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Some Histological Observations on Postnatal Growth of Rat Adrenal Gland with Advancing Age (A HRLM Study)

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ABSTRACT

The present work elucidates postnatal cytological changes in the adrenal gland with regard to their cortical zones, cell count, nuclear size and lipid contents with advancing age. Thirty six litter mates of 9, 11, 13, 15, 17 and 19 days of age (postnatal) were used by dividing into six groups. Adrenals were dissected free, fixed in glutaraldehyde, post fixed in osmic acid and then stained with 1% toluidine blue solution to study under the light microscope. The adrenal gland which is surrounded by a well developed fibrous capsule, showed a decrease in connective tissue fibers with a steady increase in capsular cells from day 9 to 19. The capsule showed a decrease in the mitotic activity with advancing age. The cortical tissue showed three cortical zones, i.e., zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa was well developed. The radial columns in zona fasciculata and zona reticularis became more distinct on 13th day onwards. The medullary cells in groups were found to invade from capsular region to the zona glomerulosa and then to zona fasciculata. Predominance of dark cells over the light cells was observed in all groups. There was an overall little increase in size of the inner zones, which might be attributed to the disappearance of many of the medullary cells. The intracellular lipid content in the zona glomerulosa was significant. In the zona fasciculata, the lipid content was little with very meager amount in the juxtamedullary portion too. The medulla appeared distinctly on day 9 of postnatal life covering a larger area. There was predominance of dark cells over the light cells in the medulla throughout the study. It is concluded that functional zonation which characterizes the cortex of the adult rat extends back to neonatal period and that the cells in both the zona glomerulosa and fasciculata actively secret in the infant adrenal.

Key Words: Postnatal growth; Adrenal gland; Rat; Histology

INTRODUCTION

Structurally and functionally, the adrenal cortex and medulla are distinct from each other but together they constitute a single topographical entity (William *et al.,* 1995). In an attempt to elucidate the mechanism through which the functional adrenal cortex is established, various immunohistochemical techniques are used showing that separation of the cortex and medulla, and development of functional zonation in the cortex begun at around the time of birth (Mitani *et al.,* 1999).

In respect to growth, the adrenal is a dynamic organ that requires constant stimuli from pituitary derived pro-opiomelanocortin (POMC) peptides to maintain its tonic state since either hypo-physectomy or dexamethasone treatment results in rapid adrenal atrophy (Bicknell, 2002). It has been observed that the growth of rat adrenal gland is relatively slower than general body growth. However, the growth of the cortex precedes that of medulla (Janjua & Khan, 1992).

The specific development of the human fetal adrenal gland requires cell proliferation, migration, apoptosis, and zone-specific steroidogenic activity. Primary cultures of human fetal adrenal cells grown on collagen IV, laminin, or fibronectin revealed that cell morphology was affected by environmental factor (Chamoux *et al.,* 2002).

Classically, the production of glucocorticoids by the adrenal gland is thought to be controlled exclusively by adrenocorticotropic hormone (ACTH). However, there are several examples in stressed humans and animals of increased plasma glucocorticoids without increase in plasma ACTH, suggesting that additional, non-ACTH mechanism(s) may contribute to the control of glucocorticoids production (Bornstein *et al.,* 1992). The hypothesis that the splanchnic innervation of the adrenal gland represents an additional physiological mechanism to control stress-induced adrenal cortical responses *in vivo* has been confirmed by Ulrich-lai *et al.* (2002).

Although, considerable work has been done regarding the development of pre-pubertal and adult adrenal gland with special reference to its medullary component (Long & Jones, 1967; McNutt & Jones, 1970; Kerr & Weiss, 1991; Kon *et al.,* 1991; Bornstein *et al.,* 1992; Janjua & Khan, 1992; Mitani *et al.,* 1999; Mughal & Janjua, 2000). However, little attention has been paid to the developmental aspect of adrenal cortex. The present work elucidates the postnatal cytological changes in the adrenal gland with the advancing age.

MATERIALS AND METHODS

The albino rats were used for this experimental study. These rats were obtained from Animal House of Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. The study was carried out in Punjab Medical College and Independent Medical College, Faisalabad in collaboration with University of Agriculture, Faisalabad.

A total of 36 litter mates of ages 9, 11, 13, 15, 17 and 19 post natal days were included in the experimental study by dividing the animals into six groups, 'A' to 'F', each group comprising six animals. On the day of sacrifice the animals were sacrificed by a hammer blow on the head, their abdomen opened through a ventrally placed midline incision, peri-adrenal fat removed and the adrenal glands adhering to the kidneys were rapidly dissected out using a dissecting microscope and then fixed in the glutaraldehyde fixative.

Sections were cut 0.5 µm thick on Servall Porter Blum Ultramicrotomy using glass knives. The sections obtained were picked by a fine hairpin loop onto the clean glass slide for study. These sections stained with 1% toluidine blue solution were studied under light microscope. Ten observations of each parameter given in performa were recorded for each animal and their mean was taken as a reading for that animal.

The measurements of different cortical zones were recorded with the help of ocular micrometer. Differential cell count was done in dark and light cortical cells. Medullary cells were also counted. Zonal distribution of lipid content was estimated. Chromatin granules, mitotic activity and staining character of the cytoplasm were also recorded in the field examined for cell count. The data collected was analyzed statistically using statistical software package SPSS.

RESULTS

No histological difference between male and female, right and left adrenal glands was observed. Two types of cells were observed i.e. dark and light cells according to the staining character. No zona inter-media was observed as described by the previous workers.

Capsule. It was well defined with elongated spindle shaped connective tissue cells having fine granular cytoplasm, oval nuclei and abundant connective tissue fibers (Fig. 1). The blood vessels usually arteries were present in the capsule but venous channels were also seen. Occasionally myelinated and unmyelinated nerve bundles were seen in 17^{th} and 19^{th} day age groups. The thickness of the capsule was observed variable in different groups (Table I).

Cortex. The functional element or the parenchyma of the cortex consists of polygonal cells, which comprise great bulk of the tissue. On the basis of difference in arrangement of the parenchymal cells, the cortex was divided into three zones, named as zona glomerulosa, fasciculate and reticularis (Fig. 2). Myelinated and unmyelinated nerve fibers appeared in groups of variable size in between the cortical cells. These bundles also accompanied the medullary cells.

Fig. 1. Photomicrograph of 0.5 µm thick, EPON embedded and toluidine blue stained section of the adrenal gland from 11 days old rat adrenal showing capsule (CAP), venous channels (VC) and nuclear infolding (arrow head) in the cell of zona glomerulosa (Mag. 3600x)

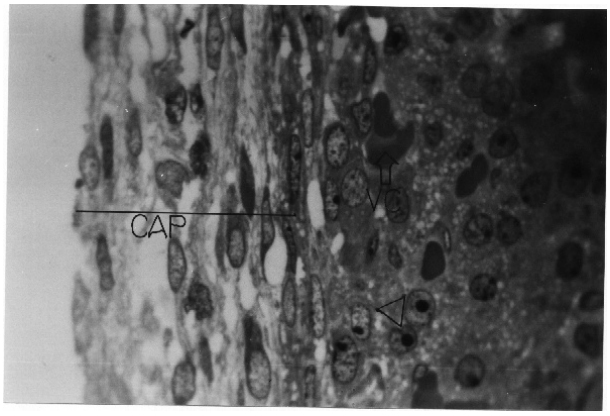
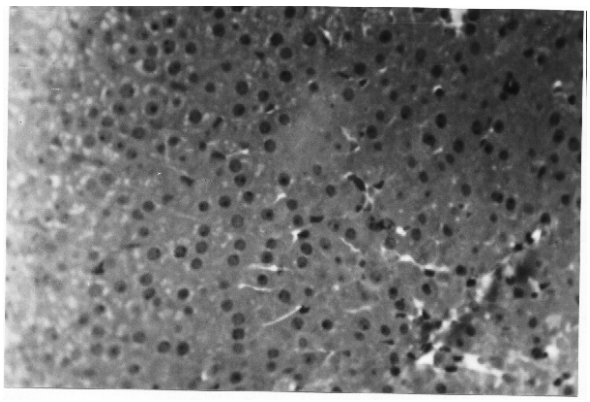


Fig. 2. Photomicrograph of 0.5 µm thick, EPON embedded and toluidine blue stained section of the adrenal gland from 15 days old rat adrenal showing general arrangement of the cells in three zones of cortex (Mag. 900x)



Zona glomerulosa. Initially the cells were small and possess few vacuoles. Later the cells in this region were more organized into small balls and arcs supported by connective tissue trabeculae. The arterioles entering the capsule break up into long sinusoids, which run radially between the columns of the cells. The cell cytoplasm showed two types of staining character i.e., dark and light. The predominance of the dark cells was observed irrespective of age. The light cells were comparatively few

Table I. Analysis of variance and means ± S.E of various parameters in different age groups of rats

Analysis of Variance (Mean squares)										
Source	**d. f**	**Width of Z.G. (in µm)**	**Width of Z.G (in µm)**	**Dark cell count in Z.G/ Unit area**	**Light cell count in Z.G/ Unit area**	**Size of Nuclei in Z.G (in µm)**	**Width of Z.F (in µm)**	**Dark cell count in Z.F/ Unit area**	**Light cell count in Z.F/ Unit area**	**Size of nuclei (in µm)**
Group	5	1.44**	53.0*	57.79***	0.46*	0.34*	1239.3***	4.77*	0.99*	0.35**
Error	12	0.33	52.1	4.56	0.61	0.14	68.7	4.56	0.33	0.09
Groups					**(Mean ± S.E)Age in days**					
A----09 days		12.92±0.42 C	39.58 ±0.42	23.33 ±1.76 C	01.67 ±0.33	07.08 ±0.42	205.33 ±2.91 C	33.00 ±0.58	±0.00	07.08 ± 0.22 AB
B----11 days		13.30 ±0.35 BC	42.67 ±0.40	26.33 ±1.45 BC	01.67 ±0.33	07.50 ±0.14	212.00 ±3.06 C	33.00 ±1.53	01.33 ±0.33	06.83 ± 0.17 AB
C----13 days		13.67 ± 0.22 BC	44.67 ±0.36	25.33 ±0.88 BC	01.33 ±0.33	07.50 ±0.25	214.67 ±3.53 C	33.67 ±1.86	±0.58	06.58 ±0.22 B
D----15 days		13.58 ±0.30 BC	45.52 ±0.30	27.67 ±0.66 B	02.00 ±0.58	07.75 ±0.00	231.00 ±2.08 B	30.67 ±1.20	01.67 ±0.33	07.00 ± 0.14 B
E----17 days		14.08 ±0.17 AB	45.75 ±0.38	32.00 ±1.15 A	01.33 ±0.33	08.00 ±0.00	245.00 ±7.64 AB	32.33 ±0.88	01.67 ±0.33	07.00 ± 0.00 AB
F----19 days		14.92 ±0.44 A	46.83 ±0.30	35.00 ±1.15 A	02.33 ±0.67	07.92 ±0.17	256.67 ±6.67 A	34.33 ±0.88	01.33 ±0.33	06.50 ± 0.14 B

* = Non-significant; ** = Significant; *** Highly significant; ZG: Zona granulosa; Zona Fasiculata. Means sharing same letters or no letter are statistically non-significant.

Table II. Analysis of variance and means ± SE of different parameters in different groups of rats

Source	**d. f**	**Width of Z.G. (in µm)**	**Dark cell count in Z.G/ Unit area**	**Light cell count in Z.G/ Unit area**	**Size of Nuclei in Z.G (in µm)**	**Dark cell count in medulla/ Unit area**	**Light cell count in medulla/ Unit area**	**Size of nuclei in Medulla (in µm)**
Group	5	325.1***	17.52***	0.367*	0.68***	92.32***	0.40*	0.12*
Error	12	11.9	0.39	0.72	0.03	2.17	0.50	0.13
Groups Age in days				**(Mean ± S.E)**				
A----09 days		62.67 ± 1.45 E	09.00 ± 0.00 E	01.33 ± 0.67	06.42 ± 0.08 A	29.67 ± 0.08 A	01.33 ± 0.33	07.08 ± 0.22
B----11 days		72.00 ± 3.51 D	11.00 ± 0.58 D	00.33 ± 0.33	06.33 ± 0.08 AB	30.67 ± 0.33 A	01.67 ± 0.67	07.25 ± 0.38
C----13 days		74.67 ± 0.33 CD	12.00 ± 0.00 CD	01.00 ± 0.58	06.08 ± 0.08 BC	26.67 ± 0.67 B	00.67 ± 0.33	06.83 ± 0.17
D----15 days		79.00 ± 2.08 BC	12.33 ±0.33 C	01.0 ± 0.58	05.83 ± 0.08 CD	22.67 ± 1.45 C	01.33 ± 0.33	07.00 ± 0.14
E----17 days		84.67 ± 1.33 B	14.00 ± 0.00 B	00.67 ± 0.33	05.58 ± 0.08 D	19.33 ± 0.67 D	01.67 ± 0.33	06.92 ± 0.08
F----19 days		92.67 ± 1.76 A	16.00 ± 0.58 A	00.67 ± 0.33	05.17 ± 0.17 E	16.67 ± 0.67 E	01.33 ± 0.33	06.67 ± 0.08

* = Non-significant; ** = Significant; *** Highly significant; ZG: Zona granulosa; Zona Fasiculata. Means sharing same letters or no letter are statistically non-significant

usually showing granular cytoplasm whereas the cytoplasm of the dark cells was homogeneously stained. The size of the nuclei remained nearly constant for all age groups measuring between 7.08 µm to 7.92 µm. Most of the cells showed peripheral distribution of the lipid globules, which varied slightly in size but the distribution was more or less constant. The cell cytoplasm was full of lipid globules. The diameter of the lipid globules was approximately 1.25 µm. For width of the capsule, cell count and size of the nuclei see Table I.

Zona fasciculate. The zona fasciculata was composed initially of uniformly large and vacuolated cell that arranged in radial columns having spherical nuclei with 1-2 nucleoli with advancing age. The nuclei of the outer region were stained slightly less as compared to the inner deeply stained nuclei. The predominance of the dark cells over the light cells was observed. The lipid globules were extremely numerous but less than the zona glomerulosa throughout the study period (Fig. 3). Mitotic activity was also noted. The width of this zone was broadest of the zones constituted the bulk of the cortical tissue. The width started from 205.33 µm to 256.67 µm showing statistically significant increased in the size. For width of the capsule, cells count and size of the nuclei (Table I).

Zona reticularis. The development of the organization of this zone started almost on 9th day and became more prominent in the older animals. The cells surrounded large blood sinuses. The size of the nuclei of the cells present in this zone appeared to be smaller than other cortical zones. Both dark and the light cells were seen with predominance of the dark cells. Mitotic figures were frequently observed in the zone. Fat globules varied greatly in size and shape. There was also a lipid free zone ordering medulla in 9th day group. For width, cell count and size of the nuclei see Table II.

Medulla. Both dark and the light cells were observed in all the groups. The cytoplasm was lightly stained showing many vacuoles and fine granules. The cells were rounded to polygonal arranged in groups or columns. Numerous sinusoids of variable size were found between the cells columns (Fig. 4), myelinated and unmyelinated fibers. Table II presents width, cell count and size of the nuclei.

Fig 3. Photomicrograph of 0.5 µm thick, EPON embedded and toluidine blue stained section of the adrenal gland from 09 days old rat adrenal showing lipid globules (lg) in zona fasciculate (Mag. 3600x)

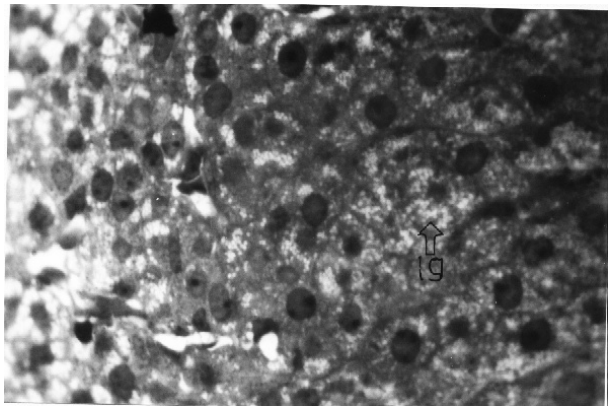
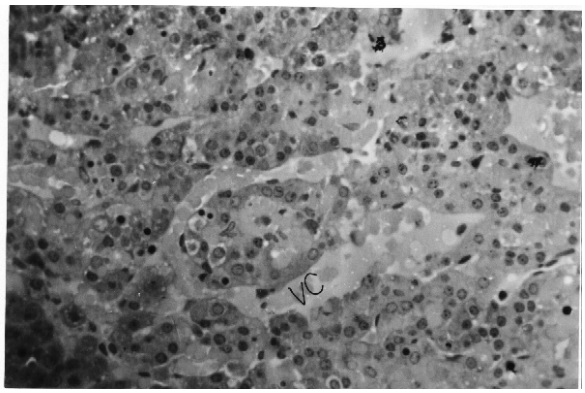


Fig 4. Photomicrograph of 0.5 µm thick, EPON embedded and toluidine blue stained section of the adrenal gland from 17 days old rat adrenal showing cells and venous channels (VC) in the medulla (Mag. 1800x)



DISCUSSION

The postnatal development of the adrenal gland in the albino rat has been studied from 9th to 19th postnatal days. The adrenal gland, which was surrounded by a well-developed fibrous vascular capsule showed different ratios between cellular and fibrous elements. In the present study a decrease in the connective tissue fibers with a steady increase in connective tissue cells (fibroblasts) was observed corresponding to the observations made on the adrenal capsule with the help of light microscope (Janjua & Khan, 1992; Mughal & Janjua, 2000), the capsule showed fair amount of chromatin granules in the nuclei and cytoplasm consisted of numerous granular portions. The cells present in the capsule of the gland appeared more closely packed during the study period. The cells alone with their nuclei were more clearly defined in early periods. A highly significant change in thickness was observed with the advancing age. These findings are in close conformity with Januja and Khan (1992), and Mughal and Janjua (2000).

The zona fasciculate appeared in radial cords and well-defined zona reticularis was also differentiated. There was significant difference between the groups in widths of the three zones.

Two types of cortical parenchymal cells were seen i.e., dark and light irrespective of age groups and the zones. The variations in the cytoplasmic densities and staining character formed the basis of differentiation between the dark and the light cells. The predominance of the dark cells was noticed irrespective of the age. The observations are consistent with the studies carried by Janjua and Khan (1992), and Mughal and Janjua (2000).

A significant increase in the dark cells count occurred in all zones of the cortex with no significant increase in the light cells count. The dark cells were presumed to be active hormone secreting cells, while the light cells are considered as inactive cells. There was decrease in the dark cells count in zona glomerulosa from 9th to 15th day, which could be due to degeneration process. The inner two zones showed a steady increase in the dark cell count, which is due to expansion of the gland. No significant statistical change in the light cells count was seen during the study period in all groups in the cortex (Janjua & Khan, 1992; Mughal & Janjua, 2000).

The over-all size of the medulla, increase with the advancing age. There was significant increase in the dark cells count during post-natal period without any significant change in the light cells count. However, striking increase was noted on 11th day (Josimovich *et al.*, 1954; Janjua & Khan, 1992; Mughal & Janjua, 2000).

In glutaraldehyde fixed and Osmic acid post fixed adrenal, the lipid globules in both the regions of cortex were well preserved. The overall size of the globules was less than 1.25 µm. All the three zones had variable distribution, with fine outline of the fat globules responsible for active secretions. These findings are in agreement with the previous study conducted Mughal and Janjua (2000).

The mitotic activity was observed both in the capsular fibroblasts and in the cortical parenchyma. Frequently, groups of big medullary cells with vesicular nuclei forming cords or sheets associated with or without nerve bundles of varying dimensions (both myelinated and unmyelinated) and venous channels were observed in the cortical zones. The observations of the medullary cells in the cortical zones revealed that these cells accompanying the nerves enter the capsule where it penetrates the cortical zones and ultimately moved down towards the medulla. The nerve fibers accompanying these cells were composed of mixture of myelinated and more unmyelinated nerve fibers. These findings have been studied in more detail with florescent tracer fast blue (Kesse *et al.*, 1988). No zona intermedia was observed as described by the earlier workers (Janjua &

Khan, 1992). Possibility is there that the zona intermedia might develop in later stages of life for different function. It, however, needs further study.

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