer glomerulosa cell structures.

ii) Zona glomerulosa

A very distinct, but thin glomerulosa, characterizes this species (Fig.7b). The constituent cells are highly columnar and are horizontally stratified to form a single cell wide cord which forms an arcade at the capsular end of the zone. The basement, fascicular end of the cords loose their structure and form undifferentiated aggregates of glomerulosal cells, or retain their cord structure and arch back toward the capsular end, depending on the histological cut. The strongly basophilic cell nuclei demonstrate two forms in their shape. At the capsular end of the cord structures the nuclei are rounded, oval, and toward the fascicular end the nuclei become progressively flattened and cigar-shaped (Fig.8a), with no nucleoli visible throughout. Wide sinusoids separate the arcade structures. In regions where the sinusoids are thinner, trabeculae from the fibrous third layer of the capsule penetrates parallel the

cords down to the fascicular zone (Fig.8a). Inside the arcade structures the area may be penetrated by groups of fascicular cells or islets of glomerulosal cells.

iii) Zona fasciculata

The cells in the outer region of the fasciculata are tightly packed and are composed of mostly undifferentiated cells with small sinuses scattered about randomly. The cell nuclei are large and lighter staining than those in the zona glomerulosa. There is some evidence of cell aggregations being oriented radially toward the medulla. In the inner, larger region of the zone, more distinct, short cords are formed which are oriented towards the medulla. The cells are more cuboidal and form single cell width columns that break-up intermittently (Fig.8b). Longer sinusoids accompany the cords, but remain thin due to the cell density. The cell cytoplasms were observed to be fairly uniform in size throughout the entire zone, and mostly one nucleolus was present.

iv) Zona reticularis

The demarcation of the start of the zona reticularis is extremely difficult to observe as there is a gradual fragmentation of the fascicular cord structure closer to the medulla. The cells are undifferentiated and formed a tightly anastomosing meshwork structure (Fig.9a). Cytoplasms are comparatively smaller due to the high density of cells. The cell nuclei is found in two forms: darker, more basophilic forms which are oval, and a lighter stained nuclei which are more rounded and possess two

or three nucleoli. Fibrocytes are found to be randomly scattered throughout the zone, as are the sinusoids which are short and rounded in appearance.

v) **Medulla**

A clear demarcation is found to separate the cortex from the medulla in the form of a pseudo-capsule. This boundary appears to be formed of a variable thickness of connective tissue resembling the innermost cortex capsular layer, and is incomplete. In many areas the reticularis borders the medulla directly with no evidence of any reticular fibers and their fibrocytes. Both an outer and inner medulla was found with the outer region consisting of mostly cuboidal cells, with highly vacoulated cytoplasms. These cells form either cord-like structures which run inward toward the direction of the medulla center, or acini formations (Fig.9b). In the case of the cords, the cells are paired with their nuclei paired centrally. The cell nuclei form aggregates where acini are observed. The inner medulla resembles the reticularis in structure and is composed mostly of dark staining nuclei, and much smaller cell sizes. This region is completely encapsulated by the outer medulla. Fibrocytes are scattered throughout this region, and to greater extent than in the outer medulla.

Figure 8 a. The third capsule of the greater kudu, penetrates as trabeculae (T) between the cord structures. At the capsular end the glomerulosal cells form an arch where cell nuclei are oval, and then become flattened at the zona fasciculata border. Magnification 250 x

b. The zona fasciculata of the greater kudu has short cords that fragment intermittently . Higher cell densities in the zone give rise to thin sinusoids. Magnification 100 x

A.

B.

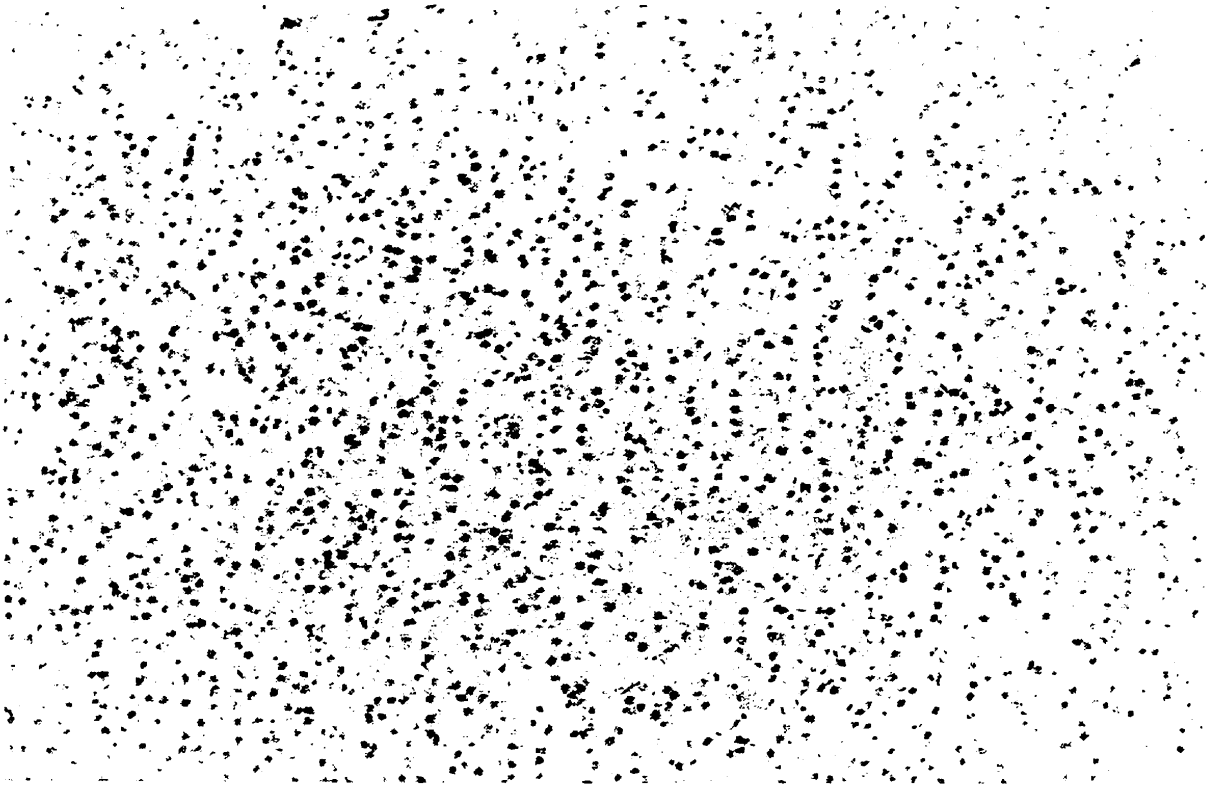
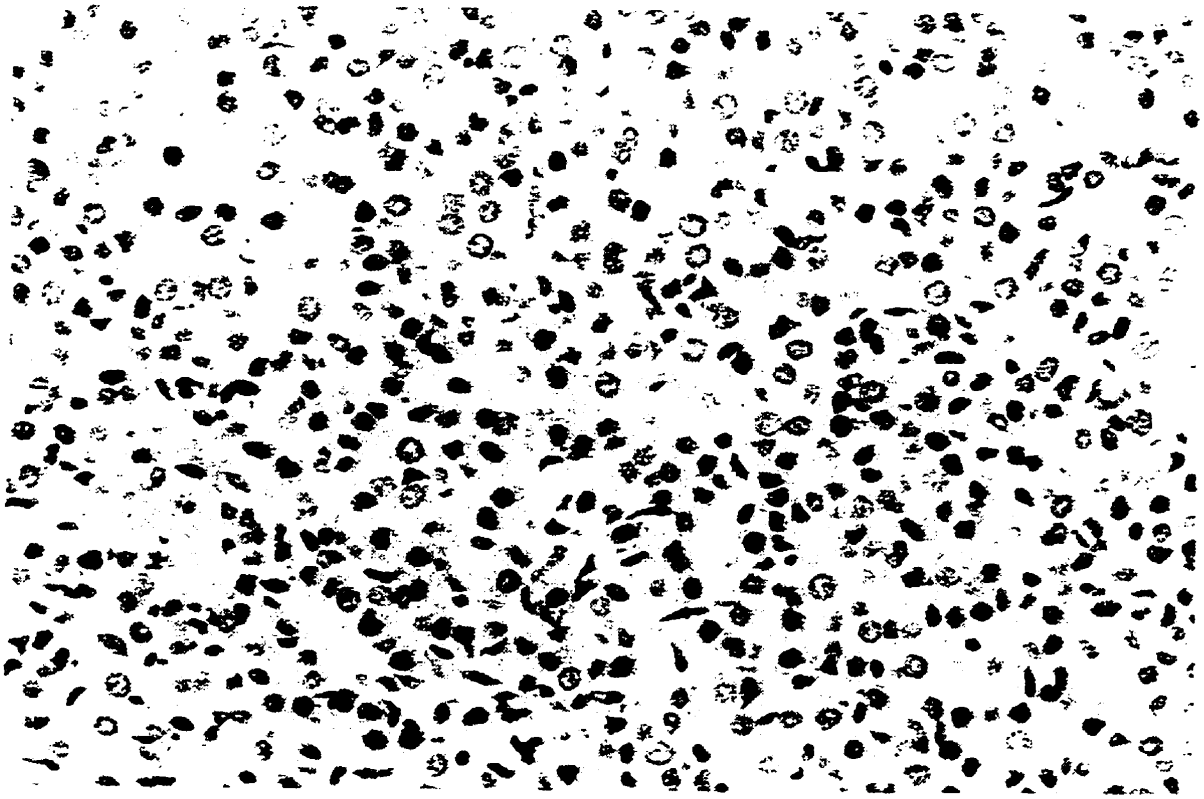


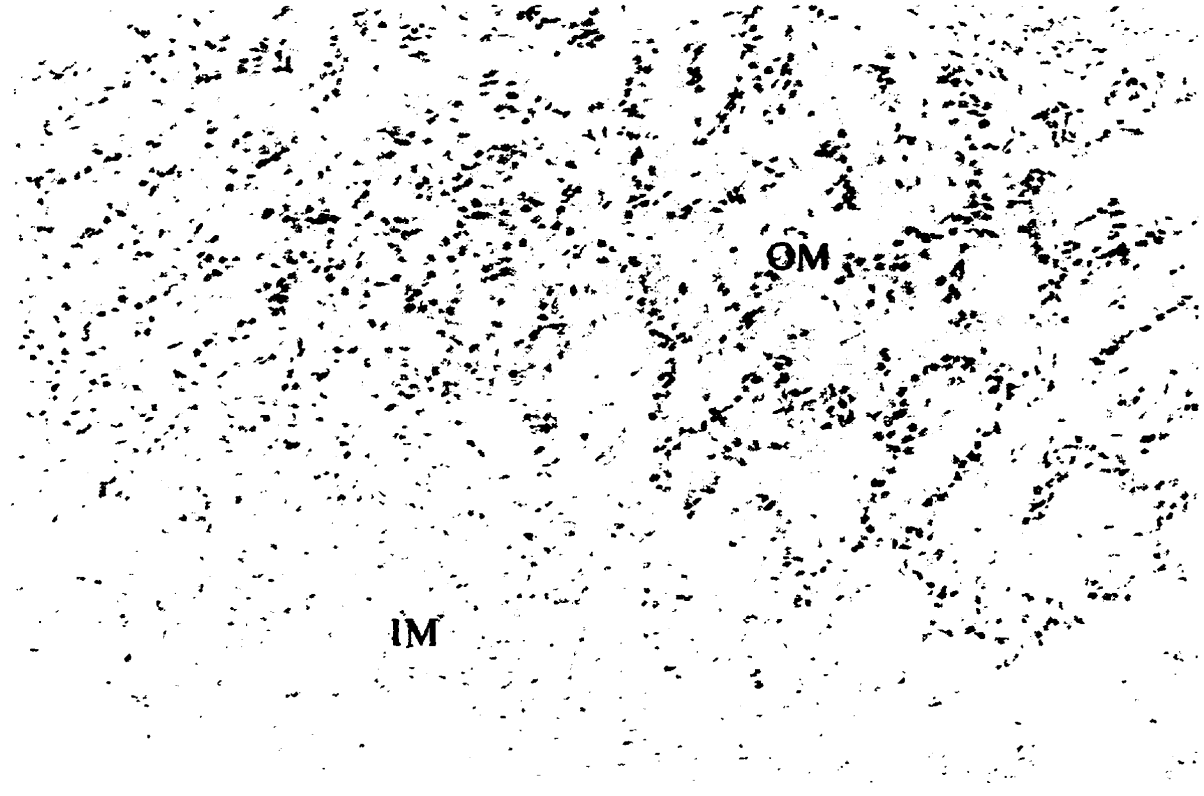
Figure 9 a. The zona reticularis of the greater kudu is characterised by the anastomosing network of cells. Both light and dark staining nuclei can be observed in this region of the cortex. Magnification 250 x

b. The outer medulla (OM) of the greater kudu forms either cords or acini formations. They are composed of large cytoplasm-rich cells, while the inner medulla (IM) consists of smaller cells with less basophilic nuclei. Magnification 100 x

A.



B.



4. Gemsbok

i) Capsule

The connective tissue capsule can be divided into two distinct layers: a thin layer made of reticular fibres and an inner larger layer of areolar connective tissue. Randomly placed, a third reticular fibrous layer is found to border the zona glomerulosa in some areas. The species is characterised by trabeculae formed of the areolar connective tissue penetrating into the zona glomerulosa (Fig.10a). In some cases the a thin reticular fibre layer accompanies it. Also, many samples studied exhibited large capsular "spikes" or trabeculae which were over 100 μm in thickness penetrate deep into the zona fasciculata, for hundreds of micrometers (Fig.11a). These enormous trabeculae were mainly composed of capsular reticular fibres.

ii) Zona glomerulosa

A well-defined glomerulosa was detected throughout all samples, but in many the outer region detached from the capsule, forming arcades of sinusoids in between the two layers. The zone is made of stratified low columnar cells. The cells are mostly found in pairs and form a narrow cord which run radially towards the medulla. These cords are frequently fragmented and form acini structures at the fascicular end of the zone (Fig.10b). Wide sinusoids are found separating many of the cords and fascicular cells penetrate into the zona glomerulosa in many areas. In many areas the cords are interconnected and form network-like structures running toward the center of the gland. The cell nuclei are large and rounded and fairly basophilic, with one or two

nucleoli usually observable.

iii) Zona fasciculata

The outer fasciculata is characterised by cells which are dark staining, due mostly to the cytoplasm. The cells form single cell width cords which are fragmented and compressed. The cells themselves are cuboidal, and only one nucleolus can be seen in some cells. In some areas the sinusoids are readily visible and clearly separate the cords. The inner fasciculata appears less compact due to the cells highly vacuolated cytoplasms. The nuclei are less basophilic and have multiple nucleoli visible. The cords are more readily visible and remain intact for longer distances and consequently the sinusoids are longer and wider (Fig.11b).

iv) Zona reticularis

The zona fasciculata border with the zona reticularis is difficult to observe. The cells lose the intact cord structures but retain the cuboidal shapes. The cell nuclei appear to be large and rounded with one or two prominent nucleoli. There are cells scattered randomly which have highly vacuolated cytoplasms. Fibrocytes are found in the short , rounded sinusoids (Fig.12a).

v) Medulla

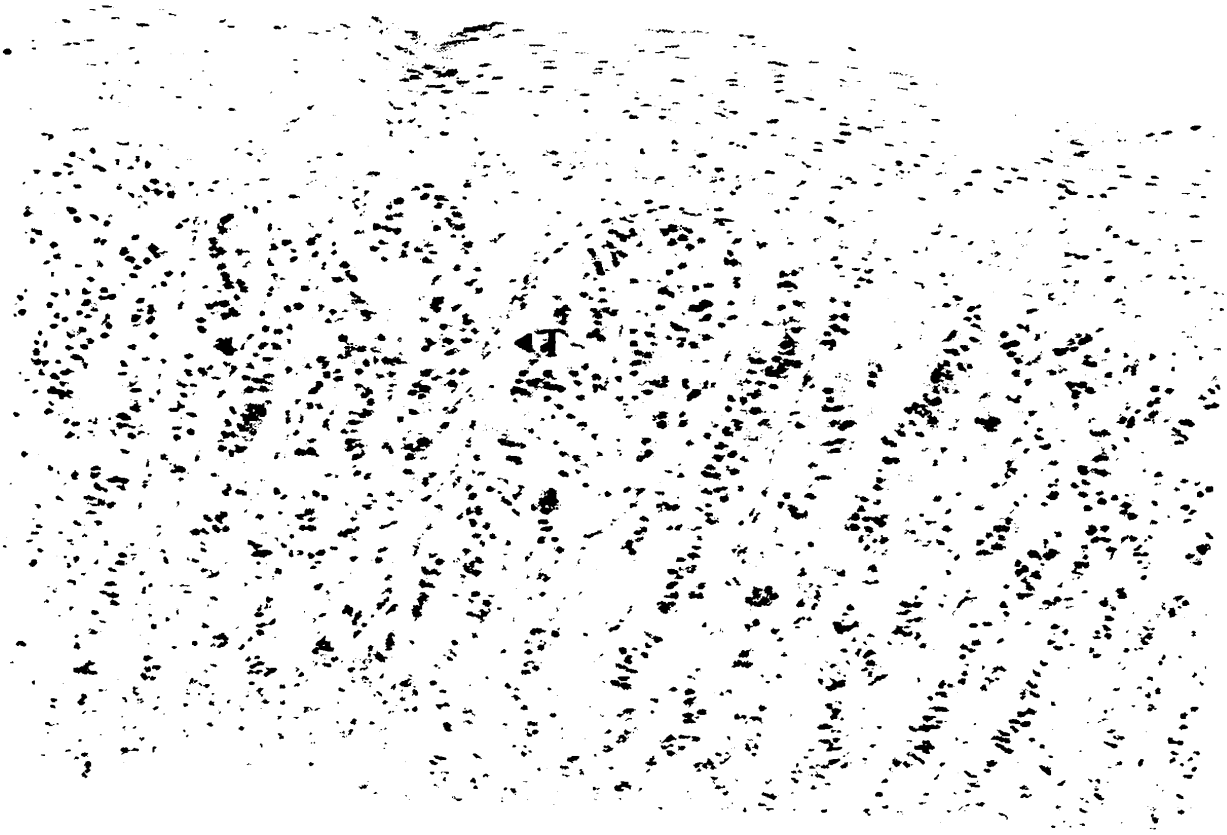
A small, incomplete reticular fibrous medullary capsule appears in different areas of the boundary between the cortex and medulla and runs parallel with the

cortex capsule. Both an outer and inner medulla is present. The outer medulla is arranged as stratified columnar with these cord-like structure fragmenting occasionally. Across this region, cord structures interchange with acini structures. The cell nuclei are found in the extreme outer or inner edge of the cells. The cord structures appear in some areas as two cell width and one in others (Fig.12b). The nuclei are dark staining and no nucleoli can be detected. The inner medulla cells are undifferentiated and are much smaller than those of the outer medulla (Fig.12b). There is a mixture of dark and light staining nuclei with only a few scattered nucleoli visible. Only small sinusoids are present throughout, while large blood vessels are scattered throughout the inner medulla.

Figure 10 a. The gemsbok capsule is composed of three layers. Trabeculae (T) formed from the secondary areolar capsular tissue penetrate between the cell cords of the zona glomerulosa. Magnification 100 x

b. A magnified view of the zona glomerulosa of the gemsbok reveals stratified low columnar cells, that are found in pairs forming narrow cords which fragment and form acini structures at the fascicular border. Magnification 250 x

A.



B.

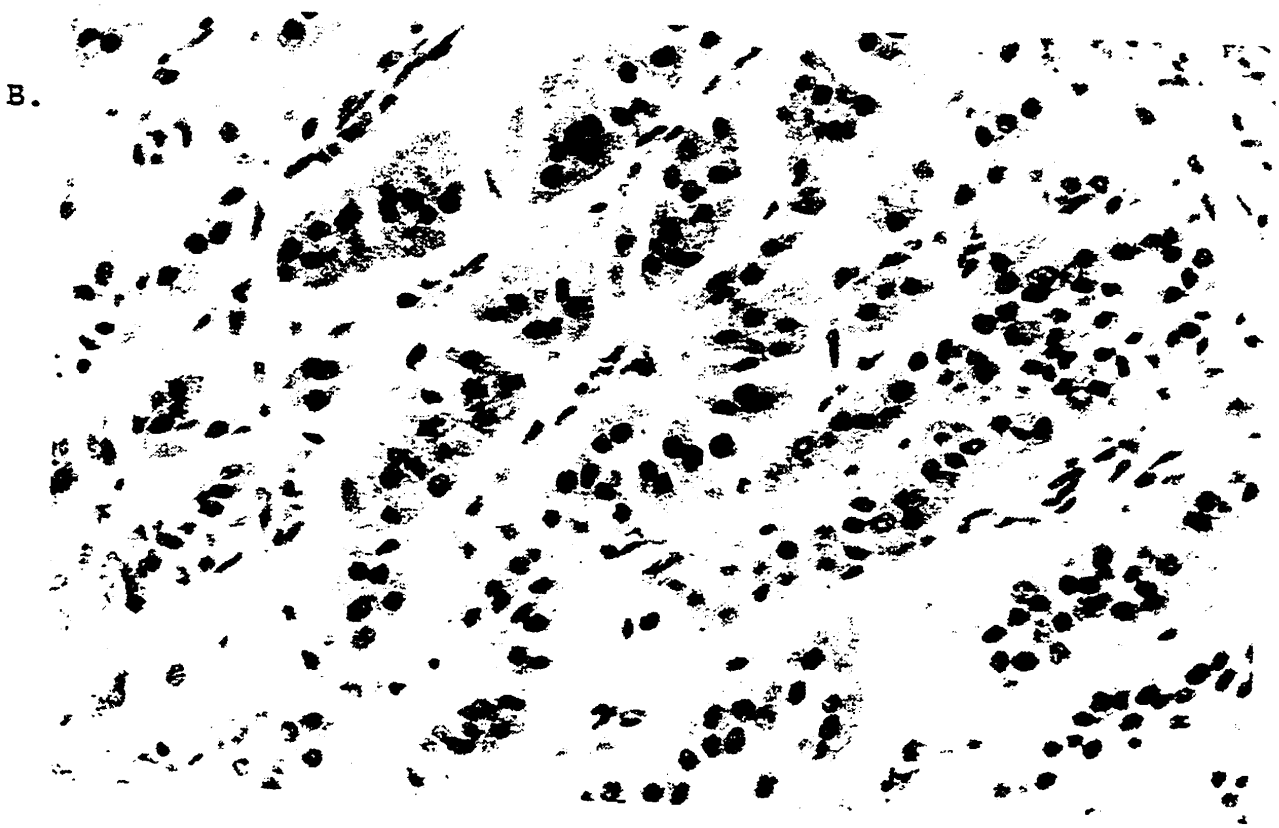
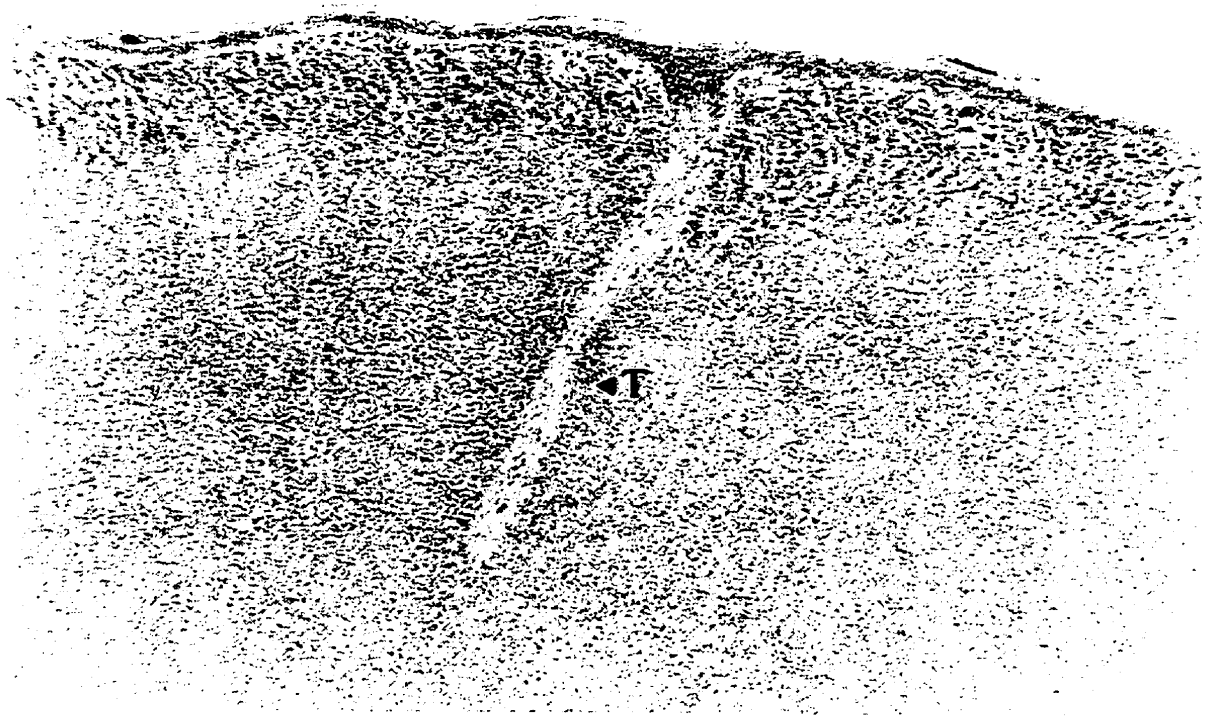


Figure 11 a. Large trabeculae from the capsule of the gemsbok, penetrate deep into the zona fasciculata. Magnification 25 x

b. The gemsbok inner zona fasciculata consists of single cuboidal cell wide cords which run intact for long distances, accompanied by wide sinusoids. Magnification 100 x

A.

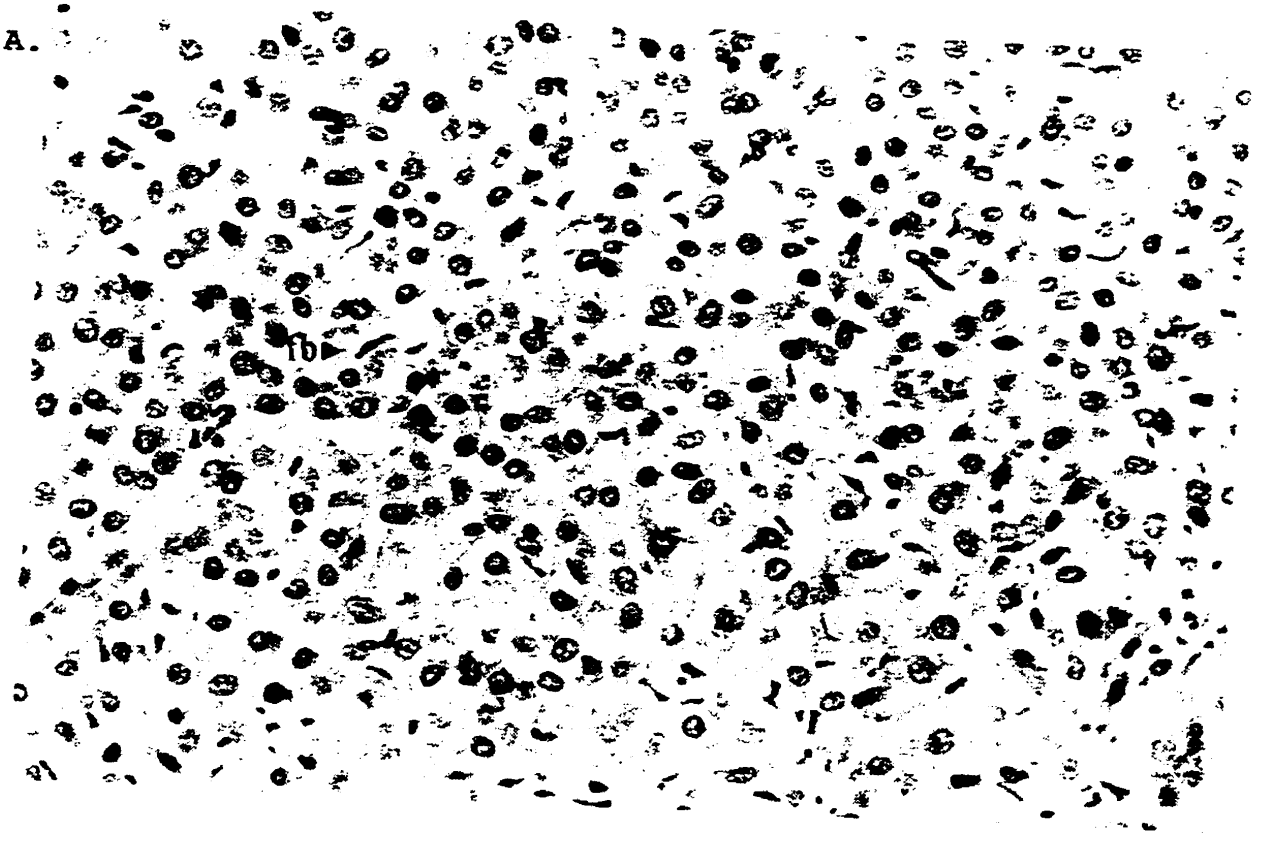


B.

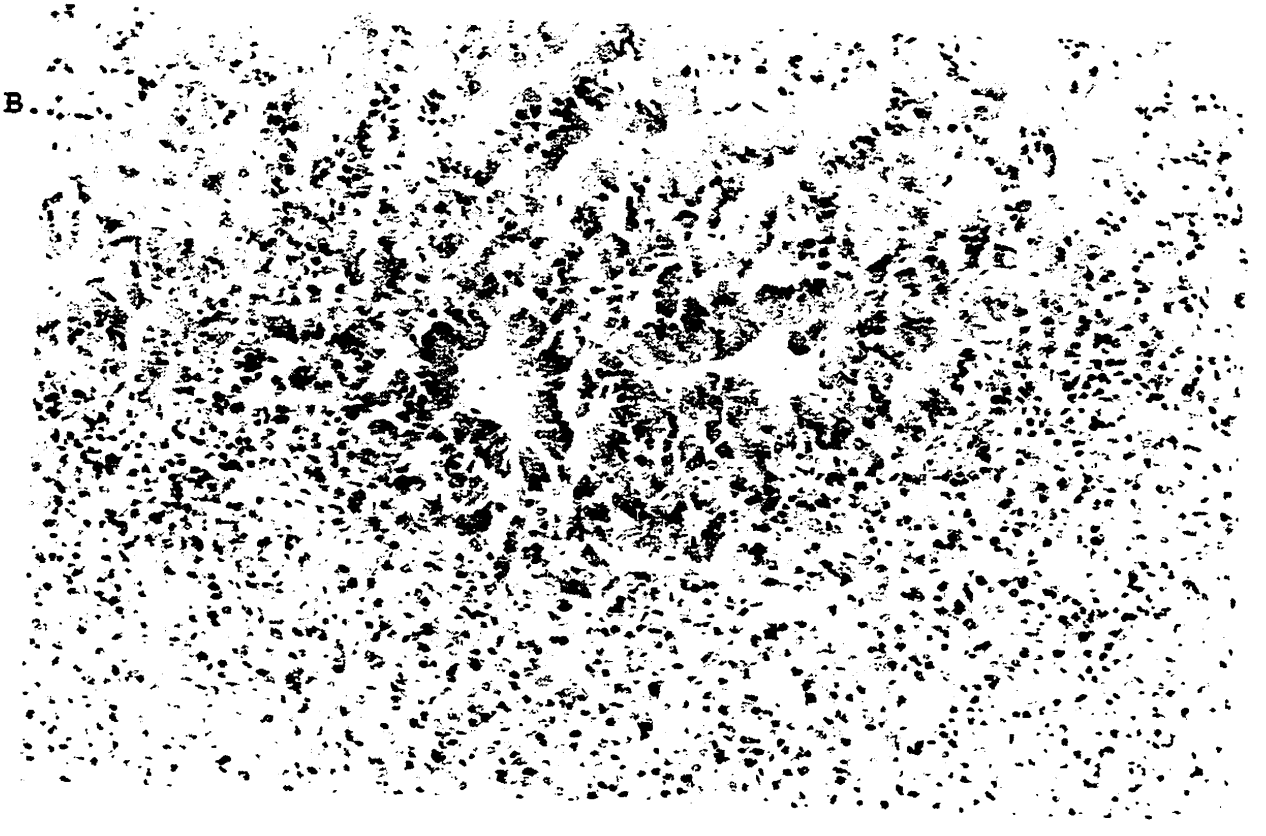
Figure 12 a. Gemsbok zona reticularis is characterised by large cell nuclei, with one or two nucleoli. The cells are randomly scattered and are highly vacuolated with fibrocytes (fb) visible inside the sinusoids. Magnification 250 x

b. The outer medulla of the gemsbok is in stratified columnar arrangement, while the inner medulla cells are undifferentiated and considerably smaller. Magnification 100 x

A.



B.



5. Cape eland

i) Capsule

A wide capsule with three layers of similar thicknesses can be detected for this species (Fig.13a). The outermost layer is thin and is of reticular fibres. The second layer is the widest and is made of areolar connective tissue. The cell nuclei are oval, and there are only a few scattered round nuclei present. The third layer is made of thin, mostly one or two cells thick, reticular fibres, which follow the contour of the zona glomerulosa beneath it. The species in general exhibits many forms of penetrating capsular tissue. Large capsular trabeculae or "spikes" are found to invade the cortex to varying depths. Some have been found to travel as far down as the zona reticularis (Fig.14a). Some trabeculae enter the cortex, deep into the zona fasciculata and circle back out to the capsule (Fig.14b). In all cases these unusual formations carry with them the zona glomerulosa region, intact.

ii) Zona glomerulosa

A clearly defined zona glomerulosa is observed throughout all samples. The layer is composed of horizontally stratified columnar cells (Fig.13a). These cells are arranged in well defined cords which run radially to the capsule. The cords are found to be either one or two cells in width, depending on the sections studied. In either case the nuclei are oriented toward the median of these structures, and can be observed all the way along the length of the cords. The cords tend to fragment heavily into small islets of glomerulosal cells at the fascicular end of the zone. At the

capsular end, where the cords are two or more cells wide, there is an arch or arcade formation. Large, clearly defined sinusoids separate the cords and arches around the capsular end, isolating the glomerulosa from the capsule. Many instances of thin reticular fibrous trabeculae travel within the sinusoids, beside the cords. The islets at the basement of the zone are in many cases totally encapsulated by these fibres. As mentioned in the previous section, with the penetration of the capsule through the cortex, the glomerulosa zone is seen to be attached to the trabeculae and retains their normal structure (Fig.14a). In the case of the circular trabeculae the glomerulosa cells appear on both sides of the fibrous penetration (Fig.14b).

iii) Zona fasciculata

The cell arrangement is uniform throughout without any signs of an outer and inner region. The cells are cuboidal in shape and are arranged in cords which run radially to the capsule. The cords are interconnecting and short due to frequent fragmentation (Fig.13b). They are separated by sinusoids which contain very few fibrocytes. Both dark and light staining nuclei are seen with the latter having one nucleolus. The cell cytoplasms do not show any significant signs of vacuolation.

iv) Zona reticularis

The zona reticularis appears to be indistinct and is difficult to observe the border with the zona fasciculata. The cells are arranged in a compact, broken network of cells. Sinusoids are short and run perpendicular to the fasciculata cords.

Both light and dark staining cells are visible with the former having one or two nucleoli. Cell forms appear to be undifferentiated and the cell density increased due to larger cytoplasm and sinsusoids (Fig.15a). Towards the medullary end of the zone reticular fibres appear to be directly abutted to the reticularis zone, and these areas appear to be infiltrated by pockets of erythrocytes.

v) Medulla

As mentioned in the above section the medulla appears to be encapsulated by a thin fibrous layer which is approximately two to three cell layers thick (Fig.15a). Very strong basophilic staining fibrocytes are evident within the fibrous tissue. The medulla itself is divided into an outer and inner medulla. The outer medulla is composed mainly of undifferentiated and cuboidal cells which form acini structures. The cells are very large and have a fairly dark staining cytoplasm. Due to the acini structures the nuclei form aggregates at the centre. This outer medulla completely encircles the inner medulla, occurring at variable thicknesses. The larger inner region has comparatively much smaller cells, due to the compact cytoplasms. In some samples, within the inner medulla, intact glomerulosa cords were observed centering around the central vein of the adrenal gland, and islets were found scattered about (Fig.15b). Small pockets of reticular fibres were also scattered within the inner medulla.

Figure 13 a. The eland capsule is of three layers. The third fibrous layer follows the contour and penetrates as trabeculae into the zona glomerulosa. The zona glomerulosa is composed of horizontally stratified columnar cells. These cells form distinct cords, one or two cells in width. Islets (Is) of glomerulosal cells are formed at the base of the zone. Magnification 100 x

b. Short interconnecting cords of cuboidal cells characterize the zona fasciculata of the eland. Magnification 100 x

A.

B.

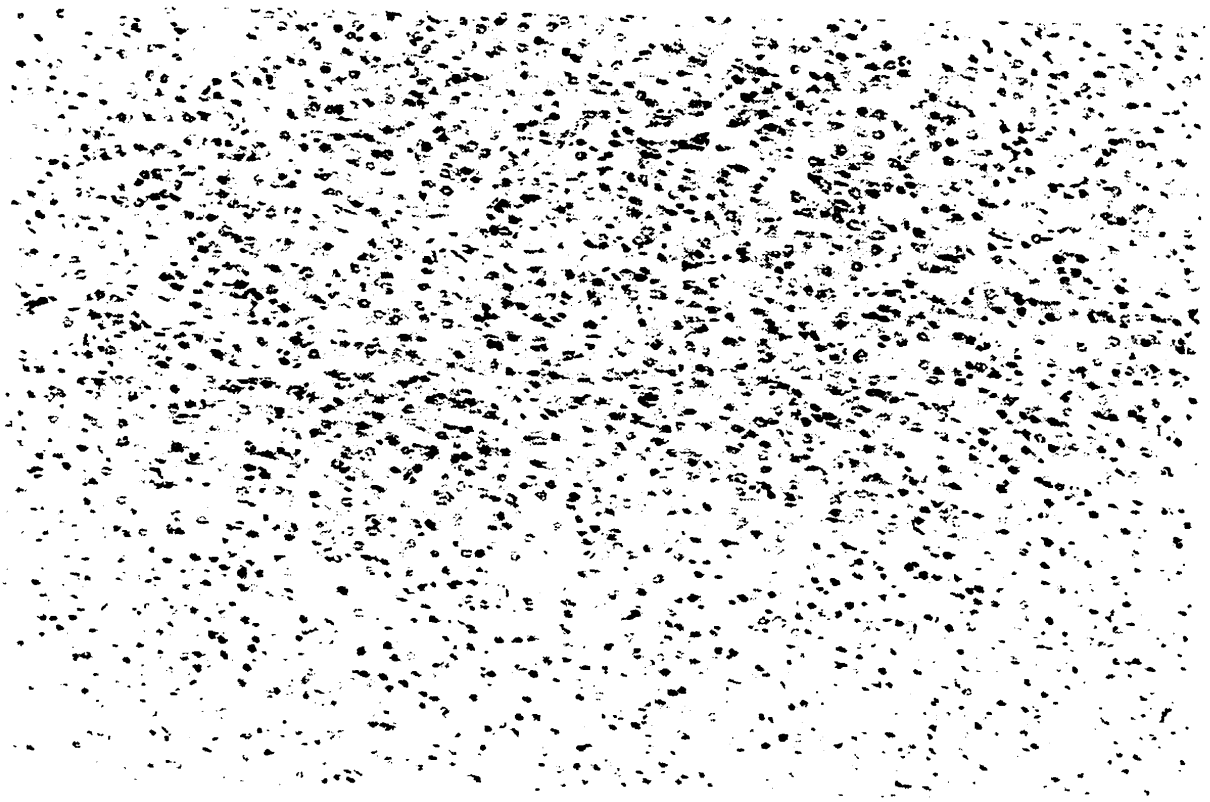
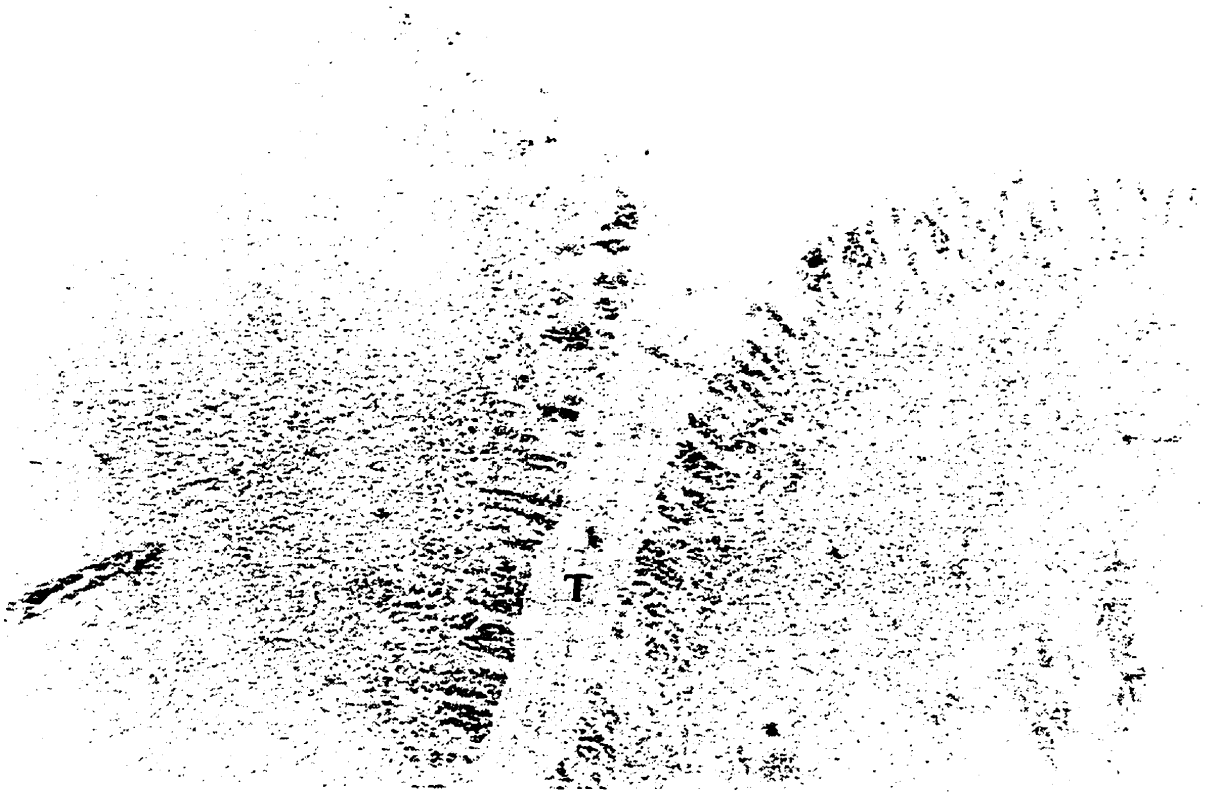


Figure 14 a. Large capsular trabeculae are found to penetrate deep into the adrenal cortex of eland, and often the zona glomeulosa is attached and carried intact with it. Magnification 25 x

b. Circular, large trabecule, composed of capsular tissues, with the zona glomerulosa, found deep inside the zona fasciculata of eland. Magnification 25 x

A.



B.

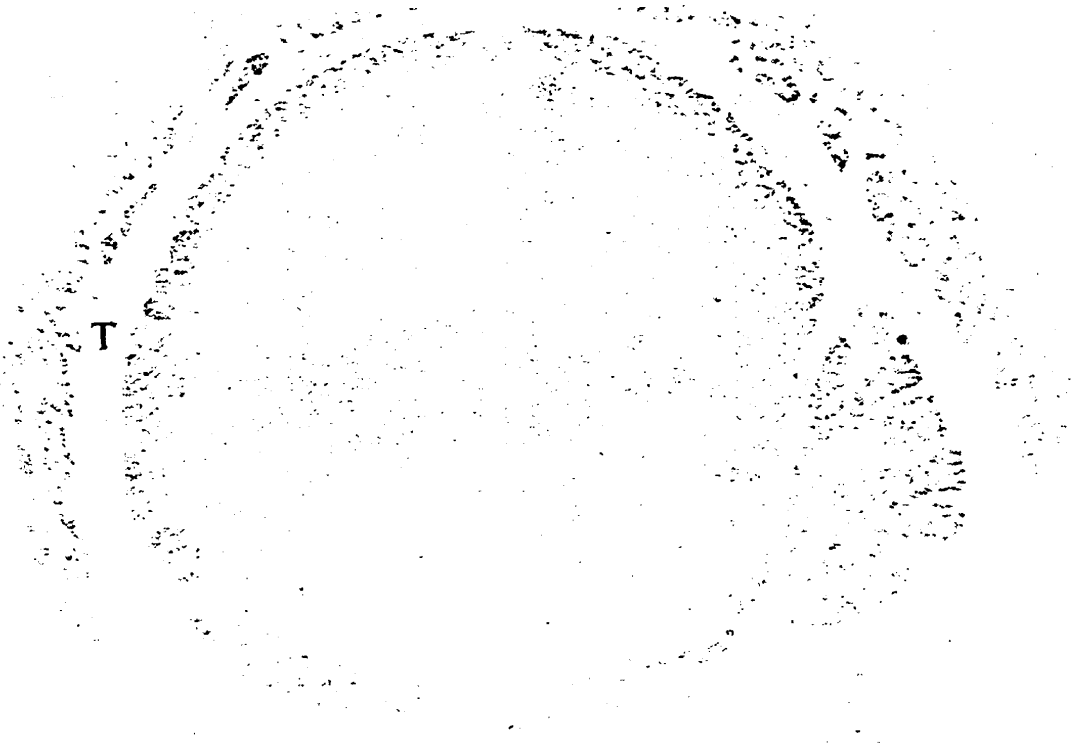
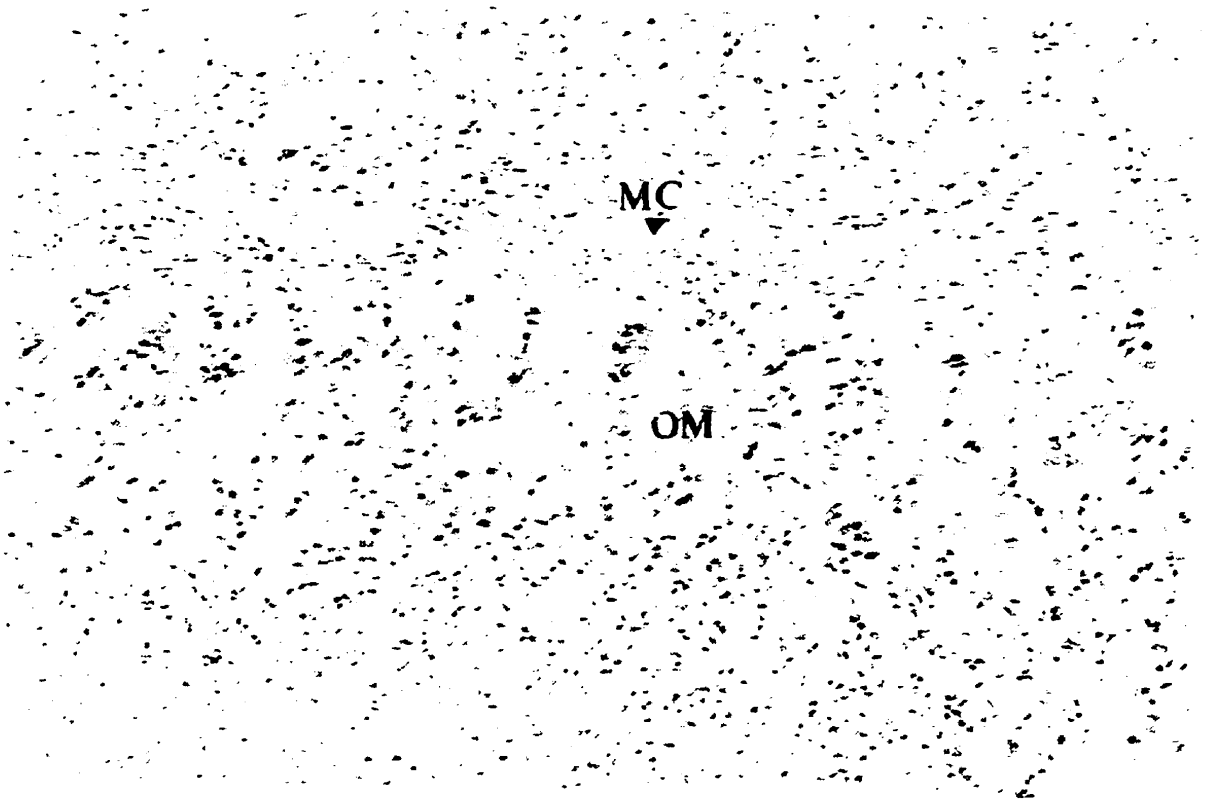


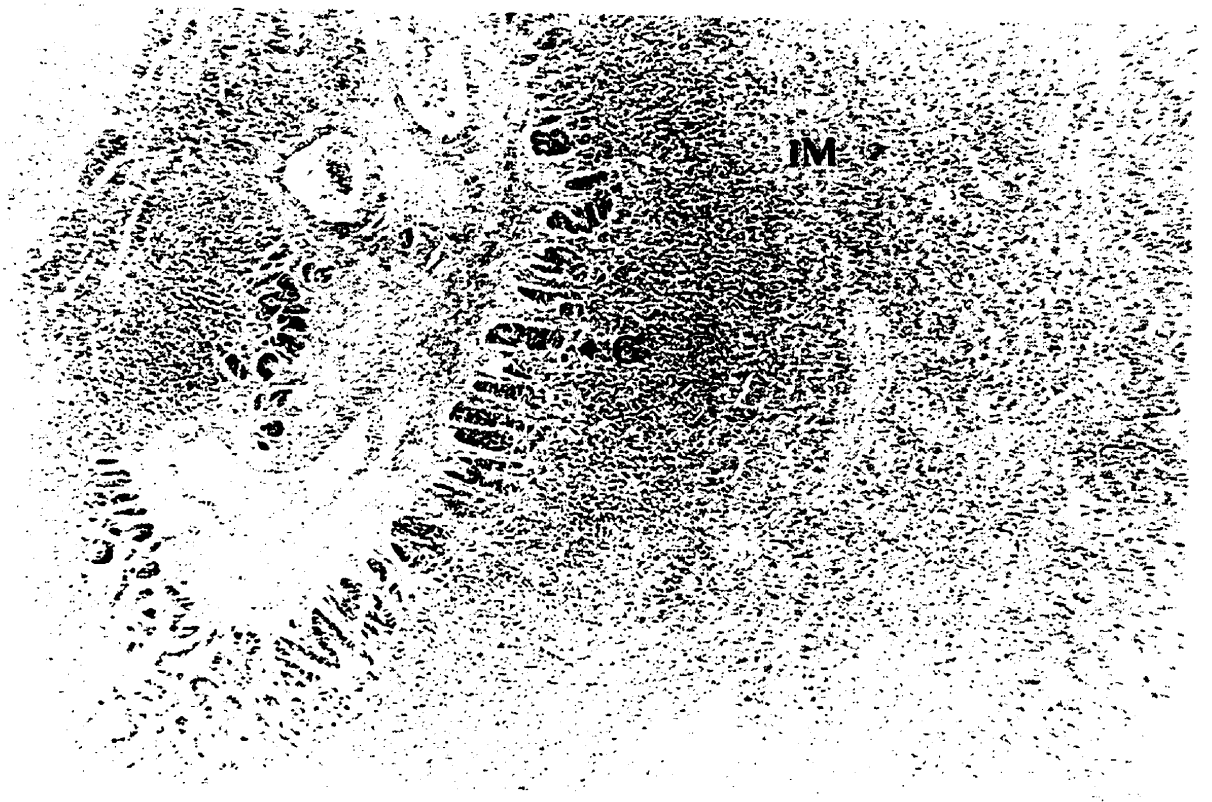
Figure 15 a. The zona reticularis of the eland appears indistinct, and is made of undifferentiated cells. The outer medulla (OM) is encapsulated by a medullary capsule (MC) of reticular fibers. Magnification 100 x

b. Within the inner medulla (IM) of the eland, intact glomerulosal cell cords (G) and islets are found around the central adrenal vein. Magnification 25 x

A.



B.



C. Quantitative histology

The quantitative histological measurements and simple statistical analysis for each species are reported in Appendices 1 through 5. The results for each species are summarized in Table 1, including univariate statistical analysis using the Bonferroni correction.

1. Left adrenal weights

The left adrenal weight of the eland was significantly higher ($P < 0.005$) than those of all the other species. The left adrenal weights of the gemsbok and greater kudu were not significantly different ($P < 0.005$), but both were significantly heavier than the red hartebeest and springbok, with the red hartebeest adrenal being significantly heavier ($P < 0.005$) than the springbok adrenal. The adrenal weight was positively correlated ($r = 0.981$) with the size of the animals, (body weights taken from Skinner and Smithers 1990). There was approximately eleven times difference in adrenal weights between the largest species studied, the eland, and the smallest, the springbok.

2. Capsule

The measurements of the capsule surrounding the cortex (Table 1) are fairly uniform throughout the species. No significant differences ($P < 0.005$) in thicknesses were observed between red hartebeest, gemsbok, and eland. The springbok and kudu capsule mean thicknesses were found to be significantly thinner ($P < 0.005$) compared

to the rest of the species, while expressing no difference between themselves.

3. Zona glomerulosa

No significant differences were found between the springbok, red hartebeest, and kudu. The zona glomerulosa of the eland and gemsbok were significantly wider ($P < 0.005$) compared to the springbok, red hartebeest and kudu. The cell densities were significantly lower for the red hartebeest ($P < 0.005$), when compared to the gemsbok and greater kudu. The eland glomerulosal cell densities were also significantly lower ($P < 0.005$) than those observed in the greater kudu (Table 1).

4. Zonae fasciculata and reticularis (combined measurements)

The combined zonae thicknesses of the gemsbok and eland were significantly higher ($P < 0.005$) than the springbok and red hartebeest. The greater kudu zonae were significantly thicker than those of the springbok. The cell densities of these two zones were measured separately at their approximate centers. The zona fasciculata density of the greater kudu was significantly higher ($P < 0.005$) from the gemsbok, red hartebeest, and springbok, while for the zona reticularis both the greater kudu and red hartebeest had significantly higher ($P < 0.005$) cell densities compared to the eland. The ratio of zona fasciculata and reticularis to zona glomerulosa was the smallest for the springbok and largest for the greater kudu (Table 1).

5. Multivariate analysis

Multivariate analysis of variance, using the morphological and histological variables of adrenal weight, all cortex measurements and densities, indicated significant differences among species groups centroids (Wilks' $\lambda = 0.021$; *d.f.* = 24; $P < 0.001$). The test for homogeneity of the covariance matrices indicated that there was a rejection of the null hypothesis of multivariate homoscedacicity ($\chi^2 = 228.37$; $P < 0.0001$).

Stepwise discriminant analysis selected all variables except the one, zonae fasciculata and reticularis combined values, as variables which contributed to the separation of the species groups, with 100% correct assignment (Table 2a) or classification of individuals to species, except for the springbok, where one individual was misclassified as red hartebeest ($P = 0.62$). Using the jack-knife procedure (Table 2b) of utilizing a randomly selected individuals from each species on 50 reps, reclassification was 100 % correct for four species, where (50/50) individual animals were correctly reclassified into their respective species of eland, gemsbok, greater kudu, and red hartebeest. For the springbok, 5 of 50 were misclassified as red hartebeest ($P = 0.10$).

Table 1. Summary of histological adrenal cortex measurements in different species.

Sp.	left adrenal wt. (g)	adrenal cortex measurements in μm^{*}					Cell Density**		
		capsule	z.glom.	z.fasc.+ z.ret.	total cortex	ratio[t]	z.glom.	z.fasc	z.ret.
S	1.2 [A] +/-0.3	115.8 [B] +/-26.5	218.1 [B] +/-40.4	1988.0 [B] +/-379.5	2311.3 +/-422.1	9.12	52.1 [ABC] +/-7.6	27.8 [B] +/-4.9	41.0 [AB] +/-5.8
H	3.0 [B] +/-0.8	158.8 [A] +/-33.9	217.2 [B] +/-35.5	2126.3 [B] +/-418.2	2502.2 +/-395.2	9.79	44.1 [C] +/-1.6	28.3 [B] +/-3.5	47.3 [A] +/-5.5
G	7.2 [C] +/-2.9	176.2 [A] +/-63.1	278.7 [A] +/-37.9	2995.1 [A] +/-569.1	3450.0 +/-568.8	10.75	52.7 [AB] +/-5.5	26.9 [B] +/-1.5	41.1 [AB] +/-4.4
E	12.2 [D] +/-1.8	185.6 [A] +/-28.0	294.4 [A] +/-45.6	3182.5 [A] +/-467.6	3625.8 +/-473.1	10.81	45.8 [BC] +/-3.9	30.7 [AB] +/-2.2	34.8 [B] +/-5.8
K	6.5 [C] +/-3.0	133.4 [B] +/-46.3	216.8 [B] +/-63.1	2643.1 [A] 282.4	2993.3 +/-272.6	12.19	54.5 [A] +/-2.4	34.5 [A] +/-2.6	48.5 [A] +/-9.3

* Each datum is a mean of all observations for a species and corresponding standard deviation

** Each datum is a mean for a species and represents the mean number of cell nuclei per unit area of 0.5625 mm^2

t Ratio of zonae fasciculata and reticularis to zona glomerulosa.

S = springbok , H = red hartebeest , G = gemsbok , E = eland , K = kudu

note: Values with same letters are not significantly different at $P< 0.005$ (Bonferonni correction; $\alpha=0.05/10$).

Table 2a. Discriminant Analysis: Percent Classification into Species.

Spp.	Number of Observations / % Classified into Species*				
	E	G	K	H	S
E	6 / 1.00	-	-	-	-
G	-	8 / 1.00	-	-	-
K	-	-	6 / 1.00	-	-
H	-	-	-	12 / 1.00	-
S	-	-	-	1/0.09	10/0.91

* Analysis carried out on complete data set

\- sign indicates no values given

E = eland , G = gemsbok , K = kudu , H = red hartebeest , S = springbok

Table 2b. Jack-knife classification of discriminant analysis.

Spp.	Number of Repetitions / % Reclassified into Species*				
	E	G	K	H	S
E	50/ 1.00	-	-	-	-
G	-	50/ 1.00	-	-	-
K	-	-	50/ 1.00	-	-
H	-	-	-	50/ 1.00	-
S	-	-	-	5/0.10	45/0.90

* Results of reclassification procedure carried out for 50 reps with each individual chosen by random selection by computer.

E = eland , G = gemsbok , K = kudu , H = red hartebeest , S = springbok

D. Corticosteroid profile

1. Qualitative results

The principal endogenous corticosteroids were examined and the findings summarized in Table 3. The results from the thin-layer chromatography indicate that all individuals from every species had 18-hydroxycorticosterone (18-OH-B) and cortisol (F) present (Appendix 6 to Appendix 10). In the greater kudu and gemsbok the mineralocorticoid, aldosterone (Aldo), was present in all individuals sampled, and in all but one specimen for the springbok group. One of the major glucocorticoids, corticosterone (B), was found present in all individuals of the eland, gemsbok, and kudu species, and again, in all but one of the springbok. Another glucocorticoid, 11-dehydrocorticosterone (A), was found in 10 of 11 specimens of springbok and 9 of 11 red hartebeest examined. There was no evidence of deoxycorticosterone (DOC) detected in any species. There was no 11-deoxycortisol (S) detected in gemsbok and eland, and only in some specimen for springbok and red hartebeest, and seemed only to be found in the greater kudu to any major extent. There was no cortisone (E) detected in the eland, and only in some specimens of springbok and red hartebeest. Only the gemsbok species had cortisone to a major extent with 6 of 8 individuals tested.

2. Quantitative results

The corticosteroid detected in the most significantly high amounts (Table 4) in all species was 18-OH-B, and the red hartebeest sample group had significantly

higher amounts of 18-OH-B than the eland ($P < 0.05$). The ratio of cortisol to corticosterone (F:B) endogenous levels were graphed for each species (Fig.16) and showed relatively no differences between springbok, kudu, and eland. For the red hartebeest there was a higher ratio and the gemsbok had a lower ratio than any other species. In Fig.17, the total corticosteroid content of all the identified steroids was graphed for each species. Total corticosteroid amounts were significantly higher ($P < 0.05$) in the springbok compared to eland. A distinct trend in increasing amounts of total steroid content may be correlated to an increase in body weight class of each species.

Table 3. Major corticosteroids detected in African antelope

Species	18-OHB	F	Aldo	E	B	S	A
Springbok	✓	✓	✓	✓	✓	✓	✓
Red hartebeest	✓	✓	✓	✓	✓	✓	✓
Greater kudu	✓	✓	✓		✓	✓	
Gemsbok	✓	✓	✓	✓	✓		✓
Eland	✓	✓	✓		✓		

note: faint marks indicate steroid detected only in some individuals

Table 4. Summary of endogenous adrenal corticosteroid levels in antelope species.

Species	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
S	6.18 * +/-1.60	3.00 +/-0.67	2.45 +/-1.46	4.50 +/-1.29	2.40 +/-1.15	3.25 +/-0.50	3.70 +/-1.42	-
H	7.45 +/-2.88	2.91 +/-1.30	1.50 +/-0.25	1.17 +/-0.26	1.36 +/-0.77	1.68 +/-0.29	2.01 +/-1.01	-
K	4.45 +/-1.97	3.23 +/-1.66	1.67 +/-0.32	1.63 +/-0.53	2.73 +/-1.40	3.28 +/-1.71	-	-
G	5.38 +/-1.19	1.56 +/-0.56	1.38 +/-0.79	1.32 +/-0.72	1.81 +/-0.65	-	1.40 +/-0.58	-
E	3.48 * +/-1.64	2.38 +/-1.37	1.38 +/-0.25	-	1.85 +/-1.28	-	-	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

S = springbok , H = red hartebeest , K = greater kudu , G = gemsbok , E = eland

* indicates values that are significantly different at $P < 0.05$ level

Figure 16 Bar graph representation of cortisol (F) to corticosterone (B) ratio in five different antelope species.

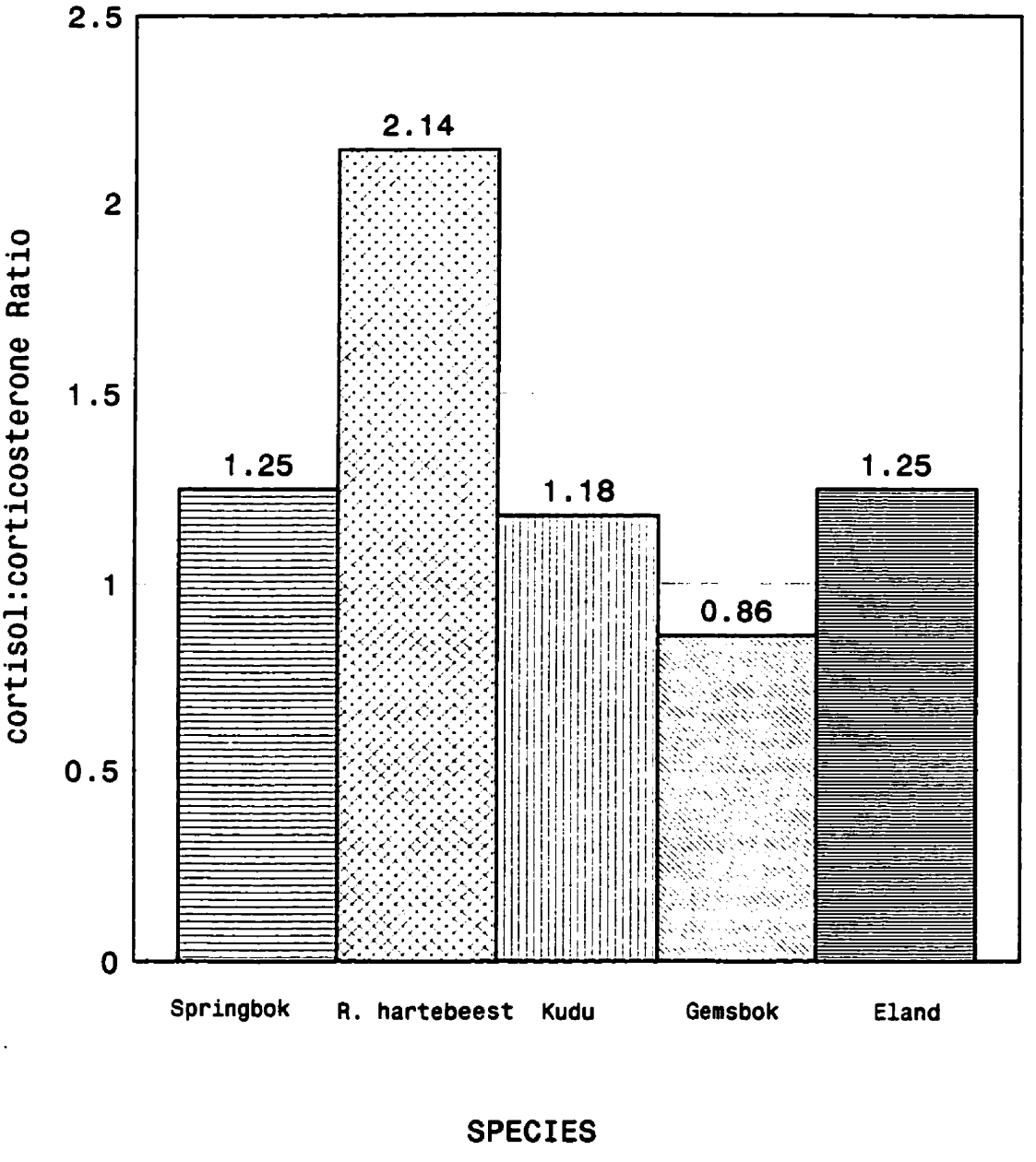
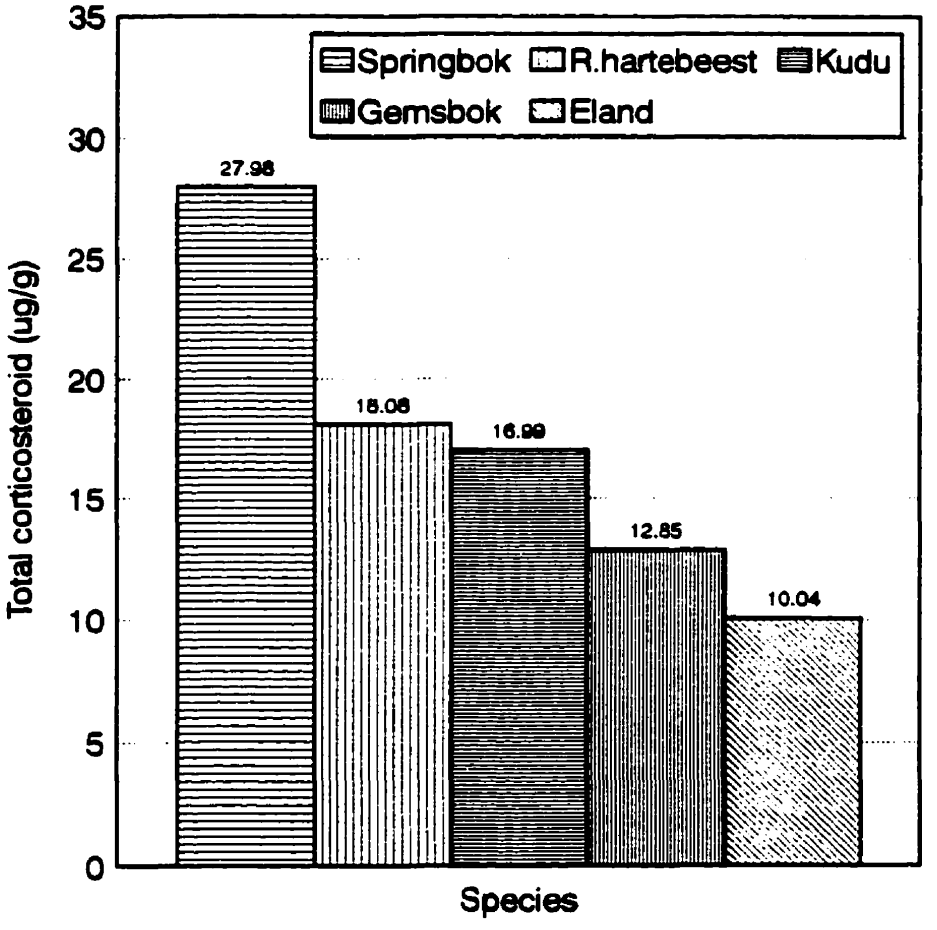

2.5
2
1.5
1
0.5
0
cortisol:corticosterone Ratio
1.25
2.14
1.18
0.86
1.25
Springbok
R. hartebeest
Kudu
Gemsbok
Eland
SPECIES

Figure 17 Bar graph representation of mean values of total identified endogenous corticosteroid content in adrenals in five different antelope species.


Springbok
R.hartebeest
Kudu
Gemsbok
Eland
27.96
18.06
16.99
12.85
10.04
35
30
25
20
15
10
5
0
Total corticosteroid (ug/g)
Species

V. DISCUSSION

A. Gross Anatomy

Previous studies have only mentioned differences between species from diverse taxonomic groups as primates, felines, rodents (Hartman and Brownell 1949, Chester Jones and Henderson 1978). This study indicates that shapes of adrenal glands can vary considerably between species of Bovidae alone. According to past literature (Bourne 1949; Dellman 1993), all members of the bovidae group were assumed to have uniform adrenal gland shapes. In all species the adrenals were dorsoventrally compressed and the apical region rounded, and embedded to varying extent in adipose tissue anterior to the kidneys.

Dimorphism between pairs of adrenals from the same individual were found in all species of antelope, as was found in the dog (Baker 1937) and the camel (Al-Baghdadi 1969). Only the left adrenal appeared to consistently demonstrate definite gross anatomical variations, with the red hartebeest possessing a lobe-like extension at right angle to the main body of the gland, while in the gemsbok the gland appeared as a flattened cylinder shape. The shape of the gemsbok adrenal resembles that of the North American porcupine which is described as being elongated rods (Hartman and Brownell 1949). Krumrey and Buss (1969) recorded a similar stout extension on the adrenals of the African elephant, as in the red hartebeest, but prominent in the right gland instead of the left, as in this study. The least distinct shape and dimorphism within the pairs of adrenals occurred in the springbok, as described for the laboratory rat by Chester Jones (1957). The results of this study

suggest that larger, more massive body weights of some species may affect adrenal gland form. The dimorphism of the left and right adrenals may also be due to the locality of the glands in the abdominal cavity and the pressures it is subjected to from surrounding organs and viscera. As mentioned by Skinner and Smithers (1990), species of antelope may have a peculiar gait and body form, as does the gemsbok and red hartebeest, both of which have a sloping back. In these cases there may be certain pressures, due to gravity and the placement of organs surrounding the adrenal glands that may affect their growth and shape.

The adrenal weights observed for each species had a direct relationship with the body weight of each species. The adrenal weights increased with the species' body weights. Indeed, Skinner and Smithers (1990) reported species weights of adult males as follows; eland, 650 kg; gemsbok, 240 kg; greater kudu, 228 kg; red hartebeest, 152 kg; springbok, 41 kg. These statistics corroborate the results of Christians (1959) who found that adrenal weight was a logarithmic function of body weight. He also stated that adrenal weights taken from captive wild animals do not reflect those in feral conditions, therefore this must be taken into account during sample acquisition.

B. Histology

The adrenal histology conforms as a whole to the typical eutherian mammalian model with some differences in the structures of different regions found in particular to the adrenal cortex. The capsule consists mostly of two layers (e.g. springbok, red hartebeest, greater kudu), with an outer thin fibrous layer a few

microns thick and an inner areolar, cellular layer, which made up the bulk of the capsule. This model is consistent with the findings of others working with various mammals (Elias 1948; Pauly 1957; Yamauchi 1961; Kramer et al. 1991). Teixeira et el. (1993) described the outer capsule of the African buffalo as consisting of collagenous fibers and the inner region of smooth muscle fibers. They also reported that the capsule of the buffalo is thinner than in the African elephant (Kramer et al. 1991) and similar to that of the impala antelope, which appears reasonable as the buffalo is in the same taxonomic family group as antelope. In this study the greater kudu and cape eland had an additional third layer, which was a very thin reticular fibrous layer. Both the gemsbok and cape eland had trabeculae intermittently penetrate into the cortex to varying depths and widths. In the gemsbok these trabeculae consisted solely of reticular fibers, and penetrated to varying depths into the zona fasciculata. In the eland there was an increase in the complexity and frequency of these structures, with areolar capsular tissue and complete glomerulosal cell aggregations incorporated into the trabeculae. Both Cupps et al. (1954) and Das et al. (1965) described in the domestic cattle trabeculae extending down to the medulla, with glomerulosal cells extending along with the trabeculae. The present study confirm the findings of Bernert (1981) that the cape eland and oryx spp. are characterised as possessing deep penetrating trabeculae. The results of this study indicate that the adrenal capsule may be more uniform and consistent in smaller species (i.e. springbok and red hartebeest and greater kudu), while having more penetrating trabeculae in larger species (ie. gemsbok and cape eland). The trabeculae

may function as a structural support system for the adrenal gland as a whole in the larger species. Therefore, the springbok and red hartebeest may not need trabeculae since the glands are considerably smaller.

In this study the zona glomerulosa in all species was the most eosinophilic of all zones in the adrenal cortex. The constituent cells ranged from cuboidal to highly columnar and were arranged in varying forms of radially oriented cord structures. This formation differs with a study on Indian buffalo (Prasad and Sinha 1984), where the zone consisted of distinct spherical groups of cells with a network of sinusoids. The cord formations of the antelope consisted of pairs of cells in the case of all species examined, excluding the greater kudu, where single cell width cords exist in a unique zonal architecture. In the springbok the cord structures remain generally intact as they descend toward the zona fasciculata. In the gemsbok the glomerulosal cords become progressively fragmented at the fascicular end. The same fragmentation occurs in the eland but with higher frequency, and forms distinct islets of glomerulosal cells surrounded by thin reticular trabeculae which encapsulates them. Therefore, these results indicate increased fragmentation of glomerulosal structure with an increase in the size of the species.

The zona glomerulosa of the greater kudu is composed of a winding cord structure which form wide arches at the capsular end. The component cells are stratified, highly columnar and have nuclei which are rounded at the capsular end of the zone and become progressively flattened towards the zona fasciculata. Large sinusoids are found to separate the meandering cords. The greater kudu zona

glomerulosa resembles greatly the descriptions of Elias (1948), Dellmann (1993), and Prasad and Yadava (1984), for equine zona glomerulosa. In equines this zone has been termed zona arcuata due to the large arches formed by two columns of cells connecting at the capsular end. Since function can be correlated often with morphology, the observed glomerulosal structures may have a role to play in cell formation in the adrenal cortex, as suggested by Banks (1986) and Teixeira et al. (1994). On this basis it can be suggested that the fragmentation distinctly observed in the eland and gemsbok are stem cells which may be responsible for supplying the pool of cells in the cortex (Vinson et al. 1992). The large contact area of glomerulosal cells to sinusoids in the larger species supports suggestions (Pauly 1957) that exchange of nutrients and metabolites may be direct and not through other cells.

The zona fasciculata in all species studied was the widest zone in the entire adrenal cortex, and was directly abutted to the zona glomerulosa. All species shared the common cuboidal cell shape forming usually single cell width cords running radially towards the medulla, and separated by extensive sinus networks. Both an outer and inner fasciculata were observed in all species except the eland where the zone was uniform and the cords were either frequently interconnecting or fragmenting. In the springbok, greater kudu and gemsbok, cord structures were more distinct and intact in the inner fasciculata, and separated by distinct sinusoids running parallel. In the red hartebeest the cells appeared significantly more vacuolated near the glomerulosal juncture, as found in the camel (Al-Bagdadi 1968). In other species a "foamy" appearance of the outer zona fasciculata is also seen to varying degrees,

and is due to the palely stained, vacuolated cells. While the inner region contains cells not as vacuolated, have a denser, more eosinophilic cytoplasm. Reports of Teixeira et al. (1993) indicated that the outer vacuolated fasciculata are more lipid rich than the inner, more compact cells. The presence of lipid droplets are a typical indication of steroid-producing cells (Nussdorfer 1986) and cholesterol is stored within these lipid-invaded, vacuolated areas of the cells. Therefore these clear cells may constitute a storage zone of steroid precursors held in reserve (Chester Jones and Henderson 1978). According to a study by Yamauchi (1965) these lipid-rich cells of the fasciculata are observed mainly in the adrenals of older individuals due to cellular inactivity brought on by increasing age.

No evidence of any zona intermedia was found in any of the antelope studied. The description of the zone is confusing in the literature as it is a well developed region in the horse, dog, and cat, and is present to a lesser degree in the cow, sheep and goat (Dellman 1993). Nicander (1952) described a broad intermediary zone for the horse, cow and rabbit. The cells of the region in question do contain undifferentiated cells in some samples of red hartebeest and greater kudu. However, the constituent cells are clearly part of the overall fasciculata architecture and do not form a distinct transitionary zone, as found in the domestic cow (Yamauchi 1965).

The zona reticularis of the antelope studied were uniform and composed of irregular network of anastomosing undifferentiated cells. No distinct border existed in any of the species between the zona fasciculata and reticularis, as found in the Indian buffalo (Prasad and Yadava 1974) , camel (Abdalla and Ali 1989), cattle

(Nicander 1952), white-tailed deer (*Odocoileus virginianus*) (Hoffman and Robinson 1966) and some East African antelope species (Bernert 1981). Hartmann et al. (1988) stated that in species where the adrenal gland is poor in lipid, the distinction between the fasciculata and reticularis is less clear. Both light, vacuolated and dark, compact staining cell cytoplasm and nuclei were found in the present study. Overall, there was more of the eosinophilic cells in the springbok species, whereas in the gemsbok the zone appeared predominated by vacuolated cell forms. The compact cells may be providing daily steroid requirements. After stimulation the large, pale staining cells which may indicate lipid richness, may transform into the compact form to give increased output of corticosteroids, therefore making the zona reticularis an actively secreting zone (Symington 1969). The zona fasciculata and reticularis may be considered as a single functional unit, where the histological appearance varies with the state of activity of the gland. Therefore, the gemsbok cells in this study may have been in a non-activity mode at the time of sampling, while the springbok may have been actively secreting.

The medulla was distinctly demarcated from the adrenal cortex in all species of antelope. In the two smaller antelope species, springbok and red hartebeest, there was no evidence of a medullary capsule observed at the border of the cortex and medulla. The cells of the cortex directly abut onto the medulla. The gemsbok and greater kudu both had evidence of a pseudo-capsule in which reticular fibers at varying thickness of two to four cell layers incompletely followed the corticomedullary junction. However, a distinct medullary capsule composed of reticular fibers identical

to those found in the cortex capsule and two to three cell layers thick, completely encapsulated the medulla of the eland. Based on these findings it is suggested that a medullary capsule is formed in species with high body weights, such as with the kudu, gemsbok and eland. These findings confirm those of Bernert (1981), where medullary capsules were seen in similar, large bodied antelope species. The two smaller species, springbok and red hartebeest have higher metabolic rates (Spinage 1986) and therfore the lack of a medullary capsule may facilitate the secretion of catecholamines. Medullary capsules were reported for the Indian buffalo (Prasad and Yadava 1974) and camel (Al-Bagdadi 1968; Abdalla and Ali 1989), but there was no evidence of a medullary capsule in the African buffalo (Teixeira et al. 1993). Findings of Hartman and Brownell (1949) support that a lack of a medullary capsule makes the cortex more easily accessible to secretions from the medulla.

In all species of antelope studied the medulla was clearly delineated into two regions. In most species both types of cells are randomly distributed in the medulla except in ruminants where there is an outer lamina of adrenaline-secreting cells (A) and an inner lamina of noradrenaline-secreting cells (NA) (Dellman 1993). Based on previous work (Zamora et al. 1967; Prasad and Yadava 1973; Dellman 1993), the outer medulla in the present antelope study was composed of columnar and cuboidal (A) secreting cells forming cords and acini structures. The inner medulla consisted of mostly undifferentiated, (NA) secrecting cells concentrated around the central vein. The outer (A) secreting region in all antelope species in this study appeared to occupy the greater proportion of the medulla. This finding corresponds to those of

Smollich (1966) who found that (A) cells were always higher in ratio and increased proportionately with advancing age. In some samples of eland antelope, intact glomerulosal cells were found in the inner medulla region around the central vein. This may augment the output of mineralocorticoids (18-OH-B, Aldosterone) in this species, and be a contributing factor in the water conserving capability (Abdalla and Ali 1989) of the semi-desert adapted eland.

C. Statistical Analysis

Due to the small sample sizes obtained for each species, and variable standard deviations, questions arose concerning the homogeneity of each species. With the use of multivariate discriminant analysis and the application of a jack-knife classification, using the histomorphometric data, this question was resolved and intraspecies homogeneity was determined to be significantly higher than the homogeneity between species. However, the univariate analysis of the measurements exposed differences to a lesser extent among species, across much of the adrenal cortex. The larger species, eland and gemsbok, had significantly wider zona glomerulosa than greater kudu, red hartebeest, and springbok due apparently to the larger body sizes. The zona fasciculata and reticularis combined measurements were again significantly wider for the three largest species. Both the greater kudu and eland contained significantly higher fascicular cell densities than the other species. The zona fasciculata appears to be the main region that can attribute to the increase in size of the adrenal cortex, while the zona glomerulosa remains mostly uniform across the

species. As stated by Bernert (1981) the smaller species have larger percentages of zona glomerulosa volume in relation to the body weight of the species. Therefore, the differences in the different variables may be attributed to the different weight classes of the antelope species. Further findings of Bernert (1981) suggest that the function and activity of the zona glomerulosa may not accompany a width increase, thereby masking the true secretory state of the region from histological examination. However, Shire (1969) reported that significant differences can exist between sub-species (i.e. breeds) in not only adrenal weights, but structure and width of zona glomerulosa, indicating that some differences can begin to be noticed at taxonomically much smaller scales.

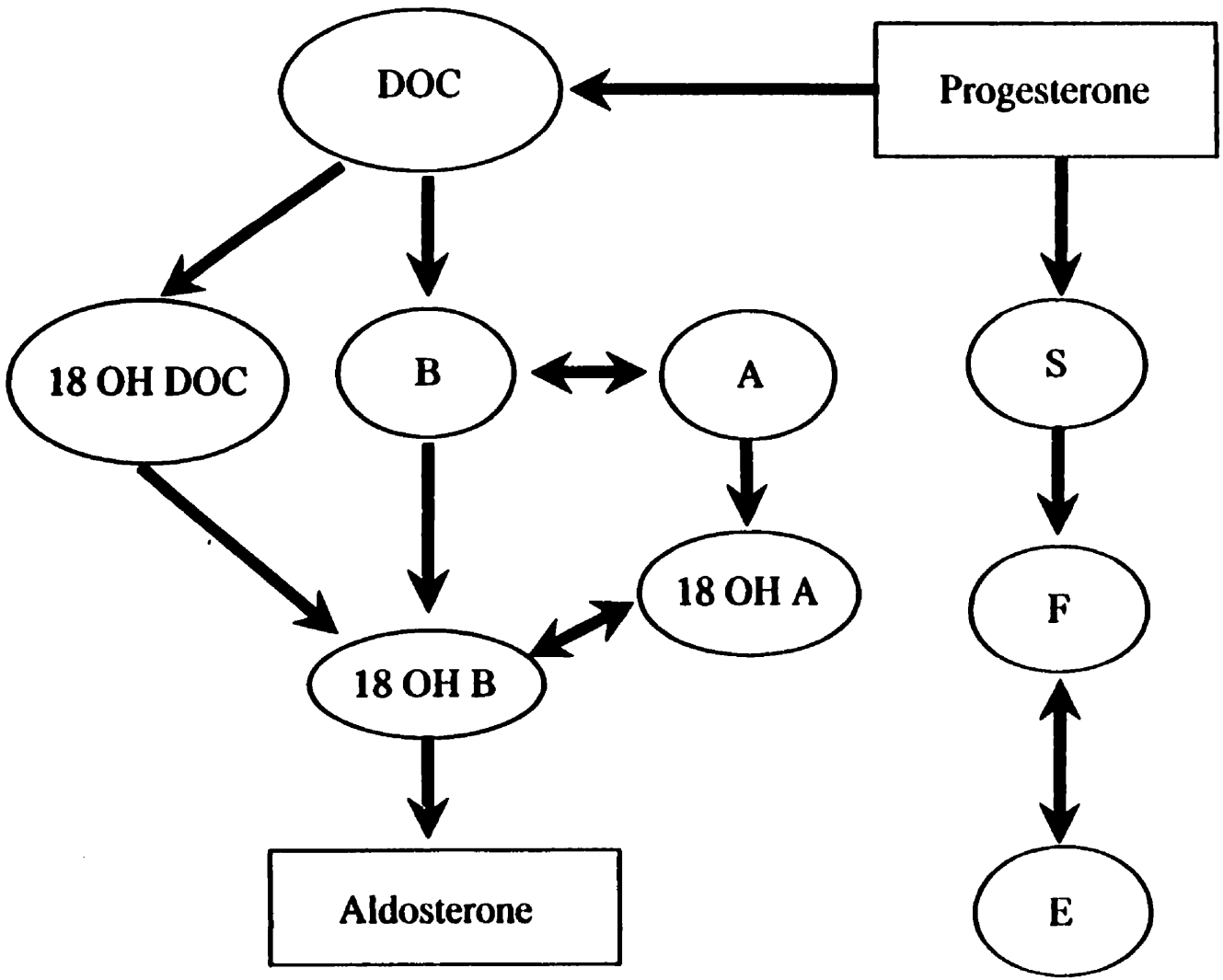
D. Corticosteroid Profile

Many major corticosteroids were present in all species of antelope, including the two most important glucocorticoids, cortisol, corticosterone and the major mineralocorticoid, aldosterone. This fit the pattern described for most mammals and other vertebrates (Chester Jones and Henderson 1978). No evidence of deoxycorticosterone was detected in any species, which may indicate that this precursor in the biosynthetic pathway leading to corticosterone and aldosterone is in rapid conversion to the next compound, and so is not stored in any significant amounts. The major biosynthetic pathways of corticosteroids are divided into two routes, with progesterone either leading to the production of cortisol and cortisone, or the other synthesizing corticosterone, 18-OH-B and aldosterone (Chester Jones

and Henderson 1978)(Fig.18).

The mineralocorticoid, 18-hydroxycorticosterone (18-OH-B), was the only hormone to be found in significantly higher amounts compared to all other corticosteroids analyzed, including aldosterone. The 18-OH-B compound has been shown to be a major precursor to aldosterone, and species differences are noted in the production of this and other 18-oxygenated steroids (Chester Jones and Henderson 1976). Chester Jones and Henderson (1976) reported further that zona fasciculata and reticularis of the ox produce 18-OH-B, but not aldosterone from corticosterone. In humans, 18-OH-B amounts secreted exceeds that of aldosterone by a factor of two. Adrenals of some monkey species and seals transformed aldosterone precursors to 18-OH-B but not to aldosterone. The significant amounts of 18-OH-B observed in this study may indicate that it is a possible reserve compound for later conversion to aldosterone to be released pending the need of the animal to deal with arid, drought conditions which may occur in the Kalahari desert. All five species of antelope studied are able to conserve water during hot, dry conditions to varying success. The gemsbok, springbok, are both the most adapted to desert conditions, while the eland and red hartebeest are able to cope with semi-desert conditions and the greater kudu being the least adapted (Spinage 1989, Skinner and Smithers 1990). Based on suggestions of Moussa et al. (1977), the relatively large amounts of 18-OH-B may cause the release of aldosterone to be slower in these antelope than in domestic bovids, but sufficient enough to maintain water and electrolyte balance in these water independent animals. No differences in

Figure 18 Major corticosteroid biosynthetic pathways suggested in the adrenal cortex of antelopes.


DOC
Progesterone
18 OH DOC
B
A
S
18 OH A
F
18 OH B
Aldosterone
E

endogenous aldosterone content was observed in any species. Stewart et al. (1972) stated that zona glomerulosa size may not be related to differences in aldosterone production, and also may not reflect a difference in capacity for aldosterone production. Therefore, the differences observed in the histological measurements of this zone in the antelope may not indicate the actual secretory state.

The low levels of steroids observed may be due to the age of the antelope. All specimens were mature adult males ,as all were trophy-hunted. Potter and Goodfriend (1987) reported for domestic cows that aging affects the endocrine system by reducing the levels of circulating hormones. The synthesis of pregnenolone from endogenous precursors was lower in old glomerulosal cells than in their younger counterparts, indicating a definite age effect.

The cortisol to corticosterone ratio varied little, except that the gemsbok adrenal contained more corticosterone than cortisol. This ratio has been observed to vary with circadian rhthymn (Chester Jones and Henderson 1976). Both aldosterone and 18-OH-B share corticosterone as an immediate precursor (Sandor 1969). Since the gemsbok is considered water independent (Skinner and Smithers 1990), aldosterone production may be of great importance, as it plays an important role in water metabolism (Wilson 1989), thereby increasing the production of its precursor, corticosterone.

Total corticosteroid content of the springbok was significantly higher than that of the eland. According to the results on all five species there is a definite trend of

a decrease in steroid content with increasing species body weight. These results may reflect the fact that smaller species, like springbok, have significantly higher metabolic rates than larger bodied species like eland (Spinage 1989), and therefore physiologically need more steroids to be synthesized, stored and circulated. There may also be a relationship of the corticosteroid content to ecology and territorial behaviour. The springbok , a sociable/territorial species known for its extensive migratory abilities, is one of the fastest of the gazelles. The males defend large territories and energetically herd, chase, and fight during mating peaks (Estes 1993). Eland are considered sociable/non-territorial and form large herds which are open and changeable. It is also the slowest antelope, and quickly tires (Estes 1993). Therefore, these behavioral traits may suggest a relation to the inherent metabolic rate of these species and so be reflected in the results seen of their comparative steroid contents.

VI. CONCLUSION

The histomorphology of the African antelope adrenal gland appears to follow the characteristic bovine pattern, with some distinct variations. The gross morphology of the adrenal glands appear to be species-specific, and may correspond to the different body weights and anatomical forms specific to each species. Future studies are needed to confirm the gross morphological differences observed within this family, including investigations into any correlation with any physiological, anatomical or environmental variables.

While the structure of the inner cortex zones differ slightly from other mammals, significant variations were found in the histological architecture of the capsule and zona glomerulosa. The clearly visible two layers of the cortex capsule often noticed appears to be a characteristic of bovids in general, with the eland and gemsbok having even three capsule layers. Extensive capsular trabeculae that penetrate deep into the cortex were found in only the largest antelope, eland and gemsbok, and are representative of these species. The zona glomerulosa appears to form species-specific variations in types of cord structures, which is formed in all antelope species examined, with greater kudu having the most unique of glomerulosal architecture, resembling those found in equine species. No zona intermedia was observed in any of the species examined. Contrary to the findings in the African buffalo and some East African antelope, no evidence of a medullary capsule was found in the springbok and red hartebeest, while an incomplete pseudo-capsule was

found in the greater kudu and gemsbok. However, the eland alone showed a distinct reticular fiber capsule which completely encircled the adrenal medulla. The adrenal medulla itself can be divided into both an outer adrenaline-secreting zone and an inner noradrenaline-secreting zone, as is characteristic of bovids in general. Intact islets of zona glomerulosa were found in the inner medulla of the eland, and running along trabeculae in the cortex indicating the possible importance of increased demands of this mineralocorticoid secretory zone to this species.

By using discriminant analysis, intraspecies homogeneity could be reported, as well as the significant heterogeneity across the five species, despite relatively small sample sizes and fairly large dispersion of different variables in each species. The quantitative measurements of the adrenal cortex were fairly uniform in all species and most of the differences reported may be attributable to the different weight classes of the species.

The corticosteroid patterns observed in the antelope appear to be typical of most mammals and bovines in particular, with cortisol being the predominant steroid. Total corticosteroid content showed interspecies differences, which appeared to be based on body size. The large amounts of 18-OH-B observed in each species may indicate that this precursor of aldosterone is a major storage reserve for later aldosterone synthesis. This may be an adaptation technique that evolved by species which inhabit dry desert regions. However, further studies are needed, using more accurate and sensitive laboratory techniques to identify and quantify the steroids present in the adrenals.

Additional studies may be carried out to study the effects of dietary sodium and possibly population density stress on the histology and endocrinology of different species. As African antelope are classified as either dependent or not on drinking water and the established importance of the adrenals in water metabolism, there may also be variations and correlations of endocrine physiology between different species found in the desert and those of forests and swamps. Based on the adrenal gland differences found in the semi-desert adapted species analyzed here and other bovids, further adrenal gland studies are warranted to answer the above hypotheses.

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Appendix 1. Springbok adrenal cortex histological measurements.

Case No.	left adrenal wt. (g)	adrenal cortex measurements in μm*				Cell Density**		
		capsule	z.glom.	z.fasc+ z.ret.	total cortex	z.glom.	z.fasc.	z.ret.
1	1.60	77.0	251.1	1692.0	2020.1	n/a	n/a	n/a
2	0.85	n/a	195.2	1528.2	1723.4	65	31	42
3	1.32	146.7	279.4	2263.2	2689.3	55	32	46
4	1.20	101.4	187.8	1916.3	2205.5	55	26	35
5	1.50	91.1	203.1	2671.7	2965.9	55	23	41
6	1.35	136.8	282.6	1940.5	2359.9	40	26	35
7	0.99	117.6	198.4	2078.4	2394.4	57	27	36
8	0.68	101.5	163.0	1457.4	1721.9	57	22	48
9	1.07	98.7	175.2	1708.4	1982.3	46	22	36
10	0.88	127.3	225.9	2204.7	2557.9	44	34	51
11	1.42	160.3	237.1	2406.8	2804.2	47	35	40
$\bar{x}^t$ SD	1.17 +/-0.30	115.8 +/-26.5	218.1 +/-40.4	1988.0 +/-379.5	2311.3 +/-422.1	52.1 +/-7.6	27.8 +/-4.9	41.0 +/-5.8

* Each datum is a mean of ten observations

** Mean number of cell nuclei per unit area of 0.5625 mm^2

t Mean +/- standard deviation

Appendix 2. Red hartebeest adrenal cortex histological measurements.

Case No.	left adrenal wt. (g)	adrenal cortex measurements in μm*				Cell Density**		
		capsule	z.glom.	z.fasc+ z.ret.	total cortex	z.glom.	z.fasc.	z.ret.
1	2.3	158.1	297.9	1520.8	1976.8	n/a	n/a	n/a
2	2.9	160.9	217.1	2434.5	2812.5	46	30	51
3	4.8	133.9	216.3	2735.1	3085.3	44	22	43
4	2.9	111.9	204.8	2869.6	3186.3	41	25	46
5	3.0	116.3	158.2	1947.6	2222.1	45	31	46
6	2.6	143.0	230.6	2260.9	2634.5	44	25	46
7	3.4	137.0	209.0	2029.4	2375.4	43	29	55
8	1.9	162.8	260.2	1597.8	2020.8	45	34	42
9	4.4	200.7	193.5	2366.0	2760.2	46	26	46
10	2.3	221.0	206.3	1891.0	2318.3	45	32	59
11	2.8	199.6	224.6	2005.7	2429.9	42	28	44
12	2.8	160.0	187.9	1856.7	2204.6	44	29	42
$\bar{x}^t$ SD	3.0 +/-0.8	158.8 +/-33.9	217.2 +/-35.5	2126.3 +/-418.2	2502.2 +/-395.2	44.1 +/-1.6	28.3 +/-3.5	47.3 +/-5.5

* Each datum is a mean of ten observations

** Mean number of cell nuclei per unit area of 0.5625 mm^2

Appendix 3. Greater kudu adrenal cortex histological measurements.

Case No.	left adrenal wt. (g)	adrenal cortex measurements in µm*				Cell Density**		
		capsule	z.glom.	z.fasc.+ z.ret.	total cortex	z.glom.	z.fasc.	z.ret.
1	5.4	115.6	221.4	2612.7	2949.7	n/a	n/a	n/a
2	8.5	106.2	228.0	2830.3	3164.5	n/a	n/a	n/a
3	4.6	143.4	156.4	2683.0	2982.8	58	36	61
4	5.0	217.4	330.1	2611.5	3159.0	53	34	50
5	11.6	83.2	160.9	2976.9	3221.0	53	37	40
6	3.9	134.6	203.7	2144.2	2482.5	54	31	43
$\bar{X}^t$ SD	6.5 +/-3.0	133.4 +/-46.3	216.8 +/-63.1	2643.1 +/-282.4	2993.3 +/-272.6	54.5 +/-2.4	34.5 +/-2.6	48.5 +/-9.3

* Each datum is a mean of ten observations

** Mean number of cell nuclei per unit area of 0.5625 mm^2

t Mean +/- standard deviation

Appendix 4. Gemsbok adrenal cortex histological measurements.

Case No.	left adrenal wt. (g)	adrenal cortex measurements in μm*				Cell Density**		
		capsule	z.glom.	z.fasc.+ z.ret.	total cortex	z.glom.	z.fasc.	z.ret.
1	5.4	168.9	301.8	2549.8	3020.5	n/a	n/a	n/a
2	8.7	120.7	275.2	2918.6	3314.5	59	28	45
3	9.4	313.0	244.6	2920.6	3478.2	57	27	42
4	12.9	146.2	267.3	4307.4	4720.9	48	24	37
5	4.0	165.4	241.5	2449.5	2856.4	51	28	45
6	6.1	117.2	307.3	2920.1	3344.6	49	27	45
7	5.5	213.0	244.1	2830.3	3287.4	59	28	40
8	5.4	165.0	348.0	3064.3	3577.3	46	26	34
$\bar{x}^t$ SD	7.2 +/-2.9	176.2 +/-63.1	278.7 +/-37.9	2995.1 +/-569.1	3450.0 +/-568.8	52.7 +/-5.5	26.9 +/-1.5	41.1 +/-4.4

* Each datum is a mean of ten observations

** Mean number of cell nuclei per unit area of 0.5625 mm^2

t Mean +/- standard deviation

Appendix 5. Cape eland adrenal cortex histological measurements.

Case No.	left adrenal wt. (g)	adrenal cortex measurements in μm*				Cell Density**		
		capsule	z.glom.	z.fasc+ z.ret	total cortex	z.glom.	z.fasc.	z.ret.
1	12.0	163.9	243.0	3102.3	3289.2	47	32	44
2	12.2	211.4	281.7	3363.0	3856.1	40	30	38
3	9.4	175.5	344.8	3545.3	4065.6	46	29	29
4	11.8	154.6	331.3	3468.6	3954.5	45	31	31
5	15.0	181.1	241.4	3336.9	3759.4	45	28	30
6	12.5	227.0	324.1	2278.8	2829.9	52	34	37
$\bar{x}^t$ SD	12.2 +/-1.8	185.6 +/-28.0	294.4 +/-45.6	3182.5 +/-467.6	3625.8 +/-473.1	45.8 +/-3.9	30.7 +/-2.2	34.8 +/-5.8

* Each datum is a mean of ten observations

** Mean number of cell nuclei per unit area of 0.5625 mm^2

t Mean +/- standard deviation

Appendix 6. Springbok endogenous adrenal corticosteroid levels.

Case No.	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
1	7.0	3.0	2.0	-	3.0	4.0	4.0	-
2	6.0	4.0	3.0	6.0	5.0	3.0	6.0	-
3	2.0	3.0	-	-	1.5	-	2.0	-
4	7.0	3.0	5.0	4.0	-	3.0	4.0	-
5	6.0	3.0	2.0	3.0	3.0	-	4.0	-
6	6.0	3.0	2.0	-	1.5	-	3.0	-
7	8.0	4.0	2.0	-	1.0	-	5.0	-
8	6.0	3.0	1.0	-	2.0	-	-	-
9	6.0	3.0	1.5	5.0	3.0	-	2.0	-
10	8.0	2.5	5.0	-	2.0	3.0	5.0	-
11	6.0	1.5	1.0	-	2.0	-	2.0	-
x^t SD	6.18 +/-1.60	3.00 +/-0.67	2.45 +/-1.46	4.50 +/-1.29	2.40 +/-1.15	3.25 +/-0.50	3.70 +/-1.42	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

t Mean +/- standard deviation

Appendix 7. Red hartebeest endogenous adrenal corticosteroid levels.

Case No.	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
1	8.0	4.0	1.5	-	3.0	2.0	-	-
2	8.0	4.0	2.0	-	1.5	1.5	-	-
3	5.0	2.0	-	1.0	0.7	1.4	0.6	-
4	10.0	3.0	1.5	1.0	1.5	-	2.0	-
5	8.0	4.0	1.5	-	-	1.5	2.0	-
6	10.0	2.0	1.0	-	1.0	-	3.0	-
7	8.0	5.0	1.5	-	2.0	-	4.0	-
8	10.0	3.0	1.5	1.0	0.5	-	1.0	-
9	10.0	3.0	1.5	1.0	1.0	-	2.0	-
10	2.0	1.0	-	1.5	1.0	-	1.5	-
11	3.0	1.0	1.5	1.5	-	2.0	2.0	-
x^t SD	7.45 +/-2.88	2.91 +/-1.30	1.50 +/-0.25	1.17 +/-0.26	1.36 +/-0.77	1.68 +/-0.29	2.01 +/-1.01	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

t Mean +/- standard deviation

Appendix 8. Greater kudu endogenous adrenal corticosteroid levels.

Case No.	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
1	3.6	2.4	1.8	-	1.8	2.4	-	-
2	3.6	1.8	1.2	-	4.8	1.8	-	-
3	7.5	6.2	2.0	1.3	3.8	6.2	-	-
4	6.0	3.0	2.0	-	1.0	3.0	-	-
5	2.0	2.0	1.5	-	2.0	-	-	-
6	4.0	4.0	1.5	2.0	3.0	3.0	-	-
x^t SD	4.45 +/-1.97	3.23 +/-1.66	1.67 +/-0.32	1.63 +/-0.53	2.73 +/-1.40	3.28 +/-1.71	-	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

t Mean +/- standard deviation

Appendix 9. Gemsbok endogenous adrenal corticosteroid levels.

Case No.	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
1	7.0	1.0	1.5	1.0	2.0	-	-	-
2	6.0	2.0	1.0	-	2.0	-	-	-
3	7.0	2.0	3.0	2.4	3.0	-	0.6	-
4	5.0	2.5	0.5	0.5	1.5	-	-	-
5	4.0	1.5	1.0	1.0	2.0	-	1.5	-
6	5.0	1.5	2.0	2.0	2.0	-	2.0	-
7	5.0	1.0	1.0	-	1.0	-	-	-
8	4.0	1.0	1.0	1.0	1.0	-	1.5	-
x^t SD	5.38 +/-1.19	1.56 +/-0.56	1.38 +/-0.79	1.32 +/-0.72	1.81 +/-0.65	-	1.40 +/-0.58	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

t Mean +/- standard deviation

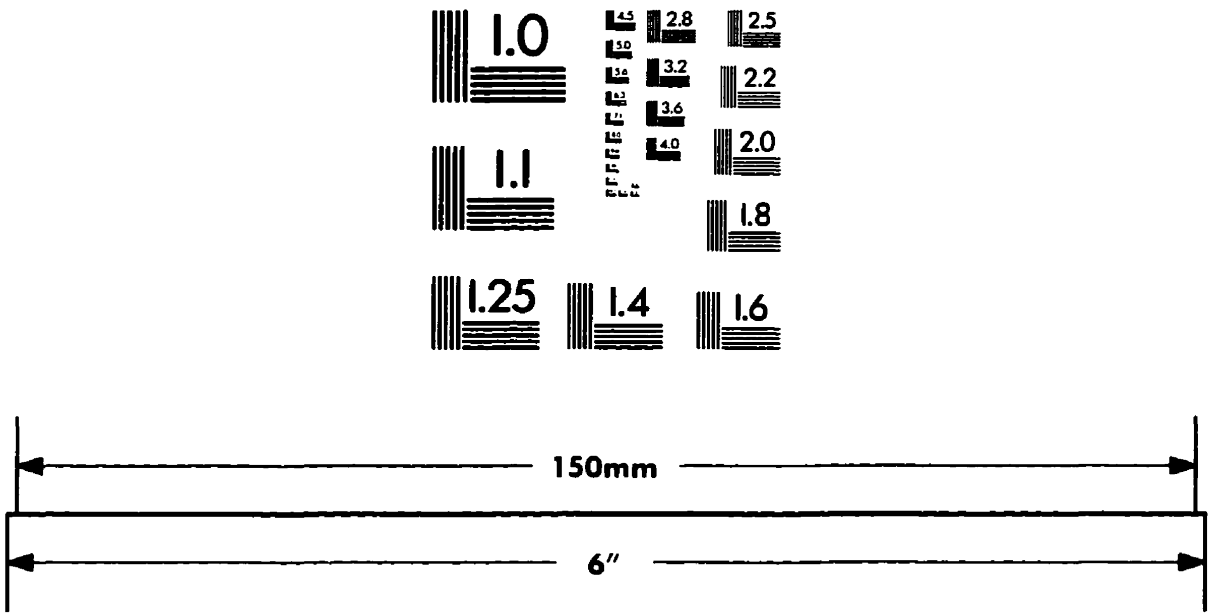
Appendix 10. Eland endogenous adrenal corticosteroid levels.

Case No.	Corticosteroid concentrations (µg/g tissue)							
	18-OHB	F	Aldo	E	B	S	A	DOC
1	2.4	4.8	-	-	4.1	-	-	-
2	1.5	1.0		-	0.5	-	-	-
3	6.0	3.0	1.5	-	3.0	-	-	-
4	4.0	1.5	1.5	-	1.0	-	-	-
5	5.0	3.0	1.5	-	1.5	-	-	-
6	2.0	1.0	1.0	-	1.0	-	-	-
x^t SD	3.48 +/-1.64	2.38 +/-1.37	1.38 +/-0.25	-	1.85 +/-1.28	-	-	-

note: cells with (-) sign indicate no steroid was detected for that specimen.

t mean and standard deviation

IMAGE EVALUATION TEST TARGET (QA-3)



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