

AGE RELATED CHANGES IN THE RAT ADRENAL CORTEX

Pages with reference to book, From 89 To 94

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ABSTRACT

The present study revealed that the growth of the fat adrenal gland was relatively slower than general body growth. However, growth of the cortex preceded that of the medulla. The parenchyma showed three usual zones but a fourth inconstant lipid-poor, zona interfascicularis between outer glomerulosa and middle fasciculata was also observed. In all cortical zones, the predominance of dark cells over the light cells occurred irrespective of the age groups studied. The advancing age replaced capsular cellular elements with fibrous ones whereas the mitotic activity of the parenchymal cells decreased and an increase in the intracellular lipid in the outer and ascorbic acid in the inner cortex was observed. The glycogen which was restricted mainly to the inner cortex remained unaffected by aging process while the acid phosphatase activity from the inner reticularis extended to the outer cortex in aged group (JPMA 42: 89, 1992).

INTRODUCTION

The adrenal (an organ of dual Characters) consists of a cortex and a medulla which are of different developmental origin, structure and functions. Arnold in 1866 (cited by Symington¹, 1962) described the microscopic structure of adrenal cortex to consist of three zones;-glomerulosa, -fasciculata and -reticularis. The role of adrenal gland in the maintenance of metabolism and homeostasis has been investigated for over a century. Experimental ablation of adrenal in cat and dog² and monkey³ has led to the death of the animal within 1-2 weeks if kept on ordinary diet. Administration of the suitable extracts, preferably with added sodium chloride in diet rectifies the condition and keeps the animal normal and alive for indefinite period. Withdrawal of extract is followed by death, hence it is essential for life. Adrenal cortex is concerned with a variety of body functions including maintenance of carbohydrate, fluid and electrolyte balance and certain cellular elements of connective tissue. Extensive investigations with light microscope supplemented by recent trends of electron microscopic observations supplemented by recent trends of electron microscopic observations on various species provides an ultrastructural basis for understanding of its morphological features. Although morphology and functions of the adrenal cortex have been widely studied, there are only few studies on the age related changes. The present study undertakes to study the changes in the morphology of the rat adrenal cortex with advancing age.

MATERIALS AND METHODS

The albino rats were the subject. They were obtained from Charles River Laboratory, Brooklyn, Massachusetts, USA and raised in animal colony at Jinnah Postgraduate Medical Centre, Karachi. Sixty male rats aged 25, 60 and 180 days were used in the study and they were classified into groups A, B and C respectively. The study was extended over a period of three weeks at weekly interval in each group, consisting of 20 animals. They were kept under observation before the start of experimental procedure for 2 weeks in a room maintained at temperature of 25-30°C and fed at libitum food pellets supplied by Lever Brothers (Pakistan) Ltd. On the day of sacrifice, to avoid prolonged stress, the animals received hammer blows delivered on their heads. The abdominal cavity was opened immediately and the

perirenal fat of the left adrenal gland was infiltrated with 4% glutaraldehyde solution in situ, removed from the surrounding fat, soaked in the same fixative and was cut into two equal halves. The right adrenal gland was removed, weighed, cut into two halves and each half fixed in neutral buffered formalin and alcoholic formalin respectively and processed as shown in Table I.

TABLE I. Summary of the methods of study.

Tissue	Fixative	Sectioning	Stained and purpose
Left adrenal one hemisphere	cold glutar- aldehyde followed by osmic acid (Pease ⁴ , 1964)	0.5 micron epoxy resin sections	1. toluidine blue for measuring zones and general morphology 2. Examination for lipid (osmic acid stain)
Second hemisphere	acidified silver nitrate (Barka and Anderson ⁵ , 1963)	7 micron paraffin sections	silver staining for ascorbic acid
Right adrenal One hemisphere	neutral phosphate buffered formalin (Burstone, 1958, modified by Barka ⁶ , 1960)	10 micron frozen sections	naphthol AS BI phosphate for acid phosphatase
Second hemisphere	alcoholic formalin (Humason ⁷ , 1962)	7 micron paraffin sections	1. Best's carmine for glycogen 2. PAS for PAS material

Measurement of zonation:

Cortical zones were measured with the help of ocular micrometer scale, in 0.5 micron thick selected araldite sections stained with toluidine blue. This scale having 50 divisions, each of which equivalent to 11.5 microns in low power, 2.88 microns in high power and 1.17 microns under oil immersion objective, was inserted in the right eye piece of the microscope.

Differential cell count:

Cell count per unit area of 80 microns square (6400 square microns), was carried out in each zone under oil immersion objective with the help of counting reticule inserted in the left eye piece. Cell count was done under oil immersion lens, at random in each zone. Nuclei counted were those of light and dark cortical cells. Doubtful nuclei were not taken into consideration. The observations on mitotic activity, nuclear size, cytoplasmic lipid globules and granulations in the cells were also recorded in the field examined for cell count.

Observations of lipid content:

The lipid content and globule size was studied in 0.5 micron thick araldite sections stained with toluidine blue. The tissues fixed in glutaraldehyde and postfixed in osmic acid preserved and stained the boundary membrane and also lightly stained the fat itself so the sections were examined with the help of light microscope for lipid content. Zonal distribution of the lipid content was estimated by mean

values of at least three observations in each zone. The size of lipid globules was measured with ocular micrometer scale and their distribution was graded as traces, sparse, moderate, dense and very dense.

Statistical analysis of the data:

The arithmetic mean of the observations and the standard error was calculated with the help of Casio Scientific Calculator FX- 98. The significance of difference between two means was evaluated by student's 't' test. The difference was regarded as statistically significant if the 'P' value was equal to or less than 0.05.

OBSERVATIONS AND RESULTS

Capsule:

Rat adrenal is surrounded by a well defined capsule which consists of elongated, spindle like connective tissue cells (fibroblasts) and connective tissue fibres. Lightly stained elongated capsular nuclei could be easily distinguished from spheroidal heavily stained parenchymal nuclei. In the present study, the capsular cells showed evidence of cell division in the youngest age group (Figure 1).

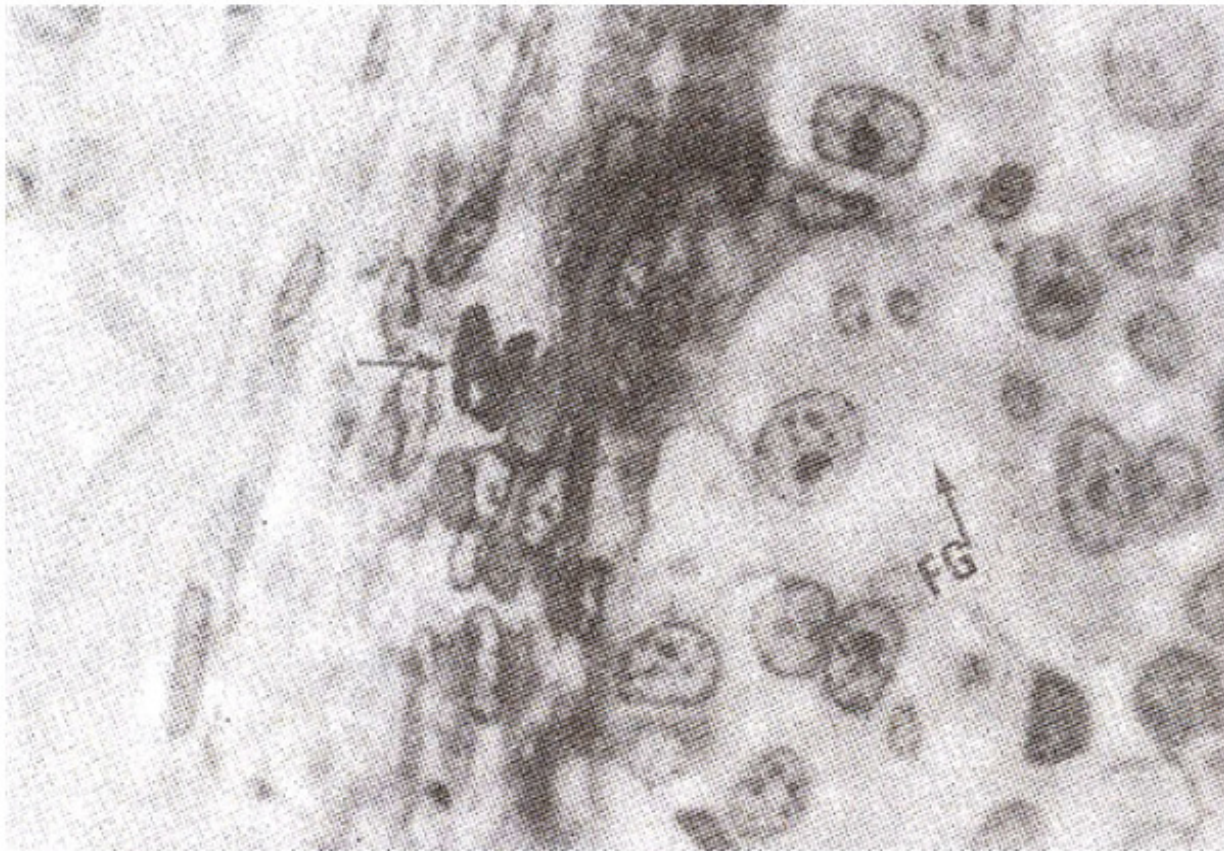


Figure 1. Section of 32 days old rat adrenal showing capsule with a mitotic figure (arrow) and adjacent zona glomerulosa with distribution of fat globules (FG) in the cells. Araldite section, toluidine blue stain. Photomicrograph X 1,500.

With advancing age the preponderant cellular elements seen in young animals were replaced by fibrous elements. The capsule was seen to contain usually blood vessels, mostly arteries, but occasionally venous channels. Both myelinated and unmyelinated nerve bundles of variable size were not uncommon in the capsule. The thickness of the capsule was not affected by age but remained constant as shown in Table II.

TABLE II. Mean* width of the four zones of adrenal cortex at different age periods.

Age (days)	Capsule thickness in microns	Cortex width in microns	Zona glomerulosa width in microns	Zona inter media width in microns	Zona fasciculata width in microns	Zona reticularis width in microns
(A) 25	16.39±1.00	533.10±28.5	27.56±1.31	12.99±2.28	320.98±19.76	149.17±06.93
32	13.49±0.47	639.80±9.20	37.40±1.67	4.08±4.08	439.94±51.95	161.00±35.00
39	15.60±0.86	619.32±45.31	42.48±0.41	14.40±8.30	385.83±51.95	161.00±40.75
46	14.89±1.26	641.81±53.73	43.88±0.42	25.20±1.81	402.33±33.19	155.33±20.24
(B) 60	13.68±4.57	697.0±78.64	52.32±4.57	32.93±1.17	458.73±66.40	145.67±30.66
67	19.68±2.67	816.69±73.90	64.32±4.18	18.96±4.92	536.33±38.16	177.40±33.55
74	13.67±1.10	634.04±70.37	60.48±8.79	21.22±7.01	404.50±58.52	134.17±19.16
81	20.85±3.97	829.31±66.84	60.72±9.07	14.40±3.32	531.50±51.93	201.83±22.25
(C) 180	20.16±1.66	915.25±72.68	69.71±12.2	17.04±3.00	529.00±35.13	276.00±23.93
187	18.09±1.04	781.09±92.62	68.16±2.55	20.17±4.98	529.00±52.69	145.67±45.19
194	17.28±1.66	782.22±2.44	60.61±8.66	14.33±3.38	517.50±33.19	172.50±33.19
201	20.16±1.66	765.19±88.23	67.44±9.48	25.92±1.66	494.50±40.38	157.17±36.56
*Mean ± standard error		*Mean ± standard error	Statistical analysis of the differences mean width between			
		25 and 67 days P < 0.05	25 and 50 days P < 0.01 highly significant increase	25 and 67 days P > 0.05 insignificant	25 and 67 days P < 0.01 highly significant	Width of the zona reticularis shows no significant increase or decrease between 25 and 201 days.pa
		67 and 201 days P > 0.05	67 and 201 days P > 0.3 insignificant	67 and 201 days P > 0.05 insignificant	67 and 201 days P > 0.05 insignificant	

Adrenal cortex:

The functional element or parenchyma of the cortex consists of polygonal epithelial cells which comprise great bulk of the tissue. On the basis of difference in arrangement of the parenchyma cells, the cortex is divided into three concentric layers from without inwards, named as zona glomerulosa, -fasciculata and -reticularis. In addition to these 3 layers usually a fourth layer named zona intermedia was also seen in almost all animals of the three age groups.

Zona glomerulosa:

The cells of the zona glomerulosa were arranged in groups supported by connective tissue trabeculae. The arterioles entering the capsule breakup into long sinusoids which run radially between the columns of cells. The cells near the capsule are most advantageously situated with respect to a fresh supply of oxygenated blood. The connective tissue stroma consists of reticular fibres which underlies the sinusoids. The cell cytoplasm showed two types of staining character, i.e., light and dark. The predominance of the dark cells was observed irrespective of the age. The light cells were comparatively few, usually showed granular cytoplasm whereas the cytoplasm of the dark cells was homogeneously stained. Cell boundaries were not demarcated in most of the cases. Both the dark and light cells were of medium size with nuclei taking up slightly deeper stain than those of the capsule and presented well defined chromatin granules. The size of the nuclei remained constant for all the age periods and measured between 5 and 6 microns. The dark cell nuclei usually showed regular outline whereas light cells occasionally showed nuclear infolding (Figure 2).

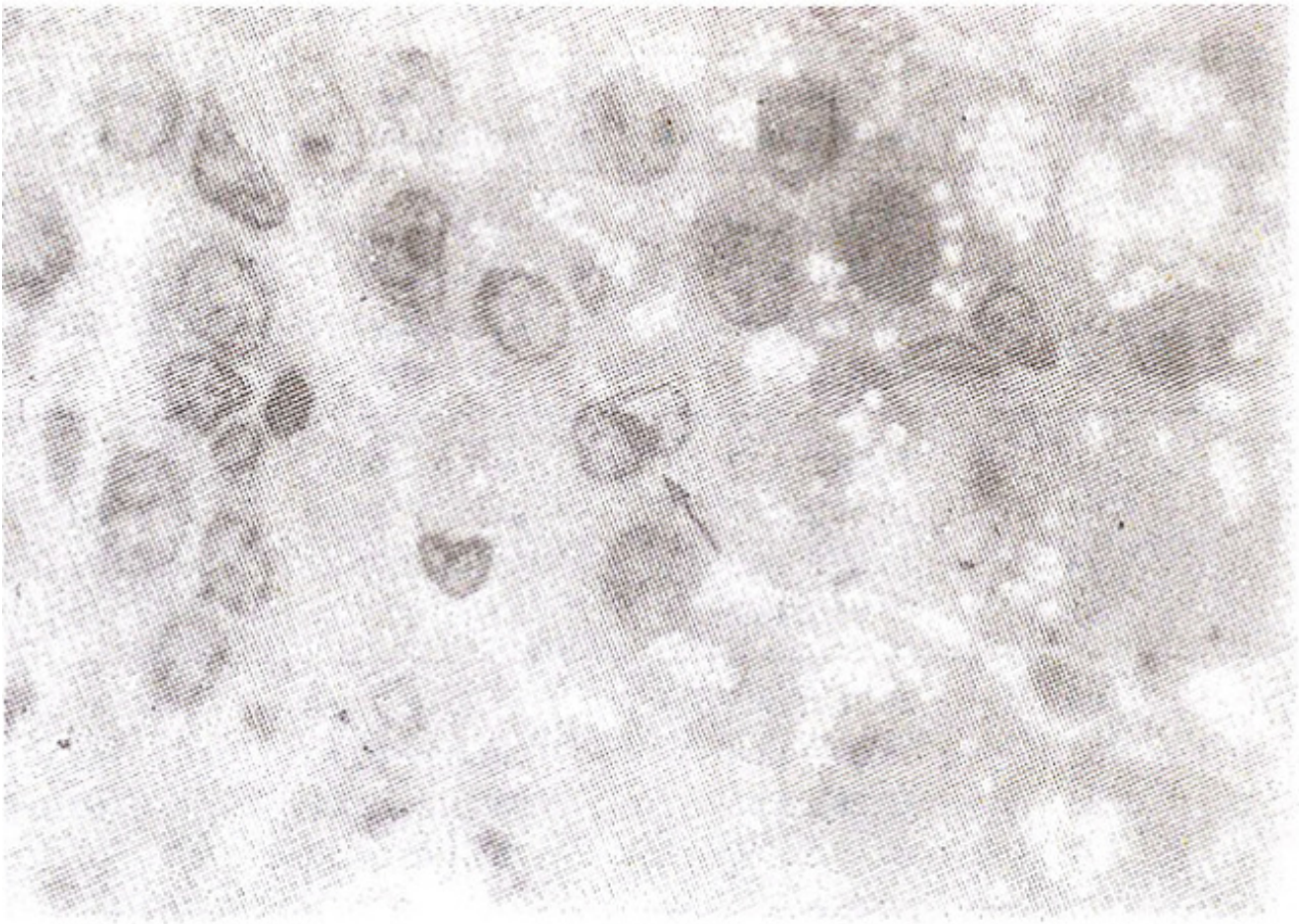


Figure 2. Section of zona glomerulosa of 27 days old male rat adrenal showing nuclear infolding in the light cell (arrow). Araldite section, toluidine blue stain. Photomicrograph X 1,500.

Most of the cells showed peripheral distribution of the lipid globules (Figure 1) which varied slightly in size, but the distribution was more or less constant. The average diameter of the lipid globules was approximately two microns. A few lipid-free cells could be demonstrated. The distribution of the lipid in this zone, generally could be graded as dense. Lipid globules were more abundant in 60 and 180 day age groups. Mitotic figures were occasionally seen in the youngest age group (Figure 2) which gradually decreased as the age advanced. The myelinated and unmyelinated nerve bundles were seen traversing this zone but actual innervation of the cortical cells could not be demonstrated. The mean width of the zona glomerulosa observed on 25th day was 27.56 ± 1.31 which showed statistically significant increase to 64.32 ± 4.18 on day 67, however, no significant change was observed thereafter to the age of 201 day. The width of this zone and comparison of light and dark cell count of different age periods is shown in Table II.

Zona intermedia:

The cells of this region, placed between glomerulosa and outer fasciculata form a zone called zona intermedia which was rather lipid-poor but not lipid free as described by some previous workers. This zone showed great variation in width for a particular age group and even in a single section it was well defined at few sites whereas ill defined or absent at the others. The observations were made at the widest part of the zone. Both light and dark cells of medium size could be demonstrated but the dark cells outnumbered the light ones. The nuclei of the cells had regular oval outline but with occasional pleomorphism, however, the average nuclear size of 5-6 microns remained almost constant as in other

zones and was not affected by age factor. The cytoplasm of the cells showed lipid globules of extremely small size which were present in traces (Figure 3).

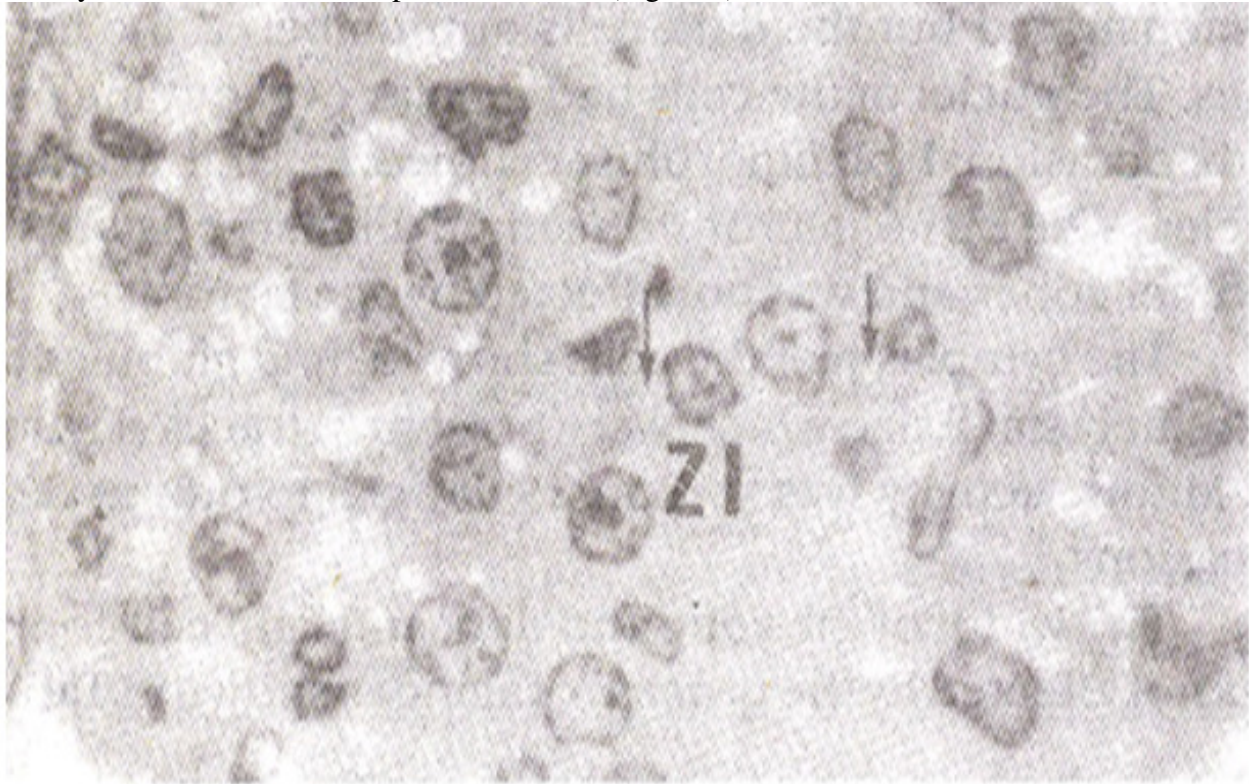


Figure 3. Section of adrenal cortex of 62 days old male rat showing zona intermedia (ZI), with extremely fine intracellular lipid globules (arrow) distributed in traces. Araldite section, toluidine blue stain. Photomicrograph X 1,500.

Mitotic activity was seen in 25 day group only at rare occasions. The width of the zone and dark and light cell count was not affected by the process of aging as shown in Table II.

Zona fasciculata:

The cell columns were arranged in radial rows with spherical nuclei with 1-2 nucleoli. The nuclei of the outer region stained slightly less as compared to the inner deeply stained nuclei. In well stained section the boundaries of the cells became apparent. The predominance of the dark cells occurred with few light cells but the size of the cells appeared larger than the superficial zones as the number of cells per unit area was reduced as compared to zona glomerulosa and zona intermedia. In the outer fasciculata the lipid globules were extremely numerous but definitely less than glomerulosa, however, their size was extremely variable. In the inner fasciculata of the younger age groups they were distinctly less numerous and somewhat smaller in size than in the outer, but in older age group the size and number of globules in both parts of the fasciculata became almost similar. The mitotic activity was observed in 25 days age group in outer fasciculata. The width of this zone was broadest of the zones and constituted the bulk of the cortical tissue. The mean width on 25th day was 320.98 ± 19.76 which increased to 536.33 ± 38.16 on 67th day, keeping pace with glomerulosa, thereafter both the zones remained almost constant, with individual variations. The mean width and cell count of the zone at various age period is shown in Table II.

Zona reticularis:

Zona reticularis starts where the radial rows of inner fasciculata ceased, became broken and disrupted. Cell cords formed an anastomosing network. The cells surround large blood sinuses and were demarcated by heavy connective tissue strands (stained with silver stain, Figure 4).

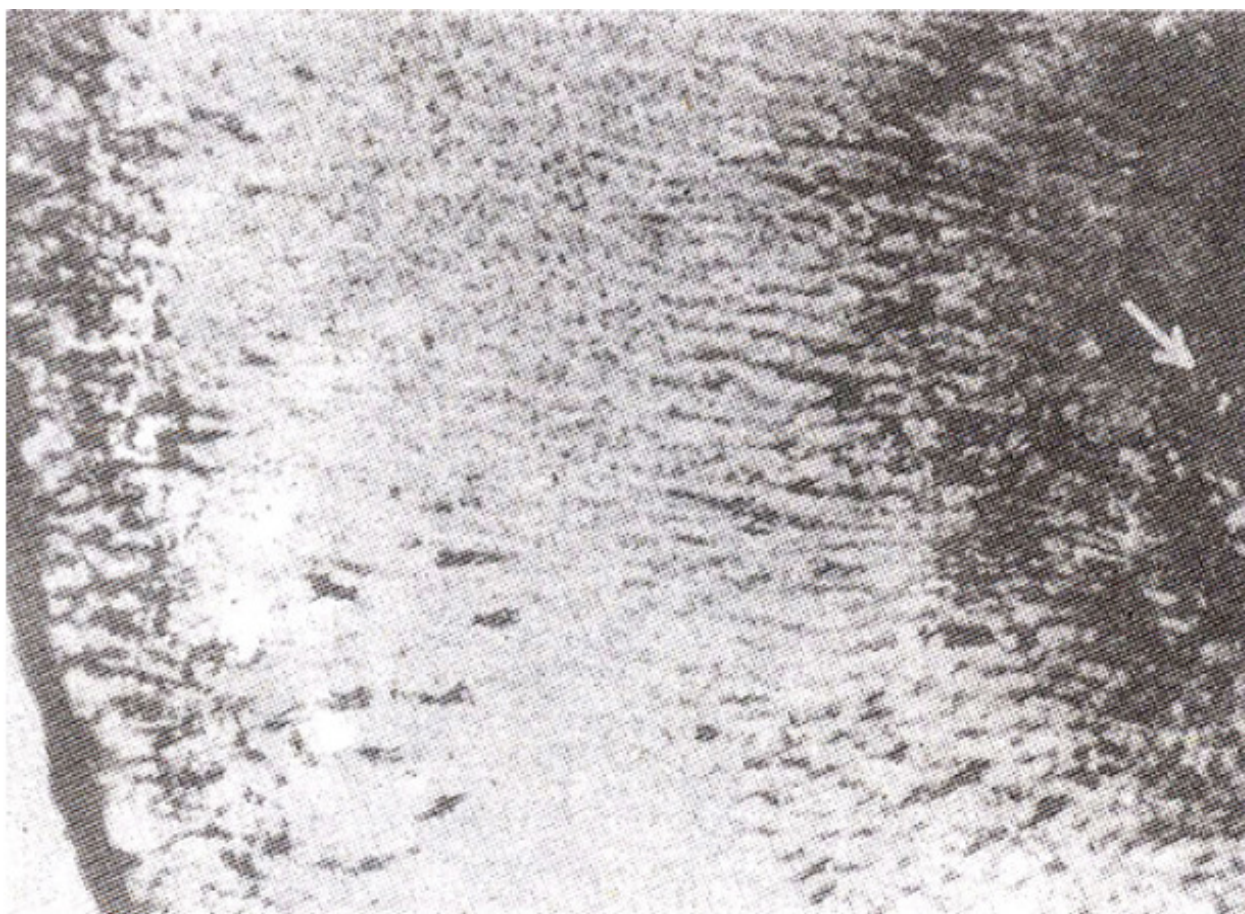


Figure 4. Section of adrenal cortex of 194 days old male rat showing zonal distribution of ascorbic acid. The blood sinuses and the connective tissue of zona reticularis shows heavy staining (arrow). Paraffin section, acidified silver stain. Photomicrograph X 150.

The cells of this zone appeared to be smaller than other cortical zones but the nuclei were comparable in size to the other zones. The cells of the inner zone vary in appearance. Some of the cells had small dark pyknotic nuclei in aged group, which might be considered degenerating cells whereas others had lighter nuclei with light or deeply stained cytoplasm. Both light and dark cells were observed but predominance of the dark cells existed. Along the cell boundaries particularly in aged group, darkstaining granules might be pigment granules. The fat globules varied greatly in size and shape were observed to be scattered sparsely in the cytoplasm. The mitotic activity was noticed only in 25 day age group. The width of this zone remained almost constant and was not affected by age factor as shown in Table II. The total width of the cortex on 25th day was 533.10 ± 28.5 which increased to 816.69 ± 73.90 on 67th day and remained constant afterwards as shown in Table.

Adrenal medulla:

Adrenal medulla was composed of two main types of medullary (chromaffin) cells which were rounded or polygonal in shape and arranged in groups or columns which anastomose in an irregular manner. The cells were surrounded by loose connective tissue which was better developed here than in cortex. Between the cell columns were numerous sinusoids of variable size, reticular fibres (revealed by silver staining) and both myelinated and unmyelinated fibres. The medullary cells showed vesicular nuclei, larger than cortical ones. Both light (with granular cytoplasm) and relatively dark cells comprised the medulla. The ratio of dark and light cells did not follow a constant pattern and was not related to age as shown in Table II. The mitotic activity could be demonstrated in the medulla on one rare occasion in 60 day group.

Adrenal weights:

A comparison of absolute and relative weights of the adrenal at different age periods showed a definite pattern. The mean absolute weight of adrenal on 25th day was 14.33 ± 4.33 mg which increased to 35.00 ± 1.00 on 81st day and remained at constant level afterwards as shown in Table III.

TABLE III. Mean* absolute and relative weights of adrenal at different age periods.

Age (days)	Absolute weight of adrenal (mg)	Relative weight of adrenal (mg/kg body weight)
25	14.33 ± 4.33	27.20 ± 2.85
32	18.50 ± 3.77	24.43 ± 4.17
39	14.67 ± 1.20	16.76 ± 2.44
46	20.83 ± 1.69	12.62 ± 0.33
60	32.33 ± 4.33	15.22 ± 1.15
67	30.33 ± 4.97	17.29 ± 2.44
74	28.00 ± 3.04	14.89 ± 0.93
81	35.00 ± 1.00	11.15 ± 0.14
180	40.33 ± 2.72	11.64 ± 1.00
187	35.50 ± 2.78	11.85 ± 0.67
194	37.17 ± 3.11	10.51 ± 0.98
201	35.33 ± 7.12	11.10 ± 1.94

*Mean $\pm$ standard error

Statistical analysis of the difference in mean width between:

Absolute weight: 25 and 81 days $P < 0.05$ significant increase

81 and 201 days $P > 0.05$ insignificant

Relative weight: 25 and 81 days $P < 0.05$ significant increase

81 and 201 days $P > 0.05$ insignificant

The relative weight of adrenal which was maximum at the age of 25 days showed a significant decline afterwards upto the age of 81 days; whereas no significant change could be detected at later life period as shown in Table III.

Histochemistry:

Intracellular ascorbic acid in the form of fine granules in zona glomerulosa was observed in traces, whereas rich granularity in the inner zones (Figure 4) was seen to increase in density and content upto 74 days age which remained almost constant afterwards till old age. Glycogen in the form of spherical granules or coalased irregular masses was demonstrated in all the zones but with a predominance in fasciculata and reticularis which increased further with advancement of age. PAS positive material was demonstrated in varying amounts in almost all the cortical zones but predominantly in the capsule and the connective tissue of the medulla (Figure 5)



Figure 5. Section of 194 days old male rat adrenal cortex showing the distribution of PAS positive material in the cortical cells with intense staining of capsule (arrow). Paraffin section, PAS stain. Photomicrograph X 375.

which increased further in aged group. Acid phosphatase activity demonstrated as red granules in the cells of medulla and juxtamedullary cortex but diffusion into the rest of cortex was not uncommon. In the advanced age group, the intracellular distribution of stain extended right upto the superficial zona glomerulosa but at no stage the capsule showed any activity of the enzyme.

DISCUSSION

The growth of the adrenal gland in male albino rat was observed to continue between 27 and 81 days but the body weight increased more rapidly during this period, so a decline in the relative weight (mg/100 gm body weight) of adrenal took place. The increase in absolute weight reflects the increase in cortical weight due to expansion of zona glomerulosa and fasciculata upto 67 days and thereafter of medulla upto 81 days. Our observations on the growth of rat adrenal are in complete agreement with findings of previous study on the growth of mouse adrenal⁸. Our findings on fibrous vascular capsule of the adrenal revealed an increase of fibrous and decrease of cellular elements with advancement of age which correspond to the earlier⁹ observations on the capsule. The increased PAS staining of the capsule in older age showed increase of connective tissue fibres and intercellular ground substance. The predominance of the dark cells irrespective of the zone or age was attributed to active hormone producing cells with dense matrix and intramitochondrial tubules whereas light cells showed the actual degenerating process in mitochondria and other organelles with lamellar bodies as observed under electron microscope. The observations of other workers¹⁰ demonstrating the predominance of the light

cells may be attributed to difference in the preparation of the materials as araldite embedded material used in the present study was not used in the previous studies. The subcellular structure of the cortical cells showed mitochondria with intramitochondrial tubular structures (Figure 6)

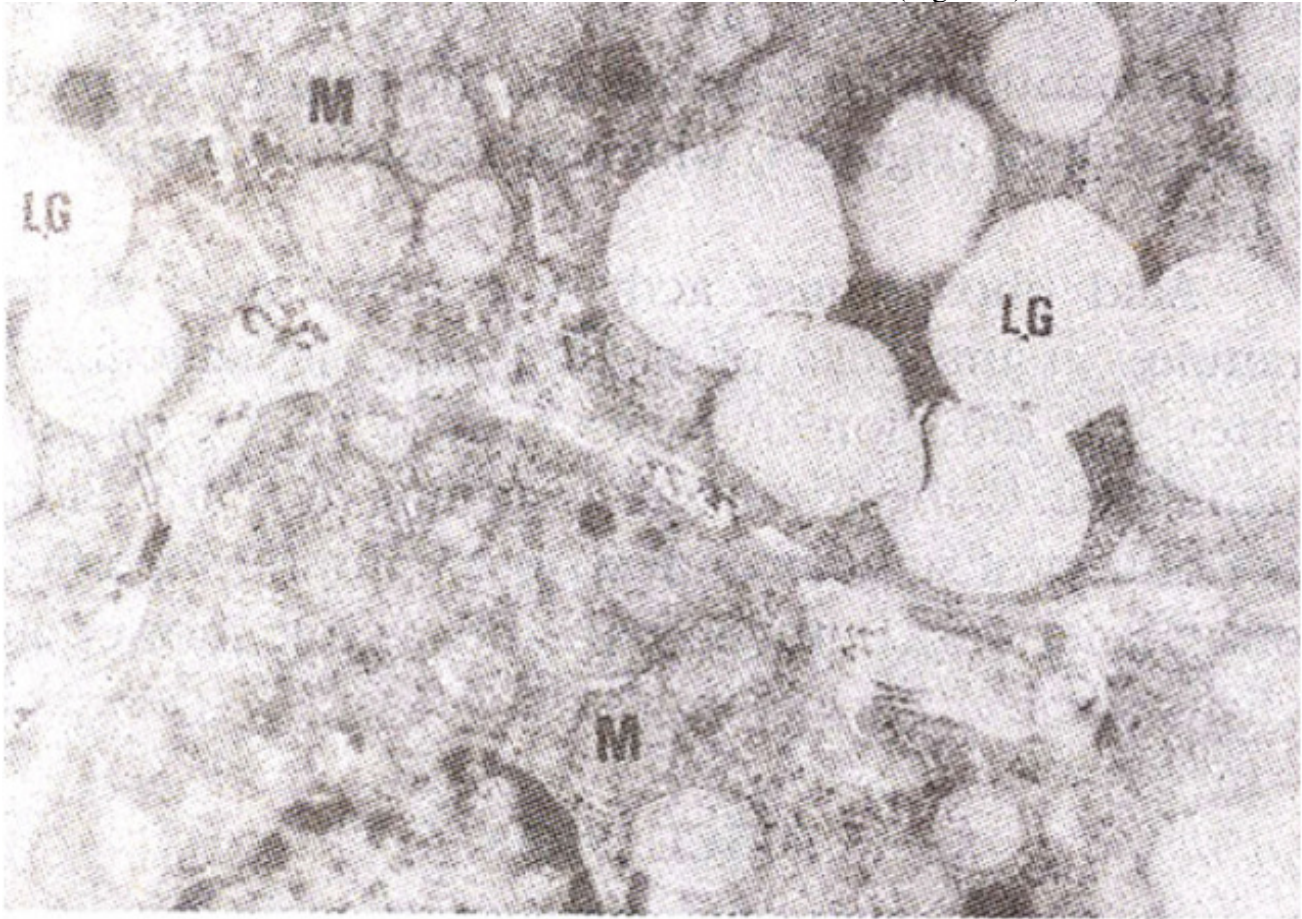


Figure 6. Section of zona glomerulosa of 182 days old male rat adrenal showing numerous mitochondria (M) of moderate electron density with intermitochondrial tubular arrangement and lipid globules (L.G.). Fixed in glutaraldehyde, postfixed in osmium tetroxide. Electron micrograph X 15,000.

which is entirely different from the usual cristae. This difference may be attributed to the formation status for the biosynthesis of steroid hormone as has already been observed in hormone producing cells e.g., interstitial cells of testis¹¹ and corpus luteum¹². Other workers¹³ showed that enzyme pregnenolone and hydrolases are localized in the mitochondrial fraction of the hormone producing cells to cause synthesis of hormone. Zona intermedia was noticed as an inconstant zone irrespective of the age groups, situated between zona glomerulosa and fasciculata. This zone previously described by some workers as lipid-free zone proved to be lipid-poor with fine intracellular localization of lipid globules in tissue fixed with glutaraldehyde and post-fixed in osmic acid. It is interesting to note that when hypophysectomized rats were treated with ACTH, the fat-free cells of zona intermedia were filled with fat and if the treatment was continued, they became indistinguishable from the fat laden cells of zona fasciculata¹⁴. It seems that zona intermedia forms a zone of reserved cells which could transform to fasciculata type of cells when required. The demonstration of ascorbic acid with silver technique and the presence of large deposits of metallic silver in the inner portion of the rat adrenal cortex were observed to increase with advancement of age upto 74 days. These observations coincide with the findings of the previous workers¹⁵. The distribution pattern of glycogen mainly in fasciculata and

reticularis and less so in glomerulosa could be attributed to the zonal activities. It can be stated that the amount of glycogen reflects the metabolic activity of the cortical cells and is inversely related to the zonal activity. The variable amount of glycogen in the capsule, cortical zones and medulla observed in the present study correspond to the earlier findings¹⁶. Histochemical demonstration of Naphthol AS BI phosphate in the present study revealed that in younger age group, acid phosphatase activity was limited to inner reticularis which spread to the outermost glomerulosa in aged group. These histochemical observations were supported by electron microscopic study which demonstrated a gradual increase in lysosomes and lipofuscin components in the inner zone extending to the outer zone with advancing age. In tissues other than adrenal, acid phosphatase activity has been demonstrated both in lysosomes and lipofuscin by other worker¹⁷. The lipofuscin (called wear and tear pigment) increased with age which can be considered as a factor contributing towards acid phosphatase activity in the older age. The increase in pigment most probably is due to decreased metabolic activities in the cells leading to senescence stage or change towards degenerative process which spread from the inner reticularis to the outer zone.

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