A Comparative Histological Survey of the Avian Adrenocortical Homologue*

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Summary. The histological characteristics of the adrenocortical tissue of twenty-two species of birds belonging to seventeen orders are described in this report. This histological survey serves to show that there are structural peculiarities associated with different species, and wide variations with regard to cytomorphism, nuclear polarity and other cyto logical features can be distinguished. On the basis of histomorphic organisation the avian interrenal can be classified into six structural categories illustrating specialisation into two zones, and a differentiation from a relatively simple to more complex types. Thus, birds like the chick, swift, crow and the snipe (type I) show no zonal specificity in their interrenal tissue. Histomorphic zonation in the periphery and the centre is first recognizable in the adrenals of the myna, sparrow, pigeon, kite and the nightjar (type II). A moderately differentiated zonal pattern is found in the kingfisher, egret, cuckoo, duck, owl, stint, cormorant, waterhen and the woodpecker (types III and IV). Types V and VI include the dove, quail, flamingo and parakeet, and represent an extreme cytological zonation in their interrenals. This investigation raises the possibility that the interrenal organ in many birds possesses a zonal specificity. No phylogenetic trends are apparent from the study of these interrenal patterns.

The adrenocortical morphology in several avian species has been described by a number of authors and attempts were also made to delineate morphologic zonation in this tissue. Histological characteristics of the adrenals have been thoroughly studied in the pigeon (MILLER and RIDDLE, 1942; SINHA, RAY and GHOSH, 1959), the chick (LATIMER and LANDWER, 1925; UOTILA, 1939; KAR, 1947a, b; SIVARAM, 1965), the domestic dove (MULLER, 1929), the brown pelican (KNOUFF and HARTMAN, 1951), the duck (BENOIT and ASSENMACHER, 1953; ASSENMACHER, 1958), the blackbird (FROMMEBOUMAN, 1962), the whitecrowned sparrow (LORENZEN and FARNER, 1964), the house sparrow (BHATTACHARYYA and GHOSH, 1965) and the eastern rosella (HALL, 1968). Some of these investigators also tried to explore histological zonation inside the adrenocortical tissue. In the fowl the peripheral and central cortical masses were found to be dissimilar in size and arrangement (LATIMER and LANDWER, 1925; MULLER, 1929). KAR (1947a, b) observed in the fowl larger cortical cells with numerous mitochondria at periphery while central cortical cells were smaller with fewer mitochondria. Similar differences were also noted in the chicken adrenals (SIVARAM, 1965). In the pigeon, peripheral interrenal cells were reportedly large with considerable

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amounts of cytoplasm; small elongated central cells had moderate quantities of cytoplasm (Miller and Riddle, 1942; Sinha et al., 1959). In the brown pelican, Knouff and Hartman (1951) claimed that cortical tissue is organised into three zones which correspond in position and appearance to three zones of eutherian adrenal cortex. Sturkie (1965) commented that avian adrenocortical tissue is not zonated except in the brown pelican.

Available literature suggests that morphologic zonation has been recognised only in a few species, and it is worth while to collect more information about this much dedated question of avian adrenocortical morphology. This study was undertaken to probe the histological organisation of the corticoadrenal component in twenty-two species of birds belonging to seventeen orders. The aim was to note the extent of variation as regards morphology and zoning of cortical cells in different birds. Assessment of basic histologic pattern and cytomorphic zonation of avian adrenocortical tissue would also help to determine the suitable types for cytophysiological experimentation.

Table 1. Synoptic classification of birds used in this survey

<table>
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<th>Common and scientific names</th>
<th>Order</th>
<th>Family</th>
<th>No. of specimens</th>
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<td>PELECANIFORMES</td>
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<td>2) Common kite</td>
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<td>3) Cattle egret</td>
<td>CICONIIFORMES</td>
<td>Ardeidae</td>
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<td>(Bubulcus ibis)</td>
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<td>7) Common chick</td>
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<td>9) Waterhen</td>
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<td>(Amurornis phoenicurus</td>
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<td>10) Common snipe</td>
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<td>11) Little stint</td>
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<td>(Calidris minutus)</td>
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<td>12) Spotted dove</td>
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<td>(Streptopelia chinensis)</td>
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Materials and Methods

Birds of both sexes trapped from nature were procured through local animal dealers. Before autopsy they were adapted to laboratory conditions for a few days. After killing the birds by cervical dislocation, the adrenals were quickly dissected out and placed in Bouin's or Bouin-Holland's fluid for 24 hrs and processed subsequently for routine microtomy. Serial paraffin tissue sections, 5 µ in thickness were cut in midsagittal plane. Most of the slides were stained with Masson's trichrome, Heidenhein's azan and hematoxylin-erythrosin-fast green sequence. Occasional slides for each specimen were stained by hematoxylin-eosin and Mallory's triple methods.

The classification of 22 species of birds representing 17 orders and 19 families (see Table 1) used for this study follows Biswas (1953). Scientific names have been checked after Whistler (1953) and Ali (1964).

Observations

The adrenal gland in birds is ensheathed by an outer connective tissue capsule and consists of two distinct elements which are homologous with the cortex and medullary counterparts of the mammalian adrenal. The adrenocortical homologue in birds has been termed in literature variously as cortical tissue or adrenal cortex or interrenal element and that corresponding to the mammalian adrenal medulla has been referred as chromaffin or "medullary" tissue (Hohn, 1961). In the bird adrenal, the two elements are intermingled to a varying degree without forming a sharply defined cortex and medulla (Barrington, 1963). The interrenal tissue is made up of cortical cords which loop against the outer connective tissue capsule. The basic unit of the cortical tissue is a solid cylindrical strand of cells which is typically composed of a double row of columnar or cuboidal cells with their long axes in the transverse plane of the strand. This basic cytological composition is, however, not uniformly maintained and deviations were noted during comparative histological study. The sequential arrangement of the species described has been made according to similarities in cytomorphic pattern.

Chick

The adrenocortical tissue is distributed throughout the gland in the form of irregularly arranged cords frequently anastomosing with one another and interspersed with groups of medullary cells. In the periphery cortical cords loop against the outer connective tissue capsule and small round or oval cortical cells do not have any regular orientation. Central cords have a random arrangement of cells and regularly oriented rows of nuclei are placed a little away from the basement membrane. Cortical cytoplasm is acidophilic and granular. Abundant numbers of nuclei are normally present in a cord. Zonal difference between subcapsular zone (SCZ) and underlying central zone (CZ) is imperceptible. The histology of the fowl interrenal has previously been described by Kar (1947a, b).

House swift

The interrenal tissue is comprised of cords with two transversely placed cellular layers. Cords are voluminous and have abundant granular cytoplasm. Nuclei
Fig. 1-6. Photomicrographs of sections of the adrenal gland of birds. 1. Crow: undifferentiated cortical tissue appearing as vacuolated deep staining masses. 2. Snipe: small tubular cortical cords similar from periphery to centre, chromaffin islets appearing as white patches. 3. Myna: cord in the form of straight tube looped beneath the capsule a part of which appears at upper left corner. Nucleoli of cortical cells very prominent. 4. Sparrow: central interrenal cords are broad masses and merging into one another, medullary islets very deep stained. 5. Cuckoo: tubular cortical cords showing zonal difference. 6. Duck: organisation of SCZ and CZ strikingly different. Capsule in each figure is present on top margin. ×170
sometimes are placed in two rows parallel to each other or randomly distributed throughout a cord. Subcapsular cortical layers are in the form of solid acini, small in size with irregularly placed nuclei. General appearance of peripheral and central cords is very much similar.

House crow

This species displays some unusual variations of the cortical parenchyma. Cortical units at the periphery are in the shape of triangular or round cellular acini, small in size with small round interrenal cells and a number of nuclei. Continuing downward are central cortical masses with dense granular cytoplasm and very large vacuoles reminiscent of lipid globules (Fig. 1, 11). Nuclei are deep staining and do not have any regular arrangement. The crow has a very undifferentiated and somewhat atypical cortical tissue.

Snipe

The interrenal tissue is comprised of cortical cords extensively intermingled with each other. Cords from periphery to centre are similar throughout (Fig. 2) and short tubular cords are continued for a small distance. Interrenal cells are small cuboidal with a round nucleus and a prominent nucleolus. Nuclei are located close to the basement membrane and also randomly dispersed in a cortical cord. Presence of cytoplasmic vacuoles in cortical cells ("clear cells") throughout the adrenal gland is a notable feature.

Myna

In subcapsular cortical cords of this bird cells are irregularly arranged while in deeper central cords they are placed in two parallel rows (Fig. 3). Cords appear dense with coarse granular cytoplasm. The nucleus is round, sharply stainable and has conspicuous nucleoli.

Sparrow

Description of the histology of the interrenal tissue component of the house sparrow has been already given by BHATTACHARYYA and GHOSH (1965). Beneath the capsule cortical strands start looping and are continued as broad cortical masses inside the parenchyma. Nuclei are flattened or crumpled almost adjacent to the basement membrane (Fig. 4, 12). Oblong or round interrenal cells have fine granular cytoplasm. Cortical cords are almost continuous everywhere merging into one another, thus forming wide bulky masses. Interrenal cells have a double layer arrangement specially in CZ. SCZ is small showing little difference from the central region.

Pigeon

The adrenal cortex of the pigeon has been extensively studied by MILLER and RIDDLE (1942), SINHA et al. (1959), BHATTACHARYYA and GHOSH (1963), BHATTACHARJEE and GHOSH (1964) and BHATTACHARYYA (1968). Cortical cords are made up of two layers of cells, generally tall columnar containing more or less granular cytoplasm. Large peripheral interrenal cells have considerable amount of cytoplasm and small
elongated central cells have moderate quantity of cytoplasm. Peripheral nuclei are larger than central ones. Large oval or rounded nuclei are usually arranged towards the basement membrane in peripheral cords; in central cords they are away from the basement membrane.

Kite

Subcapsular cords which loop down are bulging structures continuing into slender double layered cellular cords. In general, the interrenal resembles that of the pigeon.

Nightjar

Two layers of interrenal cells generally constitute a cortical cord. Beneath the capsule bilayered wide cords start looping. Nuclei are situated in a row along the basement membrane at periphery and away from the basement layer in central cords. Cells are tall columnar with well granulated sharply stainable nuclei. Morphological difference between SCZ and CZ is small.

Kingfisher

In the peripheral zone, "spongy" cells have no definite orientation and a large number of nuclei are randomly dispersed in broadened subcapsular strands. In the more central region smaller cortical cords individually continue for a small distance and merge into one another. Central cortical strands have a vacuolated core.

Egret

Massive areas of cortical tissue occupy most of the gland in this species. Peripheral cords are gigantic cellular columns which either form huge loops immediately beneath the capsule in the shape of an inverted ‘U’ or form a dense mass constituted of largely interanastomosed cortical cords. Nuclei abundant in number are situated close to the basement membrane. Further down below the cortical loops are cylindrical wide cords with two layers of cells having basally placed nuclei. Interrenal cells have a spongy vacuolated appearance particularly in the periphery. Making extensive bends cortical cords frequently anastomose with one another. Cells are tall, columnar with round well granulated nuclei.

Cuckoo

SCZ and CZ differ structurally in this species. At the SCZ a number of tubular cords are intermingled; cortical cells are low columnar or cuboidal having no definite orientation. A contingent of peripheral acini are merged parallel and close with one another and a large number of cortical loops descend simultaneously into the centre. Central loops are typically constituted of two rows of vertically placed columnar cells. Subcapsular cortical cells are clear degranulated in appearance with larger sharply stained nuclei. Central cortical cells are tall columnar with strongly acido-philic cytoplasm and round vesicular nuclei placed in a row near the basement membrane (Fig. 5, 13).
Fig. 7-10. 7. Waterhen: cellular organisation of CZ markedly differs from that of SCZ cords which enclose a lumen. 8. Woodpecker: SCZ poorly organised, CZ neatly constituted. 9. Quail: bulging SCZ continued as straight tubular cord into the centre—a conspicuous dimorphic zonation. 10. Parakeet: peripheral cords showing extreme convolutions. ×170
Duck

At the SCZ cortical cords merging into broad strands are clear in appearance, start looping beneath the capsule and are placed vertically; these are sharply demarcated from underlying CZ cords by histologic characteristics (Fig. 6). Central cords are much smaller, extensively convoluted and interwining.

Owl

A characteristic pattern in the interrenal of this species is the nuclear arrangement of cortical cells. Slender tubular cords with finely granular cytoplasm show frequent convolutions and nuclei are situated near the core of a strand in regularly oriented double rows and separated by an intervening vacuolar space. This nuclear arrangement is persistent except beneath the capsule. Subcapsular cells form bulging structures with nuclear conglomeration. Morphologic difference between SCZ and CZ can be detected by nuclear pattern and distribution.

Stint

The SCZ in this bird is a very narrow area just beneath the capsule followed by deeper central layers. SCZ is comprised of tubular round small cords containing heaps of irregularly distributed nuclei. Central cords are short, voluminous and intermingled with one another. Histologic zonation is not apparently impressive the SCZ being a very small area, while the CZ is very massive.

Cormorant

The cortical tissue constitutes the bulk of the total glandular parenchyma. Cortical cells belonging to the subcapsular region of the gland possess various shapes and are packed together into a mass. No definite arrangement of cells in the strands of this region can be found. In deeper central strands tall columnar cells constitute two regularly disposed tiers.

Waterhen

There is considerable difference between peripheral cords lying immediately below the capsule and the central cortical masses. Subcapsular cortical cords are round masses having heaps of nuclei assembled together and without having very regular cellular arrangement. Peripheral strands in the form of bulging structures often enclose a lumen, and are continued into deeper more central double layered cortical strands. Central cortical cells are very tall, columnar having nuclei in the core of a strand (Fig. 7, 14).

Woodpecker

Interrenal cells of the SCZ are low cuboidal with granular cytoplasm and present no definite orientation. Central strands are voluminous and tubular, consisting of columnar cells with nuclei situated away from the basement membrane towards the core (Fig. 8, 15).

Dove

The SCZ consists of bulging cortical masses having randomly dispersed nuclei
and irregularly scattered interrenal cells. Sometimes these cortical areas enclose an open cleft. The SCZ with one or two layers of cells continues down into acidophilic central cords comprised of two parallel rows of columnar cells. The central cells have considerable amount of cytoplasm and centrally located nuclei.

**Quail**

In this species some significant features are noted in the interrenal. Cortical strands are elongated, slender, wavy or branched except at the periphery. In the SCZ cortical loops are bulging structures consisting of a huge conglomeration of numerous cells. Low cuboidal cells of the subcapsular loops have a random arrangement giving a syncytial appearance. Vacuolated cells are commonly found in this area. Peripheral loops traverse deep into the centre forming cylindrical slender cortical tubules (Fig. 9). Central cortical cells are high columnar with nuclei placed in two double rows closely approximated at the centre. Midcortical interrenal cells have dense cytoplasm. Difference between the SCZ and CS is very pronounced.

**Flamingo**

The massive interrenal tissue of this bird can be clearly distinguished into two zones. The SCZ is very extensive occupying the major portion of the cortical parenchyma. This consists of massive compressed columns descending radially from the capsule and after continuing for a considerable distance into the centre, these form shorter cortical strands. Cortical cells of this very big SCZ are of the columnar type with numerous nuclei. Central loops are very slender, frequently convoluted and extensively inter-anastomosing, and enormous sinusoid spaces are present around their convolutions.

**Parakeet**

A conspicuous difference in structure and organisation between SCZ and CZ is found in this bird. Peripheral cords are highly convoluted and closely intermingling with several septa in between them. Convolution and anastomosis of loops lead to the formation of a distinct and massive subcapsular zone (Fig. 10). Central cortical strands are simple cylindrical structures which may interanastomose. Sometimes these cords become convoluted and form the shape of acini consisting of angular or columnar cells. Nuclei are elliptical, very abundant and dispersed throughout a cord. Central cortical cytoplasm is densely basophilic in nature. Peripheral cells have a vacuolated appearance and well marked cell membrane. Some degranulated cortical cells have a 'blown up' clear appearance (compare, snipe) (Fig. 16).

**Discussion**

Certain distinctive patterns in the organisation of cortical cords could be deduced from this comparative study. The interrenal component presents inconspicuous zonation in some birds; in others it is organised into sharply demarcated regions differing in cellular topography. Increasing grades of differentiation between SCZ and CZ and several stages of morphologic differentiation of the cortical tissue can be elucidated amongst the various species studied. As illustrated in Figure 17 the
Cytomorphologic patterns associated with the avian interrenal can be classified into several types (Table 2.)

No phylogenetic trends can be detected in these interrenal patterns obtained from the study employing twenty-two species of birds belonging to seventeen avian orders. Even within family groups morphologic disposition of interrenal tissue can be variable (columbiformes: pigeon and dove; galliformes: chick and quail). Rather a characteristic pattern seems to be typically associated with a species. The transition from a simple organisation to increasing complexity is apparently independent of the evolutionary status of the avian species concerned. Many birds belonging to Passeriformes (believed to be phylogenetically recent, see Gregory, 1957) possess an archtypal structure of the cortical tissue (i.e., crow, myna). Several primitive birds ( egret, flamingo) have morphologically complex cortical tissues. From a histological study of the reptilian adrenal cortex, HEBERD and CHARIPPER (1955) however, deduced certain phylogenetic relationships among various lizard groups. The present survey also reveals that only two zones inside the interrenal could be morphologically distinguished, and in no avian species could the three specialised regions as described in the brown pelican (Hartman et al., 1954) be recognised.

How far the morphologically divisible cellular zones are related to each other with regard to secretory capacity has been already subjected to much investigation. On the basis of chemical cytology, GHOsh (1962) detected a bizonal differentiation pattern in the distribution and concentration of lipids, cholesterol and alkaline phosphatase in subcapsular and central zones of several avian species. Differential response of the various areas of the adrenal cortex to adenohypophysectomy, corticoid therapy or salt loading has been described in the pelican, the pigeon, the cockerel, the duck, the quail, the parakeet and the myna (Hartman, Knouff and Howard, 1954; Assenmacher, 1958; Miller, 1961; Resko, Norton and Nalbandov, 1964; Sinha and GhoSh, 1964; Bhattacharjee and GhoSh, 1964; Kondics, 1965; Peczely, 1966; Frankel, Graber and Nalbandov, 1967; Miller, 1967; GhoSh and Bhattacharyya, 1970; Bhattacharyya and GhoSh, 1970, 1972), and confirms the possibility that the avian adrenal gland has regional functional zonation. Experimentation with still other avian species having various morphologic types of interrenal tissue would lead to generalisation about physiologic adrenocortical zonation.

### Table 2. Structural gradation of avian interrenal tissue

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<tr>
<th>Type</th>
<th>Morphologic grade of differentiation</th>
<th>Species</th>
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<tbody>
<tr>
<td>I</td>
<td>Primitive—no differentiation</td>
<td>Chick, Swift, Crow, Snipe</td>
</tr>
<tr>
<td>II</td>
<td>Zonal difference recognisable</td>
<td>Myna, Sparrow, Pigeon, Kite, Nightjar</td>
</tr>
<tr>
<td>III</td>
<td>Zonally differentiated</td>
<td>Kingfisher, Egret, Cuckoo, Duck</td>
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<td>IV</td>
<td>Clearly differentiated zones</td>
<td>Owl, Stint, Cormorant, Waterhen, Woodpecker</td>
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<td>V</td>
<td>Conspicuous zonation</td>
<td>Dove, Quail</td>
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<tr>
<td>VI</td>
<td>Extreme differentiation</td>
<td>Flamingo, Parakeet</td>
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Fig. 17. Diagrammatic representation of the structural types of avian interrenal tissue (see discussion for details). In each figure, upper line denotes capsule; SZ and CZ signify subcapsular and central zones respectively. Figures drawn from slides projected on the screen of a visopan microscope. ×60
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Lorenzen, L. C. and D. S. Farner: An annual cycle in the interrenal tissue of the adrenal gland of


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