Histology and histochemistry of adrenal glands of Indian mongoose
*Herpestes edwardsii edwardsii* (Geoffroy)*

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Abstract. The histology of the adrenals of the mongoose *Herpestes edwardsii edwardsii* has been studied. Three layers in the cortex, namely zona glomerulosa, zona fasciculata and zona reticularis and central medulla surrounded by the cortex have been observed.

Employing histochemical techniques it was revealed that the cortex is rich in glycoproteins, lipids and protein bound Amino groups. It has moderate amounts of proteins containing sulphydrid and disulphide radicals and tyrosine. Tryptophan has not been detected.

Negligible amounts of mucopolysaccharides were detected in the medulla. Aspects dealing with the occurrence of carbohydrates, proteins and lipids in various regions of the cortex are discussed.

Keywords. Histology; histochemistry; adrenal glands; *Herpestes edwardsii edwardsii*.

1. Introduction

Studies on the smaller terrestrial mammals have been generally confined to rodents. Carnivores seem to have been neglected probably due to the difficulties encountered in their collection and rearing. Histology of adrenal glands have been studied by Meyers and Charipper (1956), Pauly (1957), Holmes (1961), Houser et al (1962) and McKeever and Tomich (1963). Hunt and Hunt (1959) studied the glycogen content in the adrenal glands of rats at different ages and a detailed account of glycogen in adrenals was furnished by Girod (1960). Sinha and Ghosh (1961) gave information on the adrenal cortical cytochemistry in the pigeon. Prasad and Yadav (1974) made observations on the histological and histochemical details of the adrenal glands of the Indian buffalo. Recently Carole et al (1979) studied the histological details of adrenals in newborn alpacus. Our knowledge of the adrenal glands of carnivorous wild mammals is meagre. In this paper an attempt

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has been made to bring out histological and histochemical aspects of the adrenal glands of the Indian mongoose *Herpestes edwardsii edwardsii*.

2. Materials and methods

Mongeoses were obtained from villages nearby Visakhapatnam town and were acclimatized to laboratory conditions. Adrenal glands were removed from the animal and were fixed in Zenker or Bouin's or Susa or formol-calcium. After routine procedures of dehydration and embedding, 5 to 7 μ thick sections were cut. Heidenhain's Azan, Mallory's triple stains were employed to study the histological details. The histological and histochemical techniques were adopted from Gomori (1952), Lillie (1954), Carleton and Drury (1957), McManus and Mowry (1960), Gurr (1962), Barka and Anderson (1963), Humason (1965), Pearse (1968), Culling (1974) and Bancroft (1975).

3. Observations

Anatomically the adrenals of mongoose appear quite regular in shape. The left gland is relatively long and flattened whereas the right one is thick with lateroventral angular borders. Both left and right adrenals lie closely pressed to the dorsal body wall anterior to the kidneys. The caudate lobe of the liver envelopes the right gland whereas the left one is free and is lightly pressed by the pancreas and stomach.

Two regions could be distinguished in the adrenals the outer cortex and central medulla. The gland is ensheathed by thin fibrous capsule. The cortex has 3 layers, the outer zona glomerulosa, middle zona fasciculata and an inner zona reticularis.

The capsule is formed by a combination of collagenous, elastic and reticular fibres. Smooth muscle fibres are also associated with the connective tissue.

The zona glomerulosa has a cellular structure and is delineated from the capsule on the outer side and the zona fasciculata from the inner side. The cytoplasm is basophilic in nature. In this zone the cells are more columnar and arranged in vertical single rows. The cells with a single nucleus which have usually one nucleolus each but some with double nucleoli could be seen occasionally.

The zona fasciculata is the major portion of the cortex with cuboidal cells and some columnar cells. The cells are polygonal in shape and arranged in radiating columns. The cytoplasm is homogeneous and the nucleus is spherical and centrally situated. The size of the nuclei increases progressively towards the medullary part. The cells and their nuclei are larger than those of zona glomerulosa. The cells usually display a single nucleus with a nucleolus, but double nucleated cells also occur (figure 1).

The histological details of zona reticularis are in agreement with those described for other mammals. This region is interspersed with sinusoids of various sizes giving the appearance of a broken network.

To make a clear-cut demarcation between the zona fasciculata and zona reticularis is rather difficult (figure 2). Zona reticularis is well developed in adults than in young ones and a distinct demarcation between the cortex and medulla is notice-
Figures 1-2. 1. Zona fasciculata at a higher magnification showing cell (II) with two nuclei (arrow) and polygonal cell (A) (5 x 40). 2. Portion of adrenal gland showing zona fasciculata and zona reticularis (5 x 40).
Figures 3-4. 3. Medulla protruding into zona fasciculata, A—medulla; B—zona fasciculata (5 x 40). 4. Medulla (5 x 40).
Histology of adrenal glands of Indian mongoose

In adult animals, there is a mixing up of medullary cells with reticularis cells, but the extension of these cells is limited to zona fasciculata only (figure 3). The medullary cells are arranged in irregular rows and the cells are smaller than those of cortical cells. The cells are arranged in irregular groups of 2 to 10 and mainly surrounded by thick strands of interlocking connective tissues and are lightly stained with histological stains when compared to cortex (figure 4).

4. Histochemical observations

The medulla in general gives a moderate reaction with bromophenol blue and Millon's reactions but tryptophan and arginine are absent as evidenced by negative response to p-dimethylaminobenzaldehyde nitrite and Sakaguchi reactions respectively (table 1). Medulla is moderately positive to ninhydrin/Schiff and chloramine-T/Schiff when compared to other protein reactions such as KMnO₄/AB, ferric ferricyanide indicating that large amounts of protein bound amino groups rather than disulphides and sulphydryls, are present. The medulla is positive to lipids (Sudan black B) and phospholipids (copper phthalocyanin). With Congo red it stains moderately indicating the presence of glycoproteins.

Table 1. Histochemical reactions of the adrenal glands.

<table>
<thead>
<tr>
<th>Test applied</th>
<th>Medulla</th>
<th>Zona glomerulosa</th>
<th>Zona fasciculata</th>
<th>Zona reticularis</th>
<th>Capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>PAS/Acetylation</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>PAS/Deacetylation</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>PAS/Methylation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Schiff's alone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alcian blue-1 pH</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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</tr>
<tr>
<td>Alcian blue-2:5 pH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Congo red</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>BPB/Vanslykes</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Millon's reaction</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>DMAB/Nitrite</td>
<td>-</td>
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<td>Sakaguchi</td>
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<tr>
<td>KMnO₄/alcian blue</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Ninhydrin-Schiff</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Chloramine-T/Schiff</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Ferric ferricyanide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Copper phthalocyanin</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

+++ = Strongly positive; ++ = Moderately positive; + = Faintly positive; – = Negative.
Zona glomerulosa is moderately positive to all protein tests showing their presence in small quantities. Protein bound amino groups and basic proteins like tyrosine are present in little amounts, but tryptophan and arginine are absent. This is the lipid rich part of the cortex. Its intense staining with Congo red indicates that it is rich in glycoproteins. But negligible amounts of mucopolysaccharides are noticed. The zona fasciculata is rich in protein bound amino groups but tyrosine is present only in moderate amounts. This part of the cortex like other parts is devoid of tryptophan and arginine. Moderate amounts of sulphhydrils and disulphides have been localised. Phospholipids are abundant in this area. No mucopolysaccharides have been detected, but glycoprotein is present in abundance.

The reticular zone is rich in tyrosine but is devoid of mucopolysaccharides or basic proteins containing arginine and tryptophan. The protein bound amino groups, sulphhydrils and disulphides are present in moderate amounts. As in the case of adrenals of other mammals, this region displays mild amounts of lipids.

5. Discussion

In the zona glomerulosa the presence of double nucleolated cells was also observed in the Indian buffalo by Prasad and Yadav (1974). In the ferret (Holmes 1961) and in the Indian buffalo (Prasad and Yadav 1974) it was observed that zona glomerulosa took lighter stain with histological stains than the cortical layers, an observation which is in agreement with the present findings. This may be due to glucocorticoids that are secreted by the zona glomerulosa which take lighter stain.

The presence of faintly stained cytoplasm in this zone also agrees with the condition reported by Meyers and Charipper (1956) for the golden hamster, by Hewer and Foster (1966) for man, Holmes (1961) for ferret and Houser et al (1962) for Panama monkeys.

McKeever and Tomich (1963) observed an arc of cells at the capsular end in Herpestes auropunctatus in mature females but this condition could not be seen in the present study. The zona fasciculata occupies the major portion of the cortex in mongoose as is the case in bulls (Cupps et al 1954; Das et al 1965) and in Indian buffalo (Prasad and Yadav 1974). This is attributed to the fact that this may be synthesizing and secretory zone for steroidal hormones. McKeever and Tomich (1963) reported that in Herpestes auropunctatus there is a clear demarcation between inner and outer fasciculata in sexually active female, which could not be corroborated in our study on Herpestes edwardsii edwardsii, as our observations were made on females in captivity. Estrogen secreting activity may augment the bulk and reactivity of zona fasciculata which actually forms a band of cells and could be considered the estrogen secreting zone. During the course of development this estrogen secreting zone may extend into zona glomerulosa side constituting distinct zona intermedia—a condition which occurs in Indian buffalo.

Progressive increase in size of the cells in deeper parts of zona fasciculata has been reported by Copenhaver (1964) in man and by Prasad and Yadav (1974) in Indian buffalo. The larger sizes of the nuclei and cells in zona fasciculata are in
agreement with the findings in bull and bullock (Cupps et al 1954; Sohal and Chaturvedi 1962). However, Hartman (1959) found that the cells of this zone were smaller than those of zona glomerulosa in the adrenal glands of the sloth.

The zona reticularis is comparatively better developed in adult animals than in young ones, thus agreeing with the observations of Prasad and Yadav (1974) in Indian buffalo. As far as mixing up of cells in the medulla is concerned Holmes (1968) also found this condition in Macaca mulatta but Prasad and Yadav (1974) state that medullary cells migrate up to the level of zona glomerulosa.

The medulla in general is rich in lipids obviously because of the principal secretions of medulla, adrenaline and noradrenaline. A positive ninhydrin/Schiff reaction is due to free amino group in adrenaline and noradrenaline. As a whole the cortex displays a comparatively more intense reaction for proteins. After deamination with van Slykes reagent cortex as well as medulla became negative to bromophenol blue and other basic protein tests.

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