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Pattern of Distribution of Epinephrine and Norepinephrinecellsin the Adrenal Gland of Kuttanad Ducks (*Anas Platyrhynchos Domesticus*) during Post Hatch Period

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Abstract:
The study was conducted to understand the pattern of distribution of adrenaline and noradrenaline cells in the adrenal gland of Kuttanad ducks (Anas platyrhynchos domesticus) during post hatch period. Chromaffin tissue consisted of lightly and darkly stained polygonal cells. Light cells were larger in size and possessed a large spherical vesicular nucleus, while the darker cells were smaller and more in number. By Woods staining technique, large cells could be identified as the epinephrine or adrenaline cells and the small cells, the norepinephrine or noradrenaline cells. At 10 weeks of age, among the chromaffin cells, norepinephrine cells were found to be predominating. At 12 weeks, the adrenaline and noradrenaline cells showed numerous granules. At 16 weeks of age, when the birds started laying eggs, among the chromaffin cells, majority was norepinephrine cells. The norepinephrine cells possessed small condensed blue coloured nucleus without a distinct nucleolus while the brown epinephrine cells possessed larger transparent vesicular nucleus in Wood's staining and were distributed more towards the central portion of the chromaffin islet. At 18 weeks, yellow norepinephrine cells were concentrated more towards the subcapsular area and many chromaffin islets were distributed in the centre of the gland crowding around the venous sinuses. At 22 weeks, number of ganglion cells (both large and small) was greatly increased.

Keywords*:Epinephrine and norepinephrine cells, distribution, adrenal development, Kuttanad ducks*

1. Introduction

In most of the mammalian species, the major catecholamine is epinephrine. However, in avian species, the norepinephrine cells predominate over the epinephrine cells and the hormones released by these cells are responsible for short term stress response. There exist a lot of controversies regarding the avian chromaffin tissue such as whether the epinephrine or norepinephrine cells predominate and whether both hormones originate from the same cell type. In this context,study of the normal developmental anatomy of avian adrenal is much important and it will form a basis for correlating the possible functions of adrenal gland in relation to age and for further physiological, pathological and endocrinological studies.Although research has been conducted on the interrenal tissue of various species of birds such as domestic fowl and Japanese quail, information regarding the normal structure and post hatch developmental pattern of chromaffin tissue is scanty especially in ducks. Kuttanad ducks are native of Kerala and are well adapted to the climatic conditions of the state. Therefore, comprehensive study on the distribution of epinephrine and norepinephrine cells of the adrenal gland during post hatch period in Kuttanad ducks seems to be a relevant area of research.

2. Materials And Methods

Post hatch developmental pattern of epinephrine and norepinephrine cells in the adrenal gland of Kuttanad ducks was studied using 78 healthy female Kuttanad ducks of differentage groups, ranging from day-old to 24 weeks. The adrenal glands were collected from six birds in each age group at fortnightly intervals.After recording the gross features, the materials were fixed in 10 per cent neutral buffered formalin, Bouin's fluid and Formol-Dichromate at pH 4.0-4.2 (Wood, 1963).The samples were processed for paraffin embedding and sections of 5 µm thickness were taken for histological studies. Haematoxylin and Eosin (H&E) staining technique (Luna, 1968) and Wood's technique for medullary catecholamine cells (Wood, 1963) were done to differentiate the cell types of chromaffin tissue.

3. Results And Discussion

In day-old ducklings, the chromaffin tissue of the adrenal gland showed light and dark polygonal cells. Light cells were larger in size and possessed a large spherical vesicular nucleus with one or two nucleoli. Darker cells were smaller and more in number than the light cells. Nuclei of darker cells were comparatively small, but they also showed vesicular nature with a nucleolus in the centre. By Woods staining technique, the large cells could be identified as the epinephrine or adrenaline cells showing brownish, homogeneous cytoplasm and the small cells as the norepinephrine or noradrenaline cells possessing yellow, homogeneous cytoplasm (Fig. 1). Diameter of the chromaffin cells and their nuclei at different ages are given in table 1. Similar observations were made in ostrich chicks by Tang *et al.* (2009).

The epinephrine cells possessed larger nuclei than those of the norepinephrine cells in all the age groups. Among the two types of chromaffin cells, the noradrenaline cells predominated over the adrenaline cells in day-old birds. Similar trend was reported in fowl (Sivaram, 1965) and Japanese quail (Basha *et al.*, 2009) in immature age groups of both sexes.At six weeks-old birds, large number of small and large ganglion cells found associated with the chromaffin tissue. There existed intimate developmental correlation between the chromaffin cells and ganglion cells. Ganglion cells, either individually or in small groups were commonly found among the chromaffin cells as observed by Rajendranath *et al.* (2012) in emu.

At 10 weeks of age, among thechromaffin cells, norepinephrine cells were found to be predominating. Ghosh and Guha (1988) conducted light and ultrastructural studies on the avian adrenal gland and noticed that the orders with more primitive ancestry contained more norepinephrine cells, whereas the more recently evolved birds possessed more epinephrine cells in the chromaffin tissue.

At 12 weeks, the adrenaline and noradrenaline cells showed numerous granules. At 16 weeks of age when the birds started laying eggs, among the chromaffin cells, majority was norepinephrine cells. The norepinephrine cells possessed small condensed blue coloured nucleus without a distinct nucleolus (Fig. 2) while the brown epinephrine cells possessed transparent larger vesicular nucleus in Wood's staining and were distributed more towards the central portion of the chromaffin islet. Tang *et al*. (2009) observed that blood in the central zone consisted of large amount of affluent glucocorticoid which activated phenylethanolamine N-methyl transferase (PNMT) of chromaffin cells in the adrenal glands of ostrich. This enzyme is required for the conversion of norepinephrine to epinephrine. As the blood drained from periphery to centre, the central zone is supposed to be bathed in this glucocorticoid rich blood. This triggers the conversion of norepinephrine cells to epinephrine cells. This could be the reason for the concentration of more epinephrine cells in the central zone of the gland. This was supported by Wassermann and Bernard (1971) in adult fowl who reported that both adrenaline and methylating enzyme PNMT level increased significantly with corticosterone or dexamethasone treatment. They also reported the cellular transformation of norepinephrine cells to epinephrine cells.

At 18 weeks, yellow norepinephrine cells were more concentrated towards the subcapsular area. Similar observations were documented by Sivaram (1965) in fowl and Basha *et al*. (2004) in adult Japanese quail.At this stage large number of chromaffin islets was distributed in the centre of the gland crowded around the venous sinuses (Fig. 3). Large islets showed around 20 epinephrine cells and 32 norepinephrine cells while smaller islets presented about 17 epinephrine cells and 5 norepinephrine cells, on an average.

At 22 weeks, number of ganglion cells (both large and small) was greatly increased. Ganglionic transformation of chromaffin cells was reported by Basha *et al.* (2004) in Japanese quail. In the adult birds (24 weeks), the pattern of adrenaline and noradrenaline cells distribution in chromaffin tissue was similar to that of the previous age group.

4. Tables and Figures

Age	Epinephrine Cells (µm)	Nucleus of epinephrine cells (µm)	Norepinephrine cells (µm)	Nucleus of norepinephrine cells (µm)
Day-old	11.23±0.20	5.14±0.22	10.28±0.37	4.35±0.09
2 weeks	13.56±0.20	5.54±0.37	12.25±0.64	4.96±0.49
4 weeks	16.92±1.33	5.98±0.27	14.50±1.02	5.03±0.23
6 weeks	18.38±0.55	6.85±0.15	14.88±0.60	5.25±0.23
8 weeks	21.00±0.00	7.00±0.00	15.00±0.37	4.38±0.23
10 weeks	21.17±1.48	7.00±0.00	18.08±0.58	5.75±0.16
12 weeks	21.00±1.28	7.00±0.00	18.08±0.58	5.69±0.20
14 weeks	21.00±1.81	7.15±0.15	15.17±0.74	5.54±0.67

16 weeks	21.58±0.58	7.23±0.57	16.33±1.48	5.98±0.18
18 weeks	25.67±1.73	7.46±0.30	17.50±1.28	6.13±0.32
20 weeks	21.58±1.08	7.44±0.30	18.67±1.17	6.42±0.18
22 weeks	21.00±0.00	6.85±0.15	16.33±1.17	5.83±0.29
24 weeks	19.54±0.70	6.56±0.30	15.54±0.49	5.54±0.18

Table 1: Diameter of chromaffin cells and their nuclei at different ages in Kuttanad ducks (Mean ± S.E.)

- **Fig. 1 C.S. of adrenal gland showing epinephrine and norepinephrine cells (4 weeks). Wood's technique X 400**
  1. Epinephrine 2. Norepinephrine cells 3. Blood sinus 4. Interrenal tissue

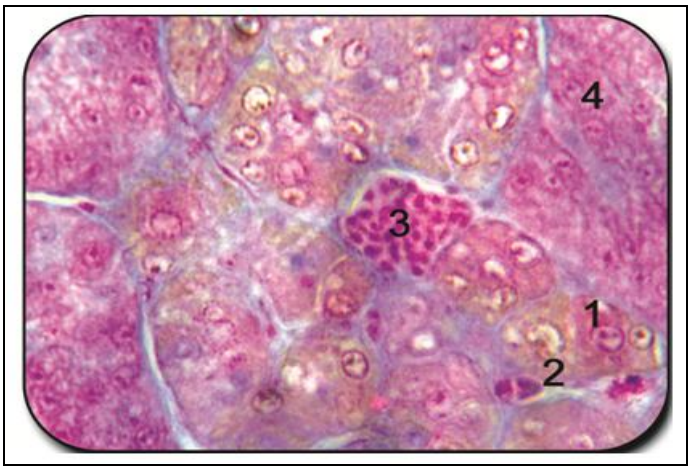

Figure 1: C.S. of adrenal gland showing epinephrine and norepinephrine cells (4 weeks). Wood's technique X 400

- **Fig. 2 C.S. of adrenal gland showing more norepinephrine cells in chromaffin islets (16 weeks). Wood's technique X 400**
  1. Epinephrine 2. Norepinephrine cells 3. Interrenal cell cords

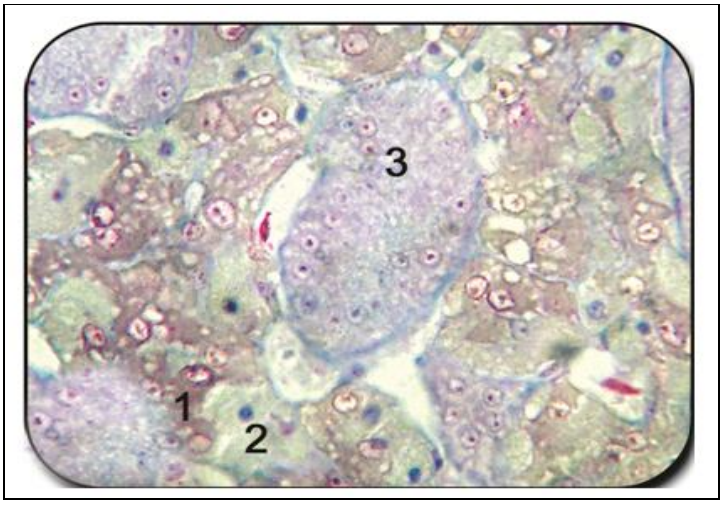

Figure 2: C.S. of adrenal gland showing more norepinephrine cells in chromaffin islets (16 weeks). Wood's technique X 400

- **Fig. 3 C.S. of adrenal gland showing chromaffin islets crowded towards the central zone (20 weeks). Wood's technique X 400**
  1. Chromaffin 2. Interrenal tissue 3. Venous sinuses

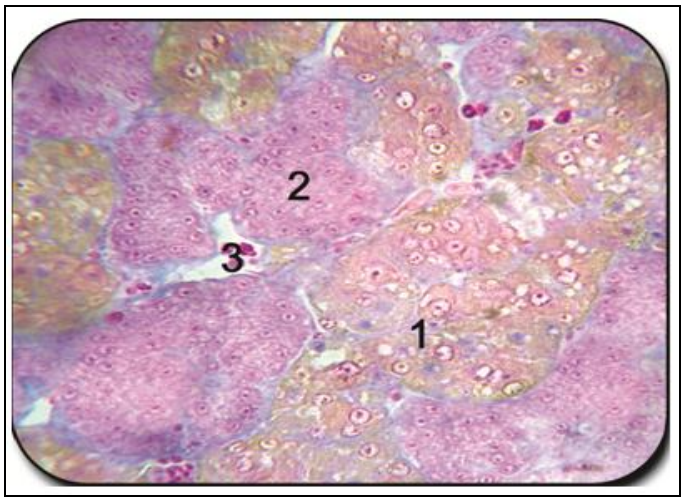

Figure 3: C.S. of adrenal gland showing chromaffin islets crowded towards the central zone (20 weeks). Wood's technique X 400

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