PART II.

SEASONAL HISTOPHYSIOLOGIC STUDY OF THE INTERRENAL OF THE HOUSE SPARROW*

INTRODUCTION

Variations have been noted in the adrenal cortex of various vertebrates due to seasonal fluctuations and a close relation has been found between reproduction and increased cortical activity [8]. There is some gross morphological evidence that the avian interrenal undergoes seasonal changes related to the sexual cycle. RIDDLE [10] observed that increased adrenal weight was coincident with the ovulation in doves and pigeons. In the brown pelican, it was noted that the largest adrenals were taken from both sexes in breeding conditions [6]. In the female mallard, the ratio of interrenal volume to total gland volume increased during the breeding season [4]. BUSHEIKIN [5] showed that, in house sparrow, the percentage volume of interrenal tissue changed from 76% in the non-breeding season to 90% in the breeding conditions.

Besides the work of FROMME-BOUMAN [2], where the interrenal activity has been assessed by karyometric methods — possibly no other report contains any data on birds based on cytological observations. In view of this, it seems desirable to reinvestigate the problem of avian gonad-interrenal relationship on a cellular level employing cytological and cytochemical techniques. We have chosen the male house sparrow

*Very recently, Köhn et al. [18] have shown that a seasonal adrenal weight cycle is apparent in both sexes of the mallard with a weight increase related to the breeding season and another increase during the autumn and winter.
Passer domesticus for this purpose, as the gonadal cycle is pretty well-known [11]. In the present study, observations were pursued throughout the year on the cytological and chemocytological changes in the suprarenal cortex of the male house sparrow and an attempt was also made to correlate the above findings to the phenomenon associated with the sexual cycle of the species.

MATERIAL AND METHODS

Observations were made from a total of 123 male sparrows during a one-year period extending from December, 1962 to December, 1963. Birds trapped from a natural population were procured and sacrificed by decapitation within a day or two after capture, approximately twice a week, after recording body weights. The adrenals were quickly dissected out, weighed in a torsion balance and fixed in appropriate fixatives for further processing. Histological preparations were made from 4-5 μ paraffin sections and stained after HEIDENHEIN’s Azan and CASSON’s staining methods. Histochemical methods used in the study were as follows: i) GOMORI’s method for alkaline phosphatase [3]; ii) Silver nitrate method for adrenal ascorbic acid [1]; iii) PAS technique for localisation of polysaccharides [9]; iv) Sudan III and IV, and SBB technique for sudanophilia [9]; v) SCHULTZ method for cholesterol [9]. Width of average cellular cords in various
months were measured with an ocular micrometer at 120 X magnification from the sections made in the largest sagittal plane of the adrenals. Sections from at least four birds from each month were measured.

RESULTS

Gravimetric changes

Average weights of the adrenals (both absolute and relative) of birds autopsied in different seasons are elucidated in Table I. A perusal of this table reveals that the variation in absolute and relative adrenal weights in the yearly cycle is not very conspicuous. The data, however, point to a retarded weight of suprarenals during the coldest months (i.e., December to February).

Seasonal changes in histochemical pattern

Seasonal changes in various microchemical cortical components have been summarised in Table III. Cortical activity on the basis of abundance and degree of staining of various histochemical reactions have been subjectively graded in the table. Detailed observations on a month-wise basis were noted as follows.
SUDANOPHILIA. Sudan-positive total lipids are present throughout the adrenocortical masses uniformly from periphery to centre; only in January, May-June and August, lipids are rarified in 2-3 subcapsular strands. Lipid droplets are usually present in a row around the basement membrane, while in the core of a cortical strand they are dispersed unevenly. Distribution of total lipids shows a maximum concentration in November through January. In February, the cortex is totally devoid of sudanophilia showing an abrupt increase in March. This increased staining pattern is more or less maintained onwards from March to July. A depletory behaviour is noted in August-September, before returning to progressively maximal values in October-November.

CHOLESTEROL. The distribution and concentration of cortical cholesterol are essentially similar when compared to that of sudanophilic lipids. A zonal distribution of this chemical moiety (i.e., low concentration in subcapsular and high in mid-cortical) is also noticed in certain months (January, May and June).

ALKALINE PHOSPHATASE. Large amounts of histochemically discernible cortical glycerophosphatase are present in the months of September through January. Cortical enzymatic activity becomes low in February, positive throughout March to July, with minor fluctuations within this period. In early August, there is a decline in phosphatase activity, being
augmented in late August. September represents again a steady increase in enzymatic pattern. An absence of cytoplasmic phosphatase from the subcapsular region in the months of January, February and July gives a picture of enzymatic zonation.

**P A S R E A C T I O N.** The cytochemical pattern of PA-Schiff reaction in the sparrow suprarenal cortex does not vary too markedly throughout the span of a twelve-month period. In general, the cortical cytoplasm gives a moderately intense, homogeneous staining reaction. The latter, however, appears to be decreased in the months of June-July and October through December. PAS positive granules presumably belonging to Golgi zone do not manifest any change as a response to seasonal and/or reproductive factors. Basement membrane and reticular connective tissue show purple colour reaction throughout the year but stain brilliantly in March to May and again in August-September.

**A S C O R B I C A C I D.** Following fixation in alcoholic silver nitrate solution, the adrenocortical cells of the sparrow show vitamin C granules concentrated mainly in peripheral interrenal cells. These are distributed in the form of irregularly scattered fine granules and are very sparse in central cortical cells. Barring two months (February and May), this picture is represented in all the other months studied. A negative AA reaction is observed in February and May.
Seasonal Histological Changes

The sparrow adrenocortical tissue presents a typical double cell-layer arrangement (cuboidal cells oriented in double rows). The peripheral capsular zone starts as straight looped tube which merges with more central 3-layered cortical cords. Nuclei are generally round and located a little away from the basement membrane and arranged in a characteristic double row in a single cortical loop. A histologic observation of this pattern is followed throughout the span of one year.

JANUARY - JUNE: The cortical tissue as a whole gives an appearance of high activity throughout these months and from January onwards, there is a progressive increase in the width of cortical cells and cellular cords. In January, cords appear slightly more enlarged than in December, specially at periphery (Fig. 1). This hypertrophy of the cortex is further augmented in February when the cortical cells exhibit individual outline clearly. Vesicular nuclei are larger than that in January. In March, the expanded cortical masses exhibit a granular cytoplasm with regularly arranged vesicular nuclei staining sharply. In April, May and June, the same hypertrophic cortical picture is maintained, but there is an increase in vascularity of the gland. Moreover, regular arrangement of cortical masses is lacking with frequent anastomosis of cortical cords.
JULY - SEPTEMBER: The enlarged appearance seen in the preceding months becomes lacking with the onset of July. The gland is hyperemic as seen previously, but cortical cords appear to be more conspicuously anastomosed. Nuclei are sometimes regularly arranged or scattered but they tend to have great staining affinity for acidic dyes like azocarmine and are very deep staining though the nuclear contents are not sharply distinguishable. Moreover, nuclei have lost vesicular appearance of the summer months. In September, a great compression is noted from the periphery inwards (Fig. 2). Nuclei are irregularly arranged and cytoplasm/vacuolated.

OCTOBER - DECEMBER: The compression of the cortex seen in September is relieved in October. There is a fairly regular disposition of cords looping inwards from subcapsular region and anastomosis is lacking. A gain in width of cortical cords is observed. Nuclei are vesicular, round with prominent chromatin material, arranged not in a 'typical double row' and placed nearer to the basement membrane. November and December maintain this picture with slender cords slightly anastomosing. Cytoplasm is a little granular. Nuclei are large, regular with finely dispersed chromatin and are situated in cuboidal cells. This histologic picture is maintained up to December and is progressively altered in January.
Round-the-year follow-up of the sparrow adrenal cortex reveals noticeable histological and histochemical differences in the cortical parenchyma. Variations in the cortical activity are mainly apparent in changes in vascularity, width of cellular cords, nuclear diameter (Table I), cortico-medullary ratio (Table II) and differences in chemocytologic picture (Table III). For greater part of the year (October to June), the interrenal tissue is considerably hypertrophic than it is in the sexually regressive phase. During this period, cords become narrow (about 36 μ in diameter, as against 40-47.8 μ of the breeding phase), nuclei less voluminous (11.8 - 12.6 μ in diameter as against 13.3 - 15.1 μ) and the general cortical area much diminished. The spermatogenic cycle of the common Indian sparrow, as worked out by SARKAR and GHOSH [11] has established that after testicular collapse from mid-July, there is a short quiescent period in August and September. Following is a phase of rapid spermatogenesis, giving a wide plateau of regular spermatogenic activity which ends

* A concomitant change in nuclear area and the cortico-medullary ratio with the functional status of the interrenal tissue in European black-bird has been shown by FROMME-BOUMAN [2].
eventually in testicular regression. These findings indicate that almost complete parallelism exists between adrenocortical and testicular cycles in the house sparrow (Fig. 3).

An endocrinological interpretation of our results may be given in the light of MeKEEVEER's work on Microtus adrenals. He suggested that the cessation of reproductive activity in females induces accumulation of hypophyseal FSH and LH unaccompanied by a gain in pituitary weight. This then becomes a causative factor in lowering down the ACTH titre of the pituitary. The meagre secretion of the corticotrophin finally results in a cortical atrophy. This mechanism, acting through the gonad-adrenal-pituitary axis, does not hold good in case of male Microtus. There is a fair possibility that the hormonal control of the adrenocortical atrophy in Passer following a reproductive regression may very well be similar to that described for the female Microtus. The responsiveness of the adrenal cortex to gonadal stimulation and regression in females of this species would be an interesting item for future study.

Histochemical observations on fluctuations in cortical substances throughout the year more or less seem to corroborate other findings. However, a depletory trend of the major cortical components is noted in February, which remains unexplained at present. A possibility cannot be ruled out, however, that these secretory changes might have been influenced to an extent by seasonal climatic variations.

The research of the senior author (T.K.B.) was supported by a grant from the University Grants Commission. The valuable assistance of Dr. Ira Ghosh in determining C:M ratio of some birds is also appreciated.
Table I. Seasonal changes in adrenal weights, cortical width and nuclear diameter of the sparrow.

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<tr>
<td>Average Absolute wt. of adrenals (mg)</td>
<td>2.00</td>
<td>1.80</td>
<td>2.60</td>
<td>2.23</td>
<td>2.30</td>
<td>2.40</td>
<td>2.36</td>
<td>2.30</td>
<td>2.90</td>
<td>2.45</td>
<td>2.50</td>
<td>2.00</td>
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<tr>
<td>Relative weight (mg/100gm body wt.)</td>
<td>10.10</td>
<td>8.65</td>
<td>12.38</td>
<td>10.61</td>
<td>14.50</td>
<td>12.00</td>
<td>11.09</td>
<td>14.54</td>
<td>13.88</td>
<td>11.92</td>
<td>12.11</td>
<td>9.00</td>
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<tr>
<td>Body weight (gm)</td>
<td>19.66</td>
<td>20.80</td>
<td>21.00</td>
<td>21.00</td>
<td>20.50</td>
<td>20.00</td>
<td>20.60</td>
<td>19.80</td>
<td>19.25</td>
<td>20.83</td>
<td>20.62</td>
<td>20.50</td>
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<tr>
<td>Width of average cortical cord (in (\mu))</td>
<td>40.00</td>
<td>47.80</td>
<td>43.20</td>
<td>44.31</td>
<td>44.31</td>
<td>41.40</td>
<td>36.00</td>
<td>36.31</td>
<td>37.32</td>
<td>41.00</td>
<td>41.70</td>
<td>40.00</td>
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Table II. Seasonal variation in cortico-medullary areas*

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<tbody>
<tr>
<td>% of Cortex</td>
<td>70</td>
<td>68</td>
<td>66</td>
<td>65</td>
<td>74</td>
<td>60</td>
<td>61</td>
<td>69</td>
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<tr>
<td>% of Medulla</td>
<td>30</td>
<td>32</td>
<td>34</td>
<td>36</td>
<td>26</td>
<td>40</td>
<td>39</td>
<td>31</td>
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* Unfortunately, we have no data for the months of Feb., May-June and Nov.*
### Table III. Seasonal changes in histochemical reactivity of the adrenal cortex of the sparrow.

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<tr>
<td>anophilia</td>
<td>3 +</td>
<td>1 +</td>
<td>3 +/2 +</td>
<td>1 +/2 + (late Sept.)</td>
<td>2 +</td>
</tr>
<tr>
<td>phosphatase</td>
<td>3 +</td>
<td>1 +</td>
<td>3 +</td>
<td>1 +/2 +</td>
<td></td>
</tr>
<tr>
<td>lesterol</td>
<td>3 +</td>
<td>1 +</td>
<td>3 +</td>
<td>1 + (Aug.)/</td>
<td>1 +/2 +</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 + (early Sept.)/</td>
<td></td>
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<tr>
<td>orbic acid</td>
<td>2 +</td>
<td>1 +</td>
<td>2 +/1 +</td>
<td>1 +/2 +</td>
<td>1 +</td>
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Legends: ± * very weak or nearly negative reaction; 1 + : weakly positive; 2 + : moderate to strong; 3 + : very strong; Cyt: Cytoplasm; CT: Connective tissue.
1. Histological picture of the interrenal tissue of the sparrow in January. A progressively hypertrophic picture is maintained during the breeding phase. x 900.

2. The interrenal in the month of September. A picture characteristic of the non-breeding season. x 900.
3. Annual adrenocortical karyometric variation of the house sparrow in comparison with testicular rhythm. C = cortex, M = medulla.
SUMMARY

The seasonal variations in the adrenocortical activity of the common house sparrow (Passer domesticus) have been investigated by karyometry and cytochemical methods. Cytological analysis of the suprarenal (as evaluated by C:M ratio, cortical width, and nuclear volume) throughout the year reveals a considerably hypertrophic cortical tissue during the breeding phase of the sparrow, while there is a regressive trend in the non-breeding season. Chemical components of the cortex show fluctuations throughout the year that corroborate cytological picture. The close parallelism between cortical activity and sexual rhythm of the male sparrow has been indicated and discussed.

REFERENCES


ADDENDUM

Since this paper was written, an excellent paper has appeared concerning the annual interrenal cycle of a migratory passerine species, *Zonotrichia leucophrys gambelii* (Lorenzen, L. C. and Farnen, D. S.: An Annual Cycle in the Interrenal Tissue of the Adrenal Gland of the White-Crowned Sparrow, *Zonotrichia* *leucophrys* *gambelii*). Not included in original MS.
In this species, the relative fractional volume of cortical component (FCV) and the relative density of sudanophilic lipids (SI) are found to be highest in winter and spring, and lowest in the summer months. The changes in FCV and SI are also beautifully correlated with the microanatomic alterations in the interrenal tissue of the white-crowned sparrow.