The histology of the adrenal gland of the African elephant, *Loxodonta africana*

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The histology, particularly the ultrastructural cytology, of the adrenal gland of the African elephant, *Loxodonta africana*, is virtually unknown. Tissue from 14 adult male and female elephants was processed for light and transmission electron microscopy. The gland is surrounded by a thick capsule composed of an outer layer of dense connective tissue and an inner layer in which smooth muscle fibres predominate. Below the layer of smooth muscle, a continuous layer of relatively undifferentiated 'capsular' cells occur. Where the capsular cells abut on the zona glomerulosa, they appear to be differentiating into glomerulosa cells, as small lipid droplets are present in their cytoplasm. The cortex is divided into three zones as is found in the adrenal glands of other mammals. Large amounts of collagenous and reticular tissue support the secretory cells, which have a marked lipid content. With electron microscopy, the cortical cells show features typical of steroid-producing cells. The medulla is characterized by an outer region of pale-staining chromaffin-positive (adrenaline) cells and an inner region of intensely staining chromaffin-positive (noradrenaline) cells. The latter cells contain granules of different sizes and structure.

Die histologie, veral die ultrastrukturele eienskappe, van die bynier van die Afrika-olifant, *Loxodonta africana*, is bykans onbekend. Weefsel afkomstig van 14 volwasse manlike en vroulike olifante is voorberei vir lig- sowel as transmissie-elektronmikroskopie. Die klier word omring deur 'n dik kapsel bestaande uit 'n buitenste laag digte bindweefsel en 'n binneste laag wat hoofsaaklik uit gladdespier bestaan. Onder die laag gladdespier word 'n aaneenlopende laag ongedifferensieerde 'kapsulêre' selle gevind. Waar hierdie kapsulêre selle aan die zona glomerulosa grens, toon hulle tekens van differensiasie deurdat daar klein vetdruppels in die sitoplasma verskyn. Die skors is verdeel in drie lae soos gevind in ander soogdier-byniere. Die sekretoriese selle word ondersteun deur groot hoeelhede kollageen en retikulêre weefsel wat 'n hoë vetinhoud toon. Op die vlak van elektronmikroskopie vertoon die selle van die skors die tipiese eienskappe van steroïed-produserende selle. Die medulla word gekenmerk deur 'n buitenste area van lig-gekleurde chromaffien positiewe selle (adrenalien) en 'n binneste area van selle wat donker chromaffien positief kleur (noradrenalien). Laasgenoemde selle bevat granules van verskillende groottes en struktuur.

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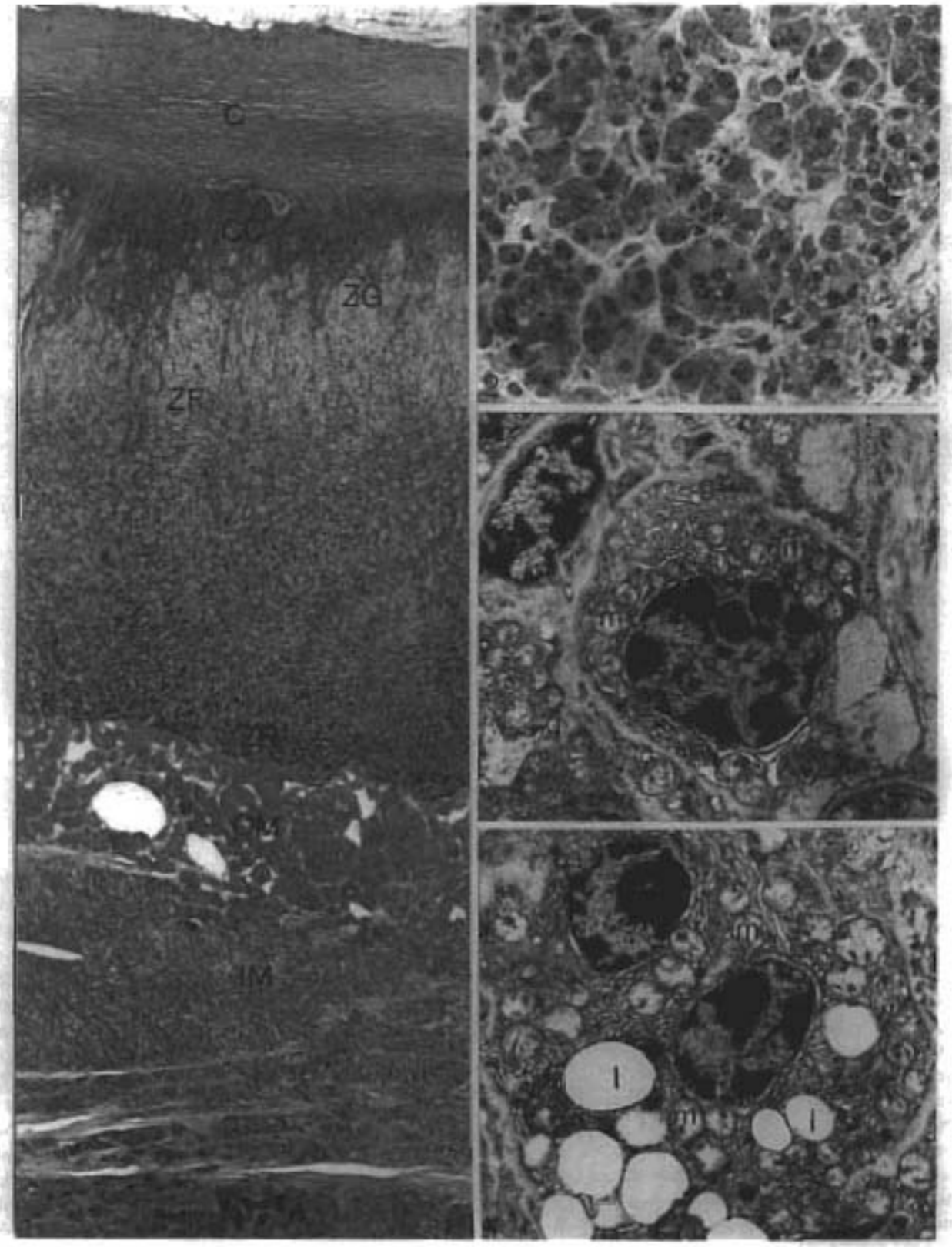
Although the gross anatomy and appearance of the elephant adrenal gland has been well described (Bourne 1949; Krumrey & Buss 1969; Baranga 1984), and some information is available on its histology (Kolmer 1918; Kohno 1925; O'Donoghue, Sykes & Turvey 1967), we were unable to find information on the ultrastructure of the gland in the literature. This information 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 this study was, therefore, to examine the ultrastructure of the gland to broaden the comparative histology database and to serve as a basis for future studies of this animal under stressful conditions.

Material and Methods

Adrenal glands from seven male and seven female adult African elephants, *Loxodonta africana* were obtained from the yearly culling programme in the Kruger National Park. The animals were herded prior to culling. Thirteen animals were culled with succinyldicholine, while one animal was brain shot.

The glands were removed 30–40 min after death. Each gland was cut into 1 mm thick transverse slices. Some slices were immediately immersed in 10% neutral buffered formalin. This tissue was routinely processed and embedded in paraffin wax for light microscopy. Sections (5 μm in thickness) were stained with either haematoxylin and eosin, Masson's trichrome stain, Gordon and Sweet's reticulum technique or the Masson-Fontana technique for chromaffin cells. (All these techniques are as described in Bancroft & Stevens 1990.) The remaining slices of tissue were fixed in 2,5% Karnovsky's fixative in phosphate buffer at pH 7,4 for 2 h at approximately 4°C. Postfixation was carried out at the same temperature, in 1% osmium tetroxide in phosphate buffer at pH 7,4 for 1 h. The tissue was routinely dehydrated and embedded in Epon-Araldite for transmission electron microscopy. Semithin (1 μm) sections were stained with 1% toluidine blue in phosphate buffer at pH 7,4. In addition some semithin sections were stained with the Masson-Fontana technique. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate and viewed in a JEM 100S transmission electron microscope.

Morphometrical assessment was carried out on the one brain shot animal, on a semi-automatic image analysis system (Kontron Videoplan). Ten random sections were measured to assess the thickness of the capsule. The diameters of 100 cells from each zone were measured to assess the size of the cells. This measurement was carried out on cells



Figures 1–4 (1) A montage of a haematoxylin and eosin stained light microscopic section through the adrenal gland of the elephant. Note the thick capsule, the capsular cells interposed between the capsule and the zona glomerulosa, and the two regions of the medulla. C = capsule; CC = capsular cells; ZG = zona glomerulosa; ZF = zona fasciculata; ZR = zona reticularis; OM = outer medulla; IM = inner medulla. ×185. (2) A semi-thin resin section of the capsular cells stained with toluidine blue. Note the presence of lipid droplets (l) in some cells. These droplets are found in cells lying close to the zona glomerulosa. ×600. (3) An electron micrograph of an undifferentiated capsular cell. There is a moderate amount of cytoplasm surrounding the rounded nucleus (n) which has condensation of the chromatin. The cytoplasm contains glycogen (g) and is filled with mitochondria (m). ×7200. (4) An electron micrograph of the capsular cells bounding the zona glomerulosa. Note the lipid droplets (l) in the cytoplasm of the cell. m = mitochondria. ×8000.

which were randomly selected from each zone and in which the nucleus could be clearly seen.

Results

The histology of the adrenal gland of the African elephant differs from other animals in the arrangement of some of its components. The gland, which is surrounded by a thick capsule, is clearly divided into an outer cortex and an inner medulla (Figure 1).

The connective tissue capsule surrounding the gland is thick, measuring 0,48 ± 0,05 mm (mean ± *SD*) in width, and is composed of collagenous and smooth muscle fibres. The collagenous fibres predominate in the outer region of the capsule, while smooth muscle cells are prominent between the collagenous fibres in the deeper regions. Large numbers of round, small eosinophilic cells measuring 0,08 ± 0,01 mm in diameter, which we have termed 'capsular' cells, are evident in the deeper aspect of the capsule, where it merges with the zona glomerulosa (Figures 1 & 2). The capsuler cells have a thin rim of cytoplasm and contain a large, round basophilic nucleus with characteristic clumping of the chromatin (Figure 3). Dense accumulations of glycogen occur in the cytoplasm of these cells, but generally they appear to be relatively undifferentiated (Figure 3). Where these cells abut on the zona glomerulosa, small lipid droplets are seen in their cytoplasm (Figures 2 & 4). No mitotic figures were observed in this layer. Dense collagenous fibres occur between the groups of capsular cells but are less prominent as the zona glomerulosa is reached. Numerous fibrous trabeculae extend at right angles from the capsule through the cortex towards the medulla (Figure 1). Bundles of collagenous fibres forming thin septa arise from the trabeculae and ramify between the secretory cells.

The zona glomerulosa cells are arranged in ovoid clusters beneath the capsular cells and contrast markedly with the latter (Figure 1). The glomerulosa cells stain palely eosinophilically and are larger than the capsular cells (0,11 ± 0,01 mm in diameter). The nuclei of the glomerulosa cells are round and basophilic. The ultrastructural characteristics of the cytoplasm of these cells are those of steroid-secreting cells. They have abundant smooth endoplasmic reticulum, tubular type mitochondria, little rough endoplasmic reticulum and a large Golgi complex. Lipid droplets are the predominant feature of these cells (Figure 5).

The zona fasciculata consists of long, radially arranged cords of cuboidal cells (Figure 1), which are the largest of the cortical cells (0,15 ± 0,2 mm in diameter). The cords are usually two cell layers thick and are often binucleate. These cells are termed spongiocytes as they appear particularly pale staining and foamy owing to the presence of numerous lipid droplets (Figure 6). The cells have the general features of steroid producing cells as described for the glomerulosa cells. Lysosomes and some lipofuscin occur in these cells. The typical arrangement and pale foamy appearance of the fasciculata cells disappear progressively as the zona reticularis is reached (see wide region in Figure 1); the cells of the fasciculata become progressively more eosinophilic and smaller, like the cells of the reticularis.

The zona reticularis forms an irregular network of anastomosing cell cords (Figure 1). The cells are polyhedral in shape and are smaller (0,12 ± 0,02 mm) in diameter than the fasciculata cells. The reticularis cells have the same morphological features as the cells of the fasciculata but appear more eosinophilic, as they have fewer lipid droplets. Lipofuscin is a common component of these cells (Figure 7). A prominent feature of this layer is the meshwork of sinusoids occurring between the cords of cells. Coarse collagenous and reticular fibres extend throughout this zone. In some specimens, these fibres run parallel to the capsule forming a layer reminiscent of a medullary capsule. This fibrous layer is not as evident with haematoxylin and eosin staining as it is with the reticulin stain.

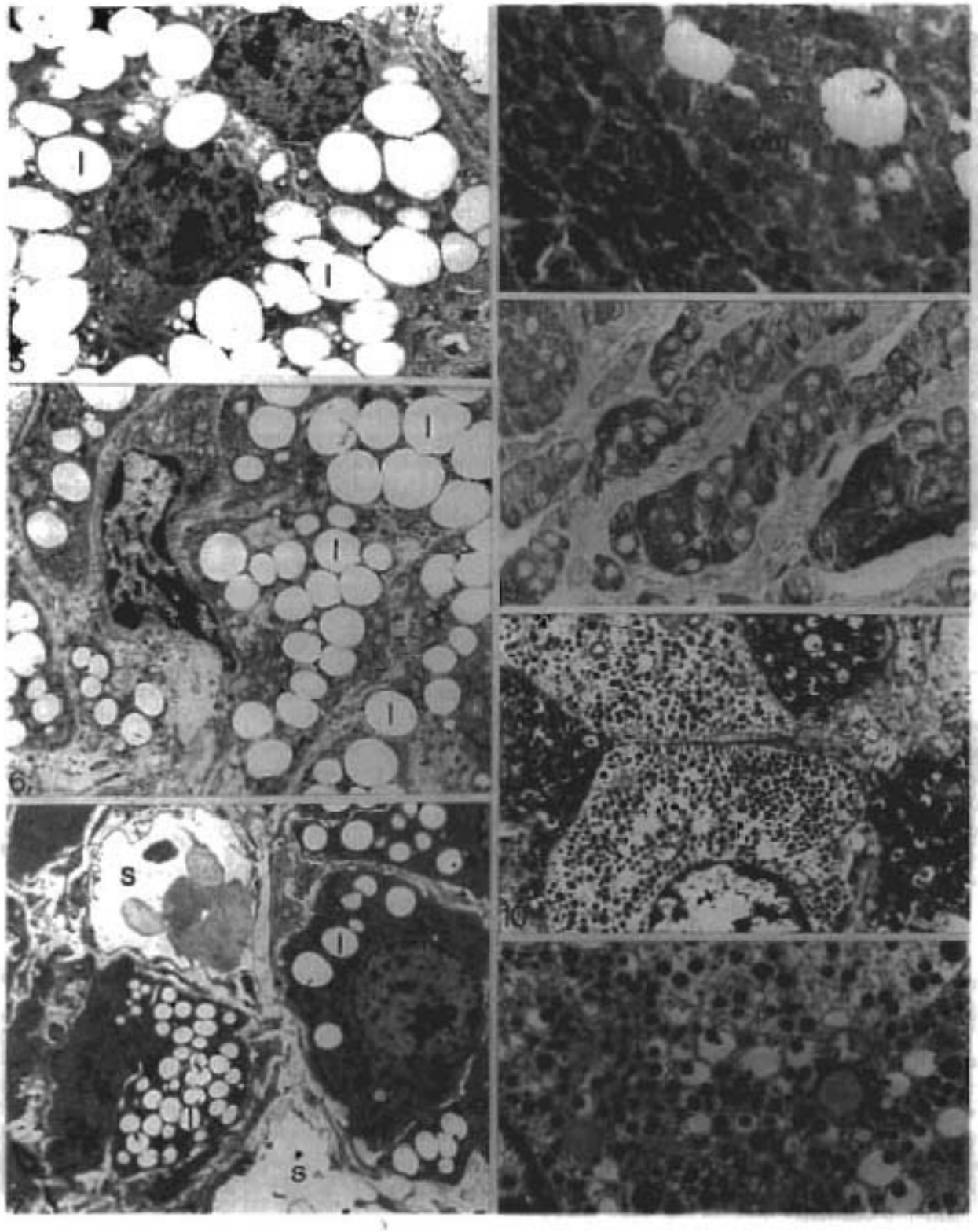
Occasionally, groups of pale staining foamy cells occur between the zona reticularis and the medulla of both male and female specimens. These cells are morphologically identical to the cells of the zona fasciculata.

The junction between the cortex and the medulla is irregular but well defined (Figure 1). The secretory cells of the medulla are arranged in two distinct zones (Figure 1). Those cells nearer the zona reticularis tend to be arranged in cords, stain intensely with all the routine stains used, and are highly granular; however they stain palely with the Masson-Fontana technique (Figure 8). They are believed to be predominantly adrenaline-secreting cells. At the ultrastructural level, these cells contain numerous granules which vary in electron density. The cells of the inner region are arranged in large rounded groups, are paler staining with routine stains but stain intensely with the Masson-Fontana technique (Figure 8). The presence of 'light' and 'dark' cells in the inner medulla is demonstrated with the Masson-Fontana technique on semi-thin sections (Figure 9) and can be seen with the electron microscope (Figure 10). All the cells contain granules with an intensely electron dense core, which are believed to be noradrenaline-containing granules. The granules 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 (Figure 11). Light and dark cells appear to be due to the relative packing of the granules within the cytoplasm.

Discussion

The abundance of smooth muscle in the inner region of the capsule is different from the paucity of these muscle fibres in this gland in other domestic animals (Dellmann & Brown 1987). Although the significance of this, and the thickness of the capsule in general, is not known, it may simply be due to the vast size of this animal and hence the larger size of all the components of the gland. It is possible, however, that the smooth muscle may aid in contraction of the gland and release of its products during times of demand.

The presence of a specific, continuous layer of cells within the capsule of the adrenal gland of the elephant has not previously been reported and is of interest. Dellmann & Brown (1987), in describing the adrenal gland of domestic animals, mention the inclusion of undifferentiated cortical cells in the capsule of these animals. O'Donoghue *et al.* (1967) reported the presence of small islets of cortical secretory tissue within the capsule of two male specimens of African elephant. The capsular cells observed in this study



Figures 5–11 (5) An electron micrograph of the cells of the zona glomerulosa. The cells are filled with lipid droplets (l). ×4800. (6) An electron micrograph of the cells of the zona fasciculata. Lipid droplets (l) are more abundant in these cells. ×4800. (7) An electron micrograph of the cells of the zona reticularis. These cells contain less lipid droplets (l) than the cells of the other two zones. In addition lipofuscin (f) is seen in the cytoplasm. s = sinusoid. ×3000. (8) A light micrograph of a section stained using the Masson-Fontana technique demonstrating the inner and outer medulla. The outer medulla (om) stains palely chromaffin positive while the inner medulla (im) is intensely chromaffin positive. ×140. (9) A semi-thin section of the inner medulla stained using the Masson-Fontana technique. Note the paler and darker stained cells. ×700. (10) An electron micrograph of the inner medulla. 'Light' and 'dark' cells are intermingled. All the granules are intensely electron dense. ×4500. (11) An electron micrograph of two of the cell types of the inner medulla. Although all the granules have an electron dense core, some are characterized by a clear space between the membrane and the granular core (x) while the remainder do not have a distinct membrane (y). ×9600.

were not secretory as described by O'Donoghue *et al.* (1967) but only showed accumulations of small lipid granules in those cells merging with the zona glomerulosa. As no mitotic figures were seen in this layer it is difficult to propose that these cells are giving rise to the zona glomerulosa; yet on morphological structure it appears as though this layer is acting as the pool of cells which differentiate into the cortical cells.

According to Banks (1986) evidence exists that the zona glomerulosa may be responsible for the proliferation and differentiation of cells within the cortex. The significance of this layer is not known, but it is our proposal that the undifferentiated and slightly differentiated cells seen in the lower regions of the capsule are, in fact, stem cells responsible for supplying the pool of cells in the cortex.

The arrangement of cells in clusters in the zona glomerulosa is like that of ruminants and man, but different from that of horses, carnivores and pigs where they appear in curved cords or arcades (Banks 1986). The progression of the cells of the zona fasciculata from foamy pale cells to smaller more intensely eosinophilic cells occurs across a relatively large distance. This region may be indicative of a transitional zone or zona intermedia. The description of a zona intermedia is confusing in the literature, for although a well-developed intermedia is found in the horse, dog and cat, it is said to be present to a lesser degree in the cow, sheep and goat (Dellmann & Brown 1987). Yet Nicander (1952) describes a broad intermediary zone to be present in the horse, cow and rabbit. The cells of this region in the elephant are not undifferentiated as is described, however, for the typical zona intermedia; thus its classification as a zona intermedia may be unjustified. Krumrey & Buss (1969) noted the occurrence of intermediary areas between cortical zones in the adrenal glands of African elephants and likened these to those found in other domestic animals. Although the region between the zona fasciculata and the zona reticularis appeared transitional in the specimens examined in this study, this is believed to be due to the amount of lipid present and not due to the presence of an undifferentiated layer of cells. Similarly, the presence of clusters of cells between the zona reticularis and the medulla could be considered an intermediary zone. Strassberger, Nel & Nel (1990) described, in one specimen from a non-pregnant, non-lactating African elephant, a zone in this region which they called an 'F-zone' and in which two parenchymal cell types occur. Although a similar zone was seen in specimens in the present study, the cells appear to be identical to those of the zona fasciculata.

The arrangement of the medullary cells into two distinct zones is established in the horse, cow, sheep and pig (Dellmann & Brown 1987) and is here described in the elephant. The identification of the granules of the chromaffin cells seen in this study has been made on morphological grounds following Coupland (1965; 1971), and Coupland & Hopwood (1966). These workers noted the loss of amine from adrenaline-storing granules during fixation in aqueous glutaraldehyde, owing to the solubility of the amine and its failure to form a precipitate. This they felt accounted for the moderate electron density of the adrenaline granules, while the noradrenaline which is precipitated *in situ* within the granule with this technique, is very electron dense. The identification of the various types of cells in the inner region of the medulla as all belonging to noradrenaline-containing cells is based on the chromaffin reaction as well as the intensely electron dense granules seen at the electron microscopic level. The appearance of light and dark cells may be due to the stage of the secretory cycle that the cell is in. However, Thureson-Klein, Harless & Klein 1984 and Gorgas & Bock 1976 have reported on a heterogeneity of noradrenaline-containing cells in the mouse medulla. These studies based the identification of different cell types on the different size and structure of the respective types of chromaffin granules. We feel it is essential to confirm these results by means of immunocytochemistry in a future study when the antisera become available.

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