The adrenal gland of the African buffalo, *Syncerus caffer*: a light and electron microscopic study

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Although the histology of the adrenal gland of many mammals, particularly domestic animals, is known, the histology of that of the African buffalo, *Syncerus caffer*, has not been described previously. Tissue from seven male and female adult buffalo was processed for light and electron microscopy. The gland is surrounded by a connective tissue capsule which contains smooth muscle fibres. The cortex of the gland is divided into three distinct zones with the cells of the outer zone being arranged in arcades rather than in glomeruli. The cells of this zona arcuata and of the outer region of the fasciculata have features typical of steroid-producing cells. The inner part of the fasciculata is a broad, less vacuolated and more intensely stained region than the outer region. Cells of the zona reticularis are arranged in freely anastomosing cords and are less vacuolated than the arcuata and the outer part of the fasciculata. The medulla is characterized by two distinct regions: an outer region of cells with granules of varying electron density which do not stain specifically with the Masson-Fontana technique and an inner zone of cells containing intensely electron dense granules which are chromaffin positive. These cells are adrenaline- and noradrenaline-secreting cells, respectively. The structure of the gland is thus typical of that of other mammals.

Alhoewel die histologie van die byniere van baie soogdiere, en veral huisdiere, goed bekend is, is dié van die buffel, *Syncerus caffer*, nog nie voorheen beskryf nie. Weefsel van sewe manlike en vroulike buffels is voorberei vir beide lig- en elektronmikroskopie. Die klier word omring deur 'n bindweefselkapsel wat gladdespiervesels bevat. Die korteks is verdeel in drie duidelike sones. Die selle van die buitenste sone is gerangskik in gange eerder as in glomeruli. Die selle in dié sone, die zona arcuata en in die buitenste gedeelte van die zona fasciculata toon eienskappe tipies van die van steroïed produserende selle. Die binneste breë sone van die zona fasciculata is minder gevakuoleerd en kleur meer intensief as die buitenste deel. Selle van die zona reticularis is gerangskik in vertakkende stringe en is minder gevakuoleerd as beide die arcuata en die buitenste gedeelte van die fasciculata. In die medulla word twee definitiewe gebiede onderskei: die buitenste sellulêre gebied met granules van verskillende elektrondigthede wat nie spesifiek kleur met die Masson-Fontana tegniek nie, en 'n binneste sone met hoë elektron-digte chromaffien-positiewe granulêre selle. Die selle van dié twee gebiede skei onderskeidelik adrenalien en noradrenalien af. Die histologie van die bynier van die buffel is dus tipies dié van soogdiere.

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Although the histology of the adrenal gland of most domestic animals has been described, little is known of the histology of tissues from animals occurring in the wild. While it would be reasonable to assume that the tissue from all mammals would be similar, differences do occur and should be documented for scientists working in the fields of comparative histology, pathology and conservation. As a case in point, the adrenal gland of the African elephant, *Loxodonta africana*, differs in a number of ways from domestic animals, e.g. it has an extensive layer of smooth muscle in its capsule and a continuous layer of undifferentiated capsular cells above the zona glomerulosa, which are not found to the same extent in any of the domestic animals (Kramer, Teixeira & Hattingh 1991). Documentation of differences could therefore be helpful to other workers in the field.

The histology of the adrenal gland of the African buffalo *Syncerus caffer* has, as far as we are aware, not been described previously. Information on glandular structure in this animal would be of value to workers in the field of capture myopathy, as it is not known whether changes in structure occur when these animals are exposed to stressors, as is the case in other species (Hartmann, Michna & Groddeck 1988). The aim of the present study was to ascertain the structure and ultrastructural cytology of the gland, in order to broaden the comparative histology database.

Materials and Methods

Adrenal glands from three male and four female adult African buffalo *Syncerus caffer*, were obtained from the yearly culling programme in the Kruger National Park. All the animals were brain shot. Approximately 30 min elapsed between the time of death and the time of tissue fixation.

Transverse sections of the gland were immersed in 10% neutral buffered formalin for light microscopy. This tissue was routinely processed through a graded series of alcohols and embedded in paraffin wax. Sections 5 μm in thickness were stained with either haematoxylin and eosin, Masson's trichrome stain, Gordon and Sweet's stain for reticular fibres or the Masson-Fontana technique for chromaffin cells (Bancroft & Stevens 1990).

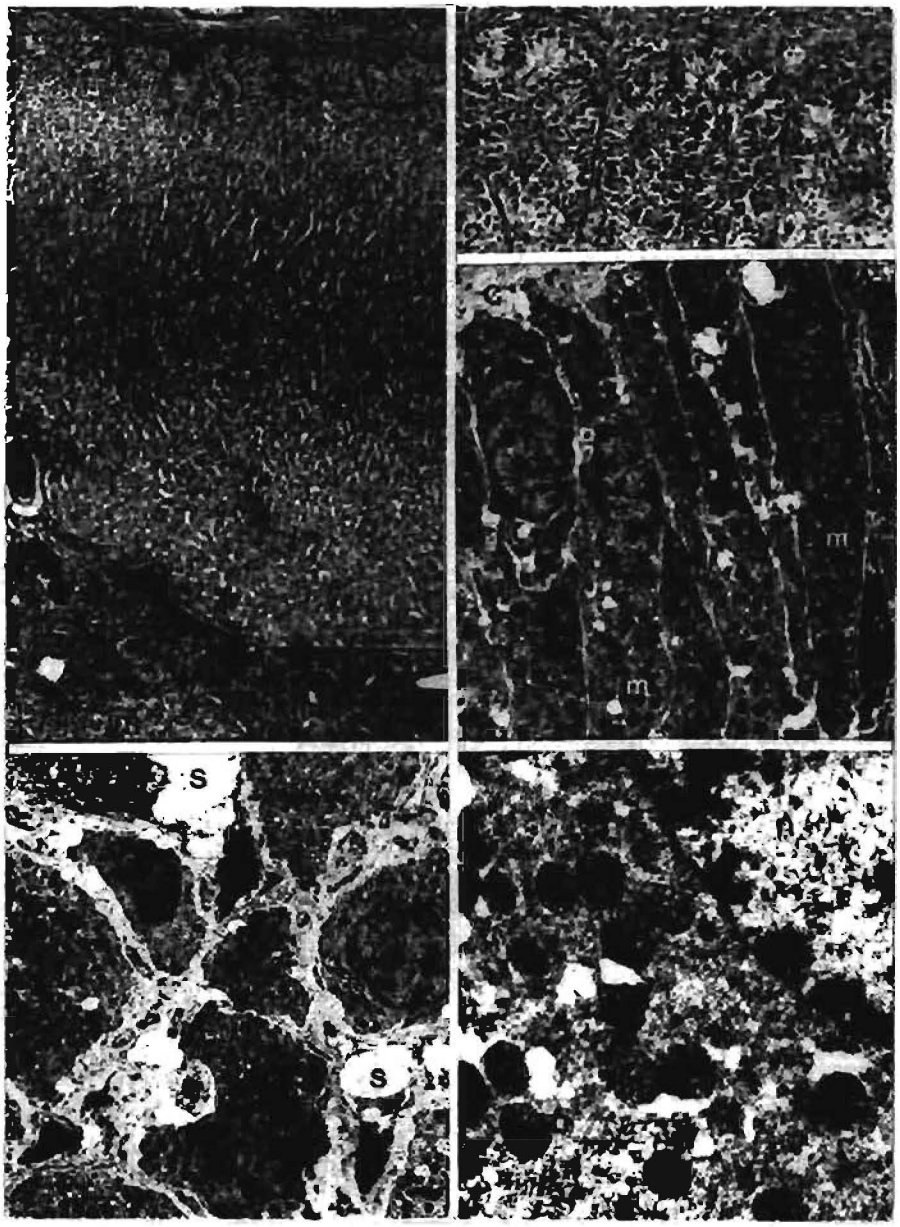
Slices of tissue not more than 1 mm thick were fixed in Karnovsky's fixative (4% paraformaldehyde and 0,5% glu-

taraldehyde in Millonig's phosphate buffer at pH 7,4) for 3 h followed by postfixation in 1% osmium tetroxide for 1 h. All fixation was carried out at approximately 4°C. In addition, some transverse sections of the gland were fixed in a mixture of 1,5% glutaraldehyde and 1,5% potassium dichromate adjusted to pH 6,8 with potassium hydroxide (after Kobayashi, Serizawa, Fujita & Coupland 1978) for the demonstration of chromaffinity. All the tissue for electron microscopy was routinely dehydrated and embedded in epon-araldite.

Tissue fixed in both the above fixatives was sectioned at 1 µm and stained with either 1% toluidine blue (Bancroft & Stevens 1990) in phosphate buffer at pH 6,8 or by the Masson-Fontana technique (Bancroft & Stevens 1990). Thin sections fixed in Karnovsky's fixative were double stained with uranyl acetate and lead citrate (Bancroft & Stevens 1990). Viewing of the sections was carried out in a JEM 100S transmission electron microscope.

Observations

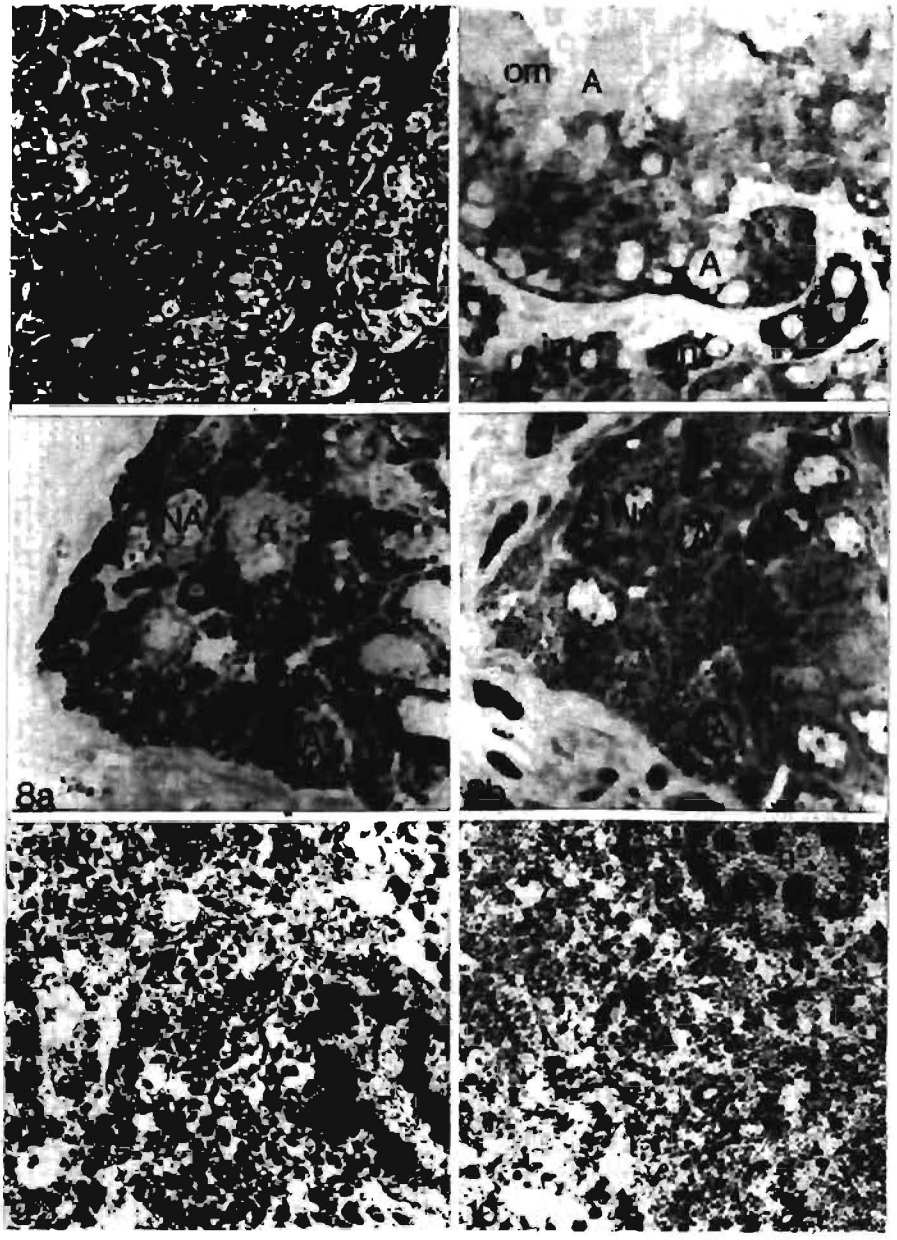
The cortex of the gland is surrounded by a well-developed capsule (Figure 1). The capsule is composed of eosinophilic



Figures 1–5. **Figure 1** A montage of a Masson's trichrome-stained light microscopic section through the adrenal gland of the buffalo. Note the relatively thick capsule, the zonation of the cortex and the two distinct regions of the medulla. c = capsule; om = outer medulla; im = inner medulla; za = zona arcuata; zf = zona fasciculata; zr = zona reticularis; ×20. **Figure 2** A light micrograph of a section stained with Masson's trichrome stain demonstrating the tall columnar, arcading cells of the zona arcuata. ×40. **Figure 3** An electron micrograph demonstrating the ultrastructure of the cells of the zona arcuata. Note the large numbers of mitochondria (m) and the small amount of lipid droplets (l) in the cytoplasm of these cells. n = nucleus; ×4000. **Figure 4** An electron micrograph of a cell of the zona fasciculata in which there is an abundance of mitochondria (m) with tubulovesicular cristae. ×15000. **Figure 5** An electron micrograph of the cells of the zona reticularis. The cords of cells are typically interwoven about a network of sinusoids (s). The reticularis cells display approximately the same amount of lipid (l) as do the cells of the zona arcuata. ×4000.

staining collagenous and smooth muscle fibres. The collagenous fibres predominate towards the outer surface of the capsule, whereas the smooth muscle fibres are more numerous closer to the cortex. Connective tissue trabeculae from the capsule penetrate into the cortex. There is a well-developed network of reticular fibres distributed throughout the gland.

The zonation of the cortical layers is clearly discernable in the buffalo (Figure 1). The subcapsular zone of the cortex is made up of curved cords or arcades of columnar cells with the convexity of the arcade directed towards the capsule (Figure 2). These cells constitute the zona arcuata (glomerulosa). They are tall columnar in shape and stain eosinophilically (Figure 3). The nuclei of the zona arcuata cells are oval, smaller, and more intensely staining than those of the zona fasciculata. The arcuata cells contain



Figures 6–10. Figure 6 Light micrograph of a section stained with Masson's trichrome stain. The cells of the outer medulla (om) stain more intensely and can be distinguished from the smaller, less intensely staining cells of the inner medulla (im). ×40. **Figure 7** Light micrograph of a section stained with the Masson-Fontana technique. There is a gradual transition from the outer medulla (om) where pale-staining A cells predominate, through a 'mixed' region of A and NA cells, to the inner medulla (im) in which intensely argentaffin NA cells predominate. A = adrenalin cells; NA = noradrenalin cells. ×160. **Figures 8a and 8b** Light micrographs of potassium dichromate and glutaraldehyde fixed, epon-araldite embedded, semi-thin sections of the inner medulla. **8a** Masson-Fontana staining. Noradrenalin-containing cells (NA) are strongly argentaffin, whereas adrenalin-containing cells (A) are not. **8b** Toluidine blue staining. The granules in the cytoplasm of the NA cells stain darkly with toluidine blue, whereas the A cells stain paler. Figures 8a and 8b are from adjacent sections. ×350. **Figure 9** An electron micrograph of an adrenalin-producing cell (A) of the outer medulla. Note the large granules of variable electron density. A = adrenalin granules; n = nucleus. ×7500. **Figure 10** An electron micrograph of a noradrenalin-producing cell (NA) of the inner medulla. The granules are intensely electron dense and are much smaller and irregular than the A granules. n = nucleus; NA = noradrenalin granules. ×7500.

numerous mitochondria with tubular cristae, and smooth endoplasmic reticulum (SER), but little rough endoplasmic reticulum. A zona intermedia is not present between the arcuata and the fasciculata.

The zona fasciculata is wide and is composed, in its outer portion, of palely stained cuboidal, vacuolated cells. These cells are arranged in distinct radial cords. Each cord consists of one or two cells separated from adjacent cords by an extensive sinus network. The cytoplasm of these cells contains a large Golgi complex, mitochondria with tubulovesicular cristae (Figure 4), and SER-organelles typical of steroid-producing cells. Although lipid granules are present in the cytoplasm, they are not numerous. The inner third to half of the zona fasciculata contains cells that are not as vacuolated as the outer zone; they have a denser, more eosinophilic cytoplasm (Figure 1). These cells contain less lipid than the outer fasciculata cells, but have well-developed SER and many mitochondria.

Freely anastomosing cords of cells occur in the zona reticularis. The cells are similar in shape and size to the cells of the fasciculata, contain less lipid than the cells of the outer zona fasciculata, and stain darker than these cells (Figure 1). Once again, mitochondria pack these cells and lipofuscin commonly occurs in their cytoplasm. The meshwork of sinusoids occurring between the cells is a prominent feature of this layer (Figure 5).

No evidence of a distinct medullary capsule is apparent in the buffalo, with the boundary between the cortex and the medulla being irregular. The cells of the cortex abut directly onto the medulla, which is clearly delineated into two zones. The outer zone is composed of large columnar cells arranged in acini (Figure 1) which stain intensely with all the routine stains used (Figure 6), but appear pale with the Masson-Fontana technique (Figure 7). These cells also appear palely stained following potassium dichromate fixation and toluidine blue staining (Figure 8b). They contain numerous granules which vary in electron density and size (Figure 9) and are here classified as adrenaline-secreting (A) cells (see Discussion).

The inner cells of the medulla are smaller and stain less intensely with routine stains (Figure 6), but the granules stain an intense dark blue with toluidine blue and are strongly argentaffin positive following the Masson-Fontana technique (compare Figures 8a and 8b). These are features of noradrenaline-secreting (NA) cells. With the Masson-Fontana technique, the cytoplasm of the NA cells is filled with granules impregnated brown to black with the ammoniacal silver in contrast to the A cells which did not show a distinct argentaffin reaction (Figure 8b). The NA cells contain small irregularly shaped granules with an intensely electron-dense core (Figure 10). The granule may sometimes be characterized by a clear space between the membrane and the granular contents, with the electron-dense core tending to be eccentrically placed within the membrane. Small-granule chromaffin (SGC) cells were not observed. Although a distinct boundary between the inner and outer zones of the medulla appears to exist after staining with routine stains, staining with the Masson-Fontana technique following potassium dichromate fixation shows a transition (Figure 7).

The parenchymal tissue of the entire gland is closely associated with an extensive vascular network while numerous prominent nerve fibre bundles course through the gland.

Discussion

A striking feature of the adrenal gland of the buffalo is the clear distinction between the adjacent zones. Whereas the zones in other animals tend to merge gradually from one region to the other making it difficult to measure their thickness, quantitative studies on zonal thickness in the buffalo would be relatively easy.

The capsule of the buffalo is thinner than that in the elephant (Kramer *et al.* 1991) and similar to that of the impala (unpublished observations). The presence of smooth muscle in the capsule is not surprising as these fibres have also been reported in adrenal glands of other mammals (Dellman & Brown 1987; Kramer *et al.* 1991).

A layer of capsular cells, as reported by Kramer *et al.* (1991) in the elephant and by Dellman & Brown (1987) in the adrenal gland of domestic animals, was not found in the buffalo. It was postulated (Kramer *et al.* 1991) that the capsular cells in the elephant were responsible for supplying the pool of cells in the cortex. It is possible that in the buffalo, as suggested by Banks (1986) in domestic animals, the cells of the zona arcuata may take over this responsibility.

The arrangement of cells in arcades in the zona arcuata is like that of horses, carnivores and pigs and unlike that of other ruminants and man (Banks 1986). There was no transitional area from the zona arcuata to the zona fasciculata, the former cells being found abutting directly on the large foamy cells of the fasciculata. The progression of cells of the zona fasciculata from pale foamy cells to more eosinophilic cells containing less lipid, is of interest. Adrenocortical cells are said to obtain cholesterol both by endogenous synthesis from acetate and by uptake of lipoproteins released by the liver (Gwynne & Strauss 1982). Only exogenous cholesterol is stored in the lipid droplets (Fruhling, Sand, Penasse, Pecheux & Claude 1973) while endogenously synthesized cholesterol is promptly utilized in steroidogenesis. Cholesterol synthesis occurs in the SER (Boyd, McNamara, Suckling & Tocher 1983); as a large amount of this organelle is found in the inner regions of the zona fasciculata it may be indicative of a high rate of endogenous cholesterol production.

The inner less foamy region of the fasciculata is not here considered to be a zona intermedia, as evidence of some lipid is always apparent in the cells. The description of a zona intermedia is confusing in the literature (see Kramer *et al.* 1991).

The prominent mitochondria with tubular to tubulovesicular cristae, the SER and presence of some lipid droplets are typical features of steroid-producing cells (Nussdorfer 1986). These structural entities can be associated with cholesterol and cholesterol esters stored in the lipid droplets (Moses, Davis, Rosenthal & Garren 1969; Sharawy, Dirksen & Chaffin 1979). These substances are transformed into definitive steroid hormones via a pathway involving enzymes located both in mitochondrial cristae and SER tubules (Tamaoki 1973).

The identification of the granules of the chromaffin cells

seen in this study has been made on both morphological grounds following Coupland (1965; 1971) and Coupland & Hopwood (1966), and on staining reactivity following Kobayashi *et al.* (1978). In the outer medulla the tall, pale chromaffin-positive cells are believed to be A cells. These gradually give way to the smaller, polyhedral, intensely chromaffin-positive NA cells of the inner medulla. This arrangement of the catecholamine-producing cells into an inner and outer zone is found in many domestic animals (Dellman & Brown 1987) and in the elephant (Kramer *et al.* 1991). Recently Al-Lami & Carmichael (1991) failed to show a distinct NA cell population in the medulla of the baboon adrenal. It was suggested that this may be due to a varied concentration of NA and A neurohormones among the vesicle population.

The distinction between the A and NA cells in the buffalo at an ultrastructural level is not as distinct as in the elephant. However, in the elephant, sub-populations of NA cells were thought to be present (Kramer *et al.* 1991). The NA cells of the buffalo appear to be a homogeneous group. Although it is not possible at present to distinguish between subpopulations of cells within a single class, Fuxe, Goldstein, Hokfeldt & Joh (1971) using specific antibodies to enzymes have been able to distinguish between A and NA cells on the basis of immunohistochemical studies.

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